Université de Liège
Faculté de Médecine Vétérinaire
Département des Sciences des Denrées Alimentaires
Service d'Analyse des Denrées Alimentaires

Use of Vietnamese plant extracts in striped catfish (*Pangasianodon hypophthalmus*) farming and processing to improve the shelf life of fish fillets

Utilisation d'extraits de plantes Vietnamiennes dans l'élevage et la transformation du poisson-chat rayé (*Pangasianodon hypophthalmus*) pour améliorer la durée de conservation des filets de poisson

NGUYEN Le Anh Dao

Thèse présentée en vue de l'obtention du grade de Docteur en Sciences vétérinaires
Année Académique 2021-2022

Université de Liège
Faculty of Veterinary Medicine
Department of Food Sciences
Laboratory of Food Analysis

Use of Vietnamese plant extracts in striped catfish (*Pangasianodon hypophthalmus*) farming and processing to improve the shelf life of fish fillets

NGUYEN Le Anh Dao

PhD thesis in Veterinary Sciences

Academic year 2021-2022

Jury members:

President:	B. Dewals (ULiège, Belgium)
Promotor:	M.L. Scippo (ULiège, Belgium)
Copromotor:	P. Nguyen Thanh (CTU, Vietnam)
Committee members:	P. Kestemont (UNamur, Belgium)
	J. Quetin-Leclercq (UCLouvain, Belgium)
	P. Tran Minh (CTU, Vietnam)
Members:	K. Raes (UGent, Belgium)
	P. Duez (UMons, Belgium)
	J.L. Hornick (ULiège, Belgium)
	J. Dommes (ULiège, Belgium)
	M. Frederich (ULiège, Belgium)
	N. Moula (ULiège, Belgium)

ACKNOWLEDGEMENTS

The road to success in PhD life is not a bed of roses, but it needs lot of hard work and efforts. No matter what challenges on PhD journey, after all, just believe that everything will be okay. On the way to PhD destination, I learnt great sense of gratitude lessons from my experiences. I am grateful to so many people who were part of my journey, had their faith in me and supported in all extent to make it successful.

First of all, I would like to express my deepest gratitude to **Prof. Marie-Louise Scippo** (ULiège, Belgium), my supervisor. I am greatly indebted to her inspiration, academic supervision and intellectual support during the course of writing this PhD thesis. Her frequent encouragement, valuable judgment and whole-hearted guidance made me develop my passion in scientific research, and the thesis progress are more enjoyable. Moreover, I am extremely grateful to my professor for supporting and understanding my situation during the two interrupted years due to the Covid 19 pandemic. Thanks to her devotion, I have an opportunity to come back ULiège and reach my destination now.

I would also like to thank **Prof. Nguyen Thanh Phuong** (CTU, Vietnam), my co-promoter in Vietnam, and **Prof. Do Thi Thanh Huong** for their considerable support during the planning, sample analysis as well as creating favourable conditions for my dream of studying in Belgium. The study was operated possible through the financial assistance of ARES-CCD project "Natural bio-active plant products for environmental friendly aquaculture production in the Mekong Delta" (AquaBioActive), and I would especially like to thank **Prof. Patrick Kestemont** (UNamur, Belgium), the project leader, for successfully coordinating the AquaBioActive project. I acknowledge the members of my thesis committee, **Prof. Joëlle Quetin-Leclercq** (UCLouvain, Belgium), **Prof. Antoine Clinquart** (ULiège, Belgium) for their successful guidance and suggestion.

It is not impossible to extend my special thanks to **Assoc. Prof. Tran Minh Phu** (CTU, Vietnam), another member of my thesis committee as well as my supporter and my brother. He is an inspirational person who enlarges my positive thinking, gives useful counsels and supports me in the intended direction.

I would like to express my sincere gratitude to the members of my thesis examination panel, **Prof. Katleen Raes**, **Prof. Pierre Duez**, **Prof. Jacques Dommes**, **Prof. Michel Frederich**, **Prof. Jean-Luc Hornick** and **Dr. Nassim Moula**, for contributing their valuable time to read my thesis, and their considerable recommendations and suggestions to improve my thesis manuscript.

I am eternally grateful to my first Belgian teacher in the lab, **Ir. Guy Degand**, for his dedicated guiding in technical practices. I will be inspired to continue to discover technical scientific prospects from his enthusiastic exemplar. I am also very gratefully to **Dr. Caroline Douny**, my Belgian sister, for her supports in sharing knowledge, technical support, writing correction, sharing her experiences of PhD life as well as giving advices on various aspects.

I would like to thank the staffs at the College of Aquaculture and Fisheries, Can Tho University (Vietnam), at the Department of Food Sciences, Laboratory of Food Analysis and Laboratory of Food Microbiology, FARAH - Veterinary Public Health, University of Liege (Liege, Belgium) where my experiments were performed. I appreciate enormously **François Brose** and **Samiha Boutaleb** for their technical assistance and kind guidance on my PhD study. Another special thank goes to our project members and team in CTU (Vietnam) **Assoc. Prof. Bui Thi Buu Hue**, **Dr. Bui Thi Bich Hang**, **Dr. Nguyen Phuc Dam**, **PhD students Le Thi Bach**, **Truong Quynh Nhu**, **Pham Thi Ngoc Nhu** for their support, encouragement and advice during my PhD work. Thank you to **Prof. Frédéric Farnir** (ULiège) and **Christine Bal** (ULiège) for their guidance in the first time I went to Belgium.

My deepest thank to **Dr. Nguyen Quoc Thinh** (CTU), my colleague and my big brother in CTU (Vietnam), for his enthusiastic support and kindness during my PhD journey. Also, I appreciate to **Dr. Tran Le Cam Tu** (CTU) for giving me information and necessary advice to finish the courses of my doctoral schools.

It is my radiant sentiment to place on record my best sense of gratitude to **Assoc. Prof. Pham Kim Dang** (VNUA, Vietnam), **Msc. Hoang Thi Dung** (VNUA, Vietnam), **Bsc. students** (CTU, Vietnam) **Truong Minh Khang**, **Pham Thanh Son**, **Huynh Thi Kim Duyen**, **Phan Nguyen Tuong Vy**, **Pham Phuc Hau**, **Tran Quang Huy**, **Pham Thi Ngoc Nhu**, who worked with me in the activities of this project for their enthusiastic support in collecting and analyzing samples throughout my PhD experiments.

Special words of thank also go to my friends **Robert**, **Soumaya**, **Adolphine**, **Herbert**, **Que**, **Berny's**, **Chimere, Dao Minh Hai, Cao Thu Thuy** for their friendliness and kindness. All of them makes me feel the warmth of friendship without borders. The experiences they have given or been with me will be unforgettable memories in my heart.

During my short stays in Liege, there were predestinations to meet two families, **Thanh Hoa - Benoit Bartiaux** and **Thu Huong – Frederic Mengotto**. I am more grateful to their cares than they will ever know. Their precious sentiments, from those Vietnamese who have been far away from our homeland, are highly appreciated.

Last but not least, I would exceptionally love to express my deepest gratitude and appreciation to my great family, my maternal grandmother, my parents and parents in laws, my elder brother and my sister in-law, my lovely younger sister, my adorable nieces and nephews, who always save unconditional affection and encouragement for myself. Most specially, I am enormously grateful to my husband, **Nguyen Minh Quan**, towards his great sympathies and unreserved sacrifice for my life. With the love and the faith he gives me, I have always been in physical and mental motivation. This achievement could not have been possible without his inspiration.

A new chapter of my life is about to begin!

Nguyen Le Anh Dao

ABBREVIATIONS

BHA	Butylhydroxyanisole
BHI	Brain Heart Infusion
BHT	Butylhydroxytoluene
BNP	Bacillary necrosis of Pangasius
BPW	Buffer pepton water
DMSO	Dimethyl sulfoxide
DPPH	2,2'-diphenyl-1-picrylhydrazyl
EC	European Commission
FDA	Food and Drug Administration
GAE	Gallic acid equivalents
GC-MS	Gas chromatography coupled to a mass spectrometer
GSO	General Statistic Office
IC50	Median inhibition concentration
LC-MS	Liquid chromatography coupled to a mass spectrometer
LC-MS/MS	Liquid chromatography coupled to a tandem mass spectrometer
MAS	Motile aeromonad septicaemia
MDA	Malondialdehyde
MIC	Minimal inhibitory concentration
MUFA	Monounsaturated fatty acid
NAFIQAD	National Agro-Forestry-Fisheries Quality Assurance Department
OD	Optical density
PCA	Plate count agar
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
QI	Quality index
QIM	Quality index method
RASFF	Rapid Alert System for Food and Feed
SFA	Saturated fatty acid
TBARS	Thiobarbituric acid reactive species
TPA	Texture profile analysis
TPC	Total phenolic compounds
TVB-N	Total volatile nitrogen
TVC	Total viable counts
VASEP	Vietnam Association of Seafood Exporters and Producer
VMARD	Vietnamese Ministry of Agriculture and Rural Development

TABLE OF CONTENTS

SUMMARY

Striped catfish (*Pangasianodon hypophthalmus*) has been farmed mostly in the Mekong Delta of Vietnam and is the dominating cultured species for exportation. Fresh fish is considered as a valuable part of human nutrition; however, it is extremely perishable and has a short shelf-life. In addition, the imprudent use of antibiotics, chemicals, plants and plant extracts in aquaculture practices is of concern. Indeed, this uncontrolled use can result in the non-compliance of fish products to international food safety regulations and quality standards, with the consequence of economic losses of the aquaculture sector, which is one of the most important activities in Vietnam (especially in the Mekong Delta). To date, more environmentally friendly prophylactic and protective solutions are developed such as natural bio-active products to enhance the immune system and health status of cultivated animals. Therefore, the assessment of natural bio-active product efficiency in fish health management, as well as of the sanitary and nutritional quality of fish flesh during the rearing phase or post-harvest have been identified among the main priorities in developing sustainable aquaculture systems.

In the situation of extensive application of chemicals and drugs in striped catfish aquaculture, the use of plant or plant products has not been widely studied at the farm level. The first part of the present work was conducted to investigate the current use of drugs, chemicals, plants and plant extract products in a total of 60 grow-out farms of striped catfish (*P. hypophthalmus*) in An Giang and Dong Thap provinces (the Mekong Delta, Vietnam).

The results of the survey indicated that farmers used enrofloxacin and ciprofloxacin, which are banned antibiotics for striped catfish farming, according to Vietnamese regulations. Various plants and plant extracts derived commercial products were used by farmers for various purposes, while the quality and the effectiveness of these products were questionable. Farmers empirically employed traditional plants in aquaculture according to the popular knowledge regarding their use for human medication but did not know which dose to apply for fish rearing. Hence, in-depth studies about the efficient on-farm use of herbals appears to be urgently needed for small farmers as well as for industrial farms.

In this perspective, selected plants were assessed for their efficiency as antioxidant and antibacterial agents for striped catfish feed preservation as well as striped catfish fillets

preservation, either following their use at the post-harvest stage or *in vivo* during striped catfish rearing.

Based on data collected from literature and the survey conducted in fish farms, 20 plants possessing potential antioxidant and antimicrobial activities were selected for *in vitro* studies of their ethanolic extracts. Five plant extracts possessed the strongest antioxidant activity in the subsequent order: *Phyllanthus amarus* > *Piper betle* > *Psidium guajava* > *Euphorbia hirta* > *Mimosa pudica*. *P. amarus* extract also showed the highest activity against two different strains of *Aeromonas hydrophila*; whereas, *P. betle* displayed moderate activity against *Edwardsiella ictaluri*. Tannins were observed as significant factors contributing to the antioxidant and antimicrobial properties of the plant extracts tested.

After this first screening of 20 plant ethanolic extracts, *Phyllanthus amarus* and *Euphorbia hirta* extracts were used in striped catfish fillets dip treatment experiments for the evaluation of their effectiveness to improve the quality of striped catfish fillets during storage. Bacterial load, lipid oxidation status and sensory properties were periodically analyzed in fish fillets during storage. Dip treatments of striped catfish fillets in an aqueous solution containing 0.04% (w/v) *P. amarus* or 0.06% (w/v) *E. hirta* extract resulted in the elongation of their shelf-life up to 8 days under ice storage, and allowed to maintain acceptable bacterial load, good sensory properties and a low level of lipid oxidation.

Plant ethanolic extracts were further studied for their capacity to prevent fat oxidation in fish feed during its preservation at ambient temperature. The results showed that ethanolic extracts of two plants (i.e. *M. pudica* and *P. guajava*) would be appropriate candidates as a natural antioxidant to preserve striped catfish feed during storage at room temperature. From these two plants, *M. pudica* ethanolic extract (at the concentration of 4 or 20 g/kg fish feed) showed the best capacity as a feed preservative as it was able to mitigate lipid oxidation in fish feed after 8 weeks of storage at ambient temperature.

Finally, two *in vivo* experiments were conducted, consisting of striped catfish supplementation with plant extracts for 2 months, one using 5 different plants extracts (i.e. *P. amarus*, *P. guajava, E. hirta*, *M. pudica* and *Azadiracha indica*) and one using two plant extracts (i.e. *P. amarus* and *P. guajava*) individually or as a mixture. After harvesting, catfish fillets were stored under ice and periodically analyzed for their bacterial load, lipid oxidation status and sensory properties. The results of the analysis of fish fillets in 16 days of ice storage showed that

E. hirta, P. guajava and *P. amarus* extracts supplementation allowed to decrease the bacterial load and the fatty acid oxidation product content of fish fillets and to improve their organoleptic quality. This positive effect was not observed when *P. guajava* and *P. amarus* extracts were given together to striped catfish. In conclusion, *P. guajava* extract used at 5 g/kg feed could be the best choice, as feed additive in aquaculture, to improve the quality of striped catfish fillet during post-harvest ice storage.

RÉSUMÉ

Le poisson-chat rayé (*Pangasianodon hypophthalmus*) est élevé principalement dans le delta du Mékong au Vietnam et est l'espèce d'élevage dominante pour l'exportation. Le poisson frais est considéré comme un élément précieux de l'alimentation humaine; cependant, il est extrêmement périssable et a une courte durée de conservation. En outre, l'utilisation imprudente d'antibiotiques, de produits chimiques, de plantes et d'extraits de plantes dans les pratiques aquacoles est préoccupante. En effet, cette utilisation incontrôlée peut entraîner la non-conformité des produits halieutiques aux réglementations internationales de sécurité sanitaire des aliments et aux normes de qualité, avec pour conséquence des pertes économiques du secteur de l'aquaculture, qui est l'une des activités les plus importantes au Vietnam (surtout dans le Mékong Delta). A ce jour, des solutions prophylactiques et protectrices plus respectueuses de l'environnement sont développées telles que des produits naturels bio-actifs pour renforcer le système immunitaire et l'état de santé des animaux d'élevage. Par conséquent, l'évaluation de l'efficacité des produits bioactifs naturels dans la gestion de la santé des poissons, ainsi que de la qualité sanitaire et nutritionnelle de la chair de poisson pendant la phase d'élevage ou après la récolte ont été identifiées parmi les principales priorités dans le développement de systèmes d'aquaculture durables.

Dans le cadre de l'application intensive de produits chimiques et de médicaments dans l'aquaculture du poisson-chat rayé, l'utilisation de plantes ou de produits végétaux comme alternatives n'a pas encore été très étudiée au niveau de la ferme. La première partie du présent travail a été menée pour étudier l'utilisation actuelle de médicaments, de produits chimiques, de plantes et d'extraits de plantes dans un total de 60 fermes d'élevage de poisson-chat rayé (*P. hypophthalmus*) dans les provinces d'An Giang et de Dong Thap (Delta du Mékong, Vietnam).

Les résultats de l'enquête ont indiqué que les agriculteurs utilisaient l'enrofloxacine et la ciprofloxacine, qui sont des antibiotiques interdits pour l'élevage du poisson-chat rayé, conformément à la réglementation vietnamienne. Divers produits commerciaux dérivés de plantes et d'extraits de plantes étaient utilisés par les agriculteurs à diverses fins, tandis que la qualité et l'efficacité de ces produits étaient discutables. Les agriculteurs ont employé empiriquement les plantes traditionnelles en aquaculture selon les connaissances populaires concernant leur utilisation pour la médecine humaine, mais ne savaient pas quelle dose appliquer pour l'élevage de

poissons. Par conséquent, des études approfondies sur l'utilisation efficace des plantes médicinales à la ferme semblent être nécessaires de toute urgence pour les petits agriculteurs ainsi que pour les fermes industrielles.

Dans cette perspective, des plantes sélectionnées ont été évaluées pour leur efficacité en tant qu'agents antioxydants et antibactériens pour la conservation de l'alimentation des poissons chats rayés ainsi que pour la conservation des filets de poisson chat rayé, soit suite à leur utilisation au stade post-récolte, soit *in vivo* lors de l'élevage.

Sur la base des données recueillies dans la littérature et de l'enquête menée en pisciculture, 20 plantes possédant des activités antioxydantes et antimicrobiennes potentielles ont été sélectionnées pour des études *in vitro* de leurs extraits éthanoliques. Cinq extraits de plantes possédaient la plus forte activité antioxydante dans l'ordre suivant: *Phyllanthus amarus* > *Piper betle* > *Psidium guajava* > *Euphorbia hirta* > *Mimosa pudica*. L'extrait de *P. amarus* a également montré l'activité la plus élevée contre deux souches différentes *d'Aeromonas hydrophila*; alors que *P. betle* a montré une activité modérée contre *Edwardsiella ictaluri*. Les tanins ont été observés comme des facteurs importants contribuant aux propriétés antioxydantes et antimicrobiennes des extraits de plantes testés.

Après ce premier criblage de 20 extraits éthanoliques de plantes, des extraits de *Phyllanthus amarus* et *d'Euphorbia hirta* ont été utilisés dans des expériences de traitement par trempage de filets de poisson chat rayé pour l'évaluation de leur efficacité à améliorer la qualité des filets de poisson pendant le stockage. La charge bactérienne, l'état d'oxydation des lipides et les propriétés sensorielles ont été périodiquement analysés dans les filets de poisson pendant le stockage. Les traitements par trempage des filets de poisson-chat rayé dans une solution aqueuse contenant 0,04 % (p/v) de *P. amarus* ou 0,06 % (p/v) d'extrait *d'E. hirta* ont entraîné l'allongement de leur durée de conservation jusqu'à 8 jours sous glace, et a permis de maintenir une charge bactérienne acceptable, de bonnes propriétés sensorielles et un faible niveau d'oxydation des lipides.

Des extraits éthanoliques de plantes ont été étudiés plus avant pour leur capacité à empêcher l'oxydation des graisses dans l'alimentation des poissons lors de sa conservation à température ambiante. Les résultats ont montré que les extraits éthanoliques de deux plantes (*M. pudica* et *P. guajava*) seraient des candidats appropriés en tant qu'antioxydants naturels pour conserver les aliments du poisson-chat rayé pendant le stockage à température ambiante. De ces

deux plantes, l'extrait éthanolique de *M. pudica* (à la concentration de 4 ou 20 g/kg d'aliments pour poissons) a montré la meilleure capacité en tant que conservateur alimentaire car il a pu atténuer l'oxydation des lipides dans les aliments pour poissons après 8 semaines de stockage à température ambiante.

Enfin, deux expériences *in vivo* ont été menées, consistant en une supplémentation de poissons-chats rayés avec des extraits de plantes pendant 2 mois, une utilisant 5 extraits de plantes différents (*P. amarus*, *P. guajava*, *E. hirta*, *M. pudica* et *Azadiracha indica*) et une en utilisant deux extraits de plantes (*P. amarus* et *P. guajava*) individuellement ou en mélange. Après la récolte, les filets de poisson-chat ont été stockés sous glace et analysés périodiquement pour leur charge bactérienne, leur état d'oxydation des lipides et leurs propriétés sensorielles. Les résultats de l'analyse des filets de poisson durant 16 jours de stockage sous glace ont montré qu'une supplémentation en extraits *d'E. hirta*, *P. guajava* et *P. amarus* permettait de diminuer la charge bactérienne et la teneur en produits d'oxydation des acides gras des filets de poisson et d'améliorer leur qualité organoleptique qualité. Cet effet positif n'a pas été observé lorsque des extraits de *P. guajava* et de *P. amarus* ont été administrés ensemble à des poissons-chats rayés. En conclusion, l'extrait de *P. guajava* utilisé à raison de 5 g/kg d'aliment pourrait être le meilleur choix, comme additif alimentaire en aquaculture, pour améliorer la qualité du filet de poisson-chat rayé pendant le stockage sous glace après la récolte.

CONTEXT OF THE STUDY

Plants are miscellaneous resources regarding biological and chemical diversification. It has been estimated that there are 250,000 to 500,000 species of plants on the Earth (Borris, 1996). People applied plants as traditional medicines for treating a variety of diseases during human history. A wide range of secondary metabolites are found in plants, which are phytochemical constituents such as phenolics, alkaloids, quinones, terpenoids, lectines, and polypeptides.

In the report of the World Health Organization (WHO, 2011), approximately 80% of the population in Asian and African countries put their faith upon traditional medicine for preliminary health care. Beside the Chinese herbals, Vietnamese medicinal plants have received interest as novel sources for substitute medication during the last decades. It is estimated that approximately 2500 species of the Southeast Asian tropical exotic herbals have been utilized in folk remedy for their natural properties (Banskota et al., 2003; Chi, 1997), such as for instance, diuretic activity (Doan et al., 1992), antioxidant activity (Thuong et al., 2006), cytotoxicity (Nguyen et al., 2005; Thu et al., 2010) and antimicrobial activity (Hue Ngan et al., 2008). Several Vietnamese medicinal plants also showed potential *in vitro* antioxidant activity throughout both DPPH (2,2'-diphenyl-1-picrylhydrazyl) and lipid peroxidation screening assays (Thuong et al., 2006).

Aquaculture production in Vietnam has been growing remarkably in the period 1995-2020, from 415 thousand tons to nearly 4.6 million tons (VASEP, 2021). Mekong delta is the main fish production area in the Southern part of Vietnam with a contribution of 70% to the national aquaculture production. Striped catfish (*Pangasianodon hypophthalmus*) is the dominating cultured species for export with an annual production of 1.1 million tons (Phu et al., 2016). Chemicals including antimicrobials have been extensively applied to control pathogens and water quality management (Phu et al., 2016; Rico et al., 2013). The imprudent use of antimicrobial to control bacterial disease in fish farming may lead to the development of antibiotic resistant bacteria, to the presence of residues of antimicrobial in fish products, to adverse effects on the environment, etc. The access to export markets is certainly warned by the risk of non-compliance to international food safety regulations and quality standards. The final outcome could be a decline of profits made by the aquaculture sector, which is one of the most important activities in Vietnam (especially in the Mekong delta).

Nowadays, more environmental-friendly prophylactic and protective solutions are claimed, and natural bio-active products are examined for enhancing the immune system and health status of cultivated animals. However, despite a great variety of wild plants available in the various regions of Vietnam and the concern of aquaculture farmers in using alternatives to antibiotics, the use of natural products in aquaculture is not yet popular in the country. This is due to the lack of information regarding the existence of bio-active products and the lack of evidence of their efficacy on fish. Therefore, the study of the evaluation of natural bio-active product efficiency in fish health management, including the sanitary and nutritional quality of fish flesh during the rearing to the post-harvest phase has been identified as one of the major issues in developing sustainable aquaculture systems.

To contribute to facing the issues mentioned above, the AquaBioactive project, funded by ARES-CCD, entitled "Natural bio-active plant products for environmentally friendly aquaculture production in the Mekong Delta", has kick-started in 2015. The project was coordinated by Prof. Patrick Kestemont (UNamur) and Prof. Nguyen Thanh Phuong (CTU, Vietnam), with the collaboration of ULiège and UCLouvain. The first activity of our project was undertaken by College of Natural Sciences (Can Tho university) and UCLouvain, throughout bibliography review of bio-active compounds from plants (antimicrobial, antioxidation activities and as immunostimulants for aquaculture), identification and collection of wild or cultivated plants potentially containing bio-active compounds in Vietnam and preparation of extracts from selected plants. Later on, integrated activities were simultaneously carried out by UNamur and ULiège for screening immunomodulatory activities and antioxidant/antibacterial activities of selected plant extracts respectively, using *in vitro* and *in vivo* approaches.

In this project, a list of useful plant extracts as a feasible alternative to the overuse of chemicals in Vietnamese aquaculture has been provided, and methods for extraction, characterization, bioactivity evaluation and formulation of high-value natural derived materials was planned to be developed for aquaculture production at affordable prices for farmers.

The two main diseases in the pangasius catfish industry were determined to be caused by aetiological agents such as *Aeromonas hydrophila* and *Edwardsiella ictaluri*. In their report of 2001, Ferguson et al. described the clinical pathology observed in sick animals as a severe multifocal necrotizing bacterial infection and named the disease as bacillary necrosis of pangasius

(BNP) (Ferguson et al., 2001). *Edwardsiella ictaluri* was identified as the aetiological agent of BNP infections in natural outbreaks (Crumlish et al., 2002). Other fish losses in Vietnamese *P. hypophthalmus* production systems were due to *Aeromonas hydrophila* outbreaks (Subagja et al., 1999). According to Newman (1993), *Aeromonas hydrophila* caused diseases in a wide range of freshwater fish species. This species is the aetiological agent of motile aeromonad septicaemia (MAS) and is often related to stressed or immunocompromised hosts (Roberts, 1993). Thus, the screening of antibacterial capacity of plant extracts against these two species is particularly important for striped catfish aquaculture.

Several studies have reported that plant extracts contain active compounds, e.g phenolic compounds, alkaloids, quinones, terpenoids, lectines, and polypeptides, which have been shown to be efficient alternatives to traditional chemotherapies, antibiotics, and vaccines (Dawood et al., 2018; Harikrishnan et al., 2011). Moreover, these compounds are often inexpensive, and act against a broad spectrum of pathogens. Also, they are easily bio-degradable and environment friendly. Beside the health effects on cultivated organisms, natural bioactive products could enhance the nutritional quality of aquaculture products, due to their protective effect against lipid or protein degradation. Indeed, at the pre-harvest stage, lipids contained in fish could be protected from oxidation by these natural products (Asimi and Sahu, 2013; Hamre et al., 2010). Furthermore, these compounds exert selective antibacterial activity, which can lead to the selection of a beneficial microflora, playing a role in lipid and protein metabolism (Fadhlaoui-Zid et al., 2012; Rodriguez et al., 2014). Moreover, an antioxidant compound used as a fish dietary supplement would seem to work with a double function, i.e., protecting the fat contained in the feed and preserving the fish flesh through the antimicrobial activity of its active constituents or their metabolites (Hernández et al., 2014). At the post-harvest stage, natural compounds ingested by the fish can partly continue to exert their protective action against lipid oxidation or protein degradation, compared to standard products. The application of the mentioned compounds could be a useful method for the sustainability of the aquaculture sector, regarding eco-friendly farming practices, high sanitary and nutritional quality products, environmental protection, and reducing use of prohibited substances. Vietnam displays an enormous geographical diversity and diverse botanical resources, which have a great potential to support high-value aquaculture and agriculture products.

OBJECTIVES OF THE THESIS

General objectives

This research aimed to study the efficiency of natural bio-active products (extracted from plants) after their application at various stages of striped catfish (*Pangasianodon hypophthalmus*) production (for fish feed preservation, during fish cultivation as well as at the post-harvest stage) to improve the safety and nutritional quality of fish fillets in laboratory as well as on-farm conditions.

Specific objectives

1. To determine (thanks to a survey) the current use of drugs, chemicals, herbals and herbal extract products in grow-out farms striped catfish (*Pangasianodon hypophthalmus*) in the Mekong Delta.
2. To determine the *in vitro* antioxidant and antimicrobial activities of 20 herbal extract samples and 3 commercial products for initial selection of potential application in aquaculture products storage.
3 To evaluate the quality changes of striped catfish fillets, after dip treatment with *Phyllanthus amarus* and *Euphorbia hirta* extracts, during ice storage to provide scientific evidence for a response to any specific demand regarding striped catfish fillets quality issues during storage.
4 To evaluate the antioxidant activity of plant ethanolic extracts in fish feed during its storage at ambient temperature.
5 To examine the impact of striped catfish dietary supplementation with herbal ethanolic extracts on the quality of fish flesh under ice storage.

1. INTRODUCTION

1.1. Striped catfish aquaculture

The striped catfish, *Pangasianodon hypophthalmus* Sauvage, 1878, is a native freshwater fish which disseminated in four countries: Thailand, Laos, Cambodia and Vietnam where Mekong and Chao Phraya rivers in the Lower Mekong basin flow through (Rainboth, 1996; Roberts and Vidthayanon, 1991). This species was domesticated in Thailand (1967) and in Vietnam (1979) (Trong et al., 2002). Vietnam is the main producer of *P. hypophthalmus* over the world, with more than 5000 hectares (ha) of farming area for 1.1 million tons of cultured fish (Hoe et al., 2016; Nguyen et al., 2015). During the last two decades, the production of striped catfish in Vietnam was continuously increasing, achieving 1.33 million metric tons, and valuing 2.36 billion USD dollars in 2018 (VASEP, 2019). Striped catfish (*P. hypophthalmus*) is one of the commercially dominating cultured species in Mekong delta as well as in Southeast Asia (Phu et al., 2016; Rainboth, 1996; Roberts and Vidthayanon, 1991). Vietnamese striped catfish was exported to 132 markets in 2018, with an export value of 2260 million USD (Figure 1) (VASEP, 2020). The huge development over the past decade of striped catfish culture in Mekong Delta was outlined as a success story of aquaculture in Vietnam (De Silva and Phuong, 2011; Phuong and Oanh, 2009).

Over the last decade, pond culture has become the predominant form of striped catfish farming in the Mekong Delta among three farming systems, including ponds (Figure 2a), cages (Figure 2b) and pens (Figure 2c). According to Phan et al. (2009), in pond farming systems, the area of water surface and of the farm ranges between 0.12 and 20 ha and between 0.2 and 30 ha, respectively, while the number of ponds per farm ranges from 1 to 17 and each pond size varies between in average between 0.08 and 2.2 ha, respectively. Phan and coworkers (2009) reported that the pond depth in striped catfish farms ranges from 2.0 to 6.0 m with the great majority of farms (69%) having pond water depths of 3.5 to 4.5 m (Phan et al., 2009). In 2011, the production of striped catfish farming system was on average 200–400 tons/ha per crop on approximately 6000–7000 ha of land (De Silva and Phuong, 2011).

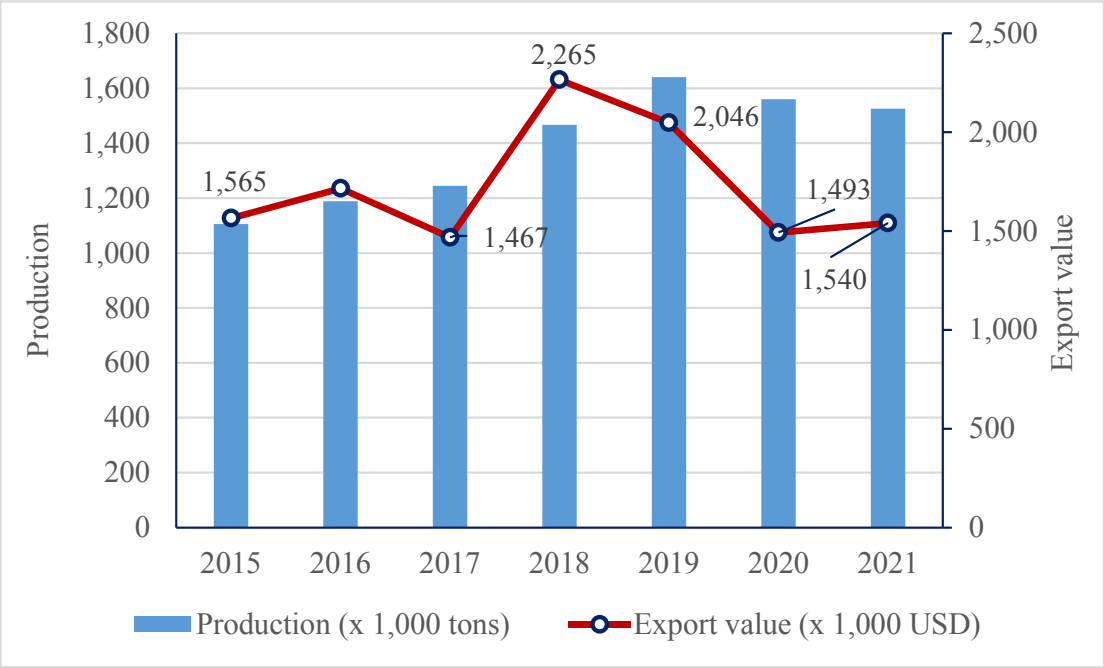


Figure 1. The production and export value of striped catfish in Vietnam (VASEP, 2020)

Figure 2. Striped catfish farming system in Mekong Delta: ponds (a), cages (b), pens (c)

After each harvest, 60 to 80% of pond water is pumped out at level of 60-80%. Because of the collapse of the dike, it is not possible to empty the pond completely. Calcium hypochlorite (0.1

mg/L) is then applied to disinfect the water. Before stocking fish, three to five days before stocking fish, the pond is filled with water and treated with the disinfectant benzalkonium chloride (0.4 mg/L) or iodine (0.1 mg/L). Copper sulphate (0.1 mg/L) and calcium hypochlorite (0.1 mg/L) were also used by some nursery and grow-out farms for external parasite control at a frequency of up to 5–7 times per crop (Phu et al., 2016).

The striped catfish production and value chain could be divided into four main steps involving seed production in hatchery (10-12 hours), nursery (1-1.5 months), grow-out (6-7 months) and processing (Phuong and Oanh, 2010). To date, striped catfish has been fed with commercial pelleted feeds (floating form). After the growing period, striped catfish are harvested at a size ranging from 0.6 to 1.5 kg (mean 1.0 kg) (Figure 3a). The fish are checked for their appearance, flesh color, chemical residues (especially for banned chemicals) by processors before purchased (De Silva, 2008). The transportation of harvested live fish to the processing companies is performed either by river in the hull of boats or by road in trucks equipped with holding tanks (Phan et al., 2009).

Figure 3. Striped catfish (*Pangasianodon hypophthalmus*) (a), Processing plant (b), fish fillet products (c)

According to the processing of striped catfish fillets described by Tong Thi et al. (2013) when arriving at the companies, bleeding, filleting and washing are done manually (Figure 3b), followed by skin removal by a skinning machine. Then, the subcutaneous fat and red muscle are trimmed manually with a knife. Next to, the fillets are sorted manually into white, pink to red and yellow groups. Each fillet is placed on a translucent table illuminated from below for detecting parasites. In the next step, the fillets are untreated or treated with additives (sodium tripolyphosphate) in tumblers according to the requirement of customers. After that, the fillets are categorized by the size classifying machines. The fillets placed in plastic bags are then cooled with flake ice before freezing. In the freezing process, the individual fillets are frozen until a central temperature of -18 °C is reached. Before packaged and labeled, the frozen fillets are glazed by dipping quickly in cold water mixed with flake ice and refrozen. This leads to a shiny appearance of fillets, lowers the lipid oxidation as well as precludes freezer-burn during storage (Figure 3c). Finally, the packed frozen products in carton boxes are stored in warehouses at -18°C.

In recent years, fish production increased due to the intensification of the farming area and fish density for maximizing farmer's profit. The intensive production of striped catfish in open farming systems has been mostly associated with the eruption of infectious diseases. Several studies evidenced that the primary pathogens that mostly caused mortality in striped catfish farms were parasites, bacteria, and fungi (Baska et al., 2009; Duc et al., 2015; Dung et al., 2008; Ly et al., 2009; Székely et al., 2009; Tien et al., 2012). Beside the parasite infestation, bacterial pathogens *Edwardsiella ictaluri*, *Edwardsiella tarda, Flavobacterium columnare*, and *Aeromonas hydrophila* have been accountable for the considerable mortality in striped catfish farms (Crumlish et al., 2010, Panangala et al., 2007, Shetty et al., 2014). To the extent of our knowledge, *E. tarda* has not been reported in striped catfish farms in Vietnam, although Shetty et al. (2014) mentioned their presence in striped catfish farms in India. Specially, *E. ictaluri* is presently the most economically serious pathogen being the causal agent of Bacillary Necrosis Pangasius (BNP) in intensively reared striped catfish from the Mekong Delta (Crumlish et al., 2002, Ferguson et al., 2001). Phan et al. (2009) reported from a survey that BNP occured in 98% of striped catfish farms and caused up to 90% mortality. The mortality caused by *E. ictaluri* was determined in all stages of striped catfish, although fingerlings and juveniles seem to be more sensitive than the adult stage (Dung et al., 2008). Similarly, *A. hydrophila* is also considered as a major pathogen, causing massive economical losses in striped catfish farms (Subagja et al., 1999) due the motile aeromonad

septicemia (MAS) (Newman, 1993). The outbreak of MAS is frequently accompanied with stress due to the poor environment or to the immunocompromised status of hosts. Moreover, *A. hydrophila* is also considered as an opportunistic pathogen, which could develop during stressful conditions, involving bacterial challenge with another pathogen (Nusbaum and Morrison, 2002). Furthermore, *F. columnare* which caused columnaris disease, is known as one of the oldest bacterial pathogens in freshwater fish including striped catfish (Hawke et al., 1981, Declercq et al., 2013). In the United States, *F. columnare* was seen as the second significant bacterial pathogen warning in the catfish culture industry after *E. ictaluri* (Shoemaker et al., 2007).

A variety of antibiotics are used for disease prevention and treatment during production stages (Phu et al., 2016). Amoxicillin is the most used antibiotic, followed by doxycycline and sulfamethoxazol + trimethoprim in striped catfish aquaculture in Mekong Delta region (Thinh and Phu, 2021). Especially, enrofloxacin has been included in the list of banned antibiotics since 2012 (VMARD, 2012), whereas ofloxacin and levofloxacin (belonging to the fluoroquinolones group) were also added to this list in 2016 by Circular No. 10/2016/TT-BNNPTNT (VMARD, 2016) due to their toxicity on abnormal development, histopathological changes (Wang et al., 2014; Yin et al., 2014), potential environmental risks (Sun et al., 2016) and common cross-resistance among the fluoroquinolones in bacteria (Wolfson and Hooper, 1985). According to a report of Thinh and Phu (2021), 12 types of antibiotics (allowed, based on the regulations of the Ministry of Agriculture and Rural Development) and 2 banned antibiotics (enrofloxacin and ciprofloxacin) were used in the striped catfish farming systems in the Mekong Delta.

Chemical residues (antimicrobials) and pathogens in striped catfish products are considered as food safety hazards. The European Rapid Alert System for Food and Feed (RASFF) is intended to inform EU member countries about non compliant food detected at EU borders. In the period 2001-2003, 76 seafood shipments which violated antibiotic residue regulations were reported in the RASFF (Phu et al., 2016). The return of these shipments to exporters resulted in a loss of USD 15 million (Thanh and Chuong, 2010). Moreover, numerous violations of Vietnamese aquaculture production related to microorganism hygiene standards and antibiotic residues were reported in 2005 in USA (Hanh, 2005). To face food safety requirements from export markets, the Vietnamese National Agro-Forestry-Fisheries Quality Assurance Department (NAFIQAD) was established to implement a domestic residue control plan (Phu et al., 2016). In addition, the use of

antimicrobials and chemicals, which were banned in importing countries but approved in Vietnamese aquaculture, was regularized by the Vietnamese Ministry of Agriculture and Rural Development (VMARD) (Chi et al., 2017). Certification requirements, e.g. Good Agricultural Practices (GAPs) (VietGAP, GlobalGAP, etc.) were developed and the aquaculture industry in Vietnam was encouraged to fulfill it. Farmers need to be regularly updated in terms of which antimicrobials are approved for use in aquaculture by the local Departments of Aquaculture (Chi et al., 2017). As a full traceability system is lacking, raw material must be regularly tested by the processors to check its quality (e.g. absence of residues of antimicrobials) prior to purchase it from independent farms (Phu et al., 2016).

Striped catfish is exported worldwide as skinless and boneless fillets (Tong Thi et al., 2013). Catfish meat is characterized by tender and white flesh, absence of fishy odour, firm cooked texture and high nutritional value as well as excellent sensory properties (Rao et al., 2013). The nutritional value and physical properties of fish meat can vary considerably between individuals of the same species (Cruz Casallas et al., 2012). A study of Orban et al., (2008) showed that striped catfish fillets exported from Vietnam were characterized by high moisture (80-85%) and low crude protein (12.6-15.6%) and lipid (1.1-3.0%) levels. The characterization of total lipids in this species showed lower cholesterol levels of 21-39 mg/100 g, when compared to those in marine fish such as Coho salmon (*Oncorhynchus kisutch*) containing 51 mg/100 g of cholesterol or rainbow trout (*Oncorhynchus mykiss*) containing 59 mg/100 g (Nettleton and Exler, 1992). According to Orban et al. (2008), the fatty acid profile of total lipids from striped catfish fillets as they are exported, was dominated by high percentages of saturated fatty acids (41.1-47.8% of total fatty acid), mainly palmitic (C16:0, 27.5–28.8% of total fatty acids) and stearic acids (C18:0, 8.9–15.4% of total fatty acids). Monounsaturated fatty acids (33–37% of total fatty acids), were mainly represented by oleic acid (C18:1, ω-9) which contributed to 90% of total monounsaturated (28.9–33.8% of total fatty acids). Levels of polyunsaturated fatty acids (PUFA) were determined low (12.5–18.8% of total fatty acids) with a higher percentage of ω-6 than ω-3 PUFA, which is a characteristic common to numerous freshwater fish species (Henderson and Tocher, 1987). Among the PUFAs, linoleic acid (C18:2, ω-6, 7–8% of total fatty acids) mainly occupied 44-59% of total PUFA, whereas the other PUFA were arachidonic acid (C20:4, ω-6, 1.5–3.6% of total fatty acids) and docosahexaenoic acid (C22:6, ω-3, 1.7–3.6% of total fatty acids). The content of fatty acids belonging to the ω-3 series was very low (sum of ω-3 PUFA: 2.6–6.7% of total fatty acids) while

docosahexaenoic acid occupying more than 60% of total ω-3 PUFA. Regarding safety aspects, the quality of striped catfish samples analyzed was in high-quality, with low residue concentrations of mercury, organochlorine pesticides, polychlorinated biphenyls as well (Orban et al., 2008). Residue monitoring program in striped catfish aquaculture is monthly carried out in the South of Vietnam by NAFIQAD. Recent results reported that chemical contaminants (such as Hg, Pb, Cd or organochlorinated pesticides) were not found in the fish muscle. Likewise, antibiotic residues (amoxicillin, ormetoprim, ivermectin, trichlorfon, praziquantel, avermectin B1a and sulfonamides group) or banned products (malachite green, leucomalachite green, nitrofurans and quinolones) were not found in tested fish (NAFIQAD, 2021).

1.2. Plant extracts application in striped catfish aquaculture

General information about plant use in aquaculture

"Medicinal plants can be defined as the plants that possess therapeutic properties or exert beneficial pharmacological effect on the human or animal body" (Namdeo, 2018). Herbal medicines proved to be the major remedy in traditional system of medicine. The therapeutic potency of a medicinal plant is due to the presence of some bioactive components. However, medicinal plants have to be used with caution and further studies need to be performed to determine the impact of medicinal plants on human health. Some plants are both medicinal and food plants, such as garlic.

Food plants are those plants that produce food that we consume in our daily life. Many food products that we eat are derived from the stems, leaves, and roots, and other parts of different plants. Food from plants is a good source of nutrients and includes carbohydrates, vitamins, minerals, proteins, and fats.

The application of medicinal plants as well as food plants in aquaculture was reported in various Asian countries (Direkbusarakom, 2004). More than 60 different plant species have been evaluated for their capacities on anti-stress, growth promotion, anti-pathogen, improving immune response in aquaculture, which were most widely investigated in folk medicine in China, India, Thailand, Korea and Vietnam (Ashraf and Goda, 2008; Bulfon et al., 2014; Bulfon et al., 2015; Francis et al., 2005; Beltrán et al., 2020; Magnadottir, 2010; Ninomiya et al., 1995; Van Doan et

al., 2019; Nhu et al., 2019a). Vietnam has a diversity of 3780 medicinal plant species (Trang Thi Huong Tran et al., 2016), which can be a great advantage to get the best for fish aquaculture and update the scientific knowledge of using medical plants in fish farming, instead of using food plants only.

1.2.1 Factors affecting the antioxidant and antimicrobial efficiency of plants and plant products

1.2.1.1 Bioactive compounds contained in plants and plant extracts

Plant active compounds can primarily be classified based on their chemical structure into alkaloids, terpenoids, polyphenols, glycosides, propyl disulfide, glucosinolates, isothiocyanates, cyanogenic glycosides, oxylipins (Lovkova et al. 2001), which have biological effects on cardiovascular, digestive, endocrine, immune, and nervous systems (Salehi et al., 2020). According to most studies, these molecules demonstrated hypotensive, antioxidant, antimicrobial, immunomodulatory, anticancer, antithrombotic, opioid, hypocholesterolemic or anti-diabetic, hypocholesterolemic and anti-inflammatory effects (Harborne, 1990; Dillard and German, 2000). The first highlighted group of secondary metabolites is polyphenols, which is characterized by the presence of aromatic rings and the hydroxyl phenolic group. Polyphenols are acknowledged as strong natural antioxidants playing a key role in a wide-range of biological and pharmacological properties such as antimicrobial, anti-inflammatory, anticancer, antiallergic, etc. The antimicrobial activity of plant secondary metabolites is correlated to the -OH group(s) attached to the phenol ring. The aromaticity can also be accountable for this effect (Brglez Mojzer et al., 2016). Terpenes can act as antioxidant compounds through direct ROS scavenging pathway and modulating the endogenous antioxidant system (Gonzalez-Burgos and Gomez-Serranillos, 2012). Terpenoids antimicrobial properties are associated to their functional groups. Specially, in phenolic terpenoids, hydroxyl groups and delocalized electrons demonstrate functions against microorganisms. These compounds can easily interact with bacterial wall impeding with the biosynthesis of its components possessing their lipophilic property. They indicate their ability in the bacterial cell penetration and breaking protein synthesis, DNA replication and repair mechanisms (Kıyama, 2017; de Oliveira-Júnior et al., 2018). Alkaloids, at molecular level, can influence membrane permeability, membrane proteins with ion channels and receptors, enzymes and other proteins, i.e

DNA, RNA and corresponding proteins, electron chain, and the cytoskeleton (Wink, 2016). An extensive range of alkaloids have effects on nervous system such as antidepressant effects mediated by serotonergic, noradrenergic, and dopaminergic intervention. Moreover, alkaloids stimulate α- and β-receptor and improve the activity of prefrontal cortex, thalamus and visual system (Perviz et al., 2016).

Regarding the use of plants in aquaculture, most studies usually do not supply any data about the chemical constituents of the plant products (extracts, oils…). However, it is believed that the antimicrobial/immunomodulatory activities could be attributed to secondary metabolites of the classes of compounds mentioned above. For instance, *Psidium guajava* ethanol extract was shown to mainly contain flavonoids and triterpenic derivatives, whereas the ethanolic extract of *Phyllanthus amarus* was shown to contain hypophyllantin, the major active compound also remaining after removing tannins (Nhu et al., 2020b). According to Nhu et al. (2020a), the oral administration of *P. amarus* ethanolic extract at the concentration of 0.5% (w/w) in feed for six weeks could diminish the impact of the infection due to the pathogen *Edwardsiella ictaluri* in striped catfish (*Pangasianodon hypophthalmus).* Previous studies reported that feeding fish with a diet containing *P. guajava* extract showed a significant capacity of improving the growth performance, antioxidant, and immune parameters in rohu *Labeo rohita* (Fawole et al., 2016; Giri et al., 2015), Mozambique tilapia *Oreochromis mossambicus* (Gobi et al., 2016) and common carp *Cyprinus carpio* (Hoseinifar et al., 2019). Abundant studies about the bulbs of garlic (*Allium sativum*), which contain an acrid volatile oil (0.25%), organo-sulfur compounds formed from alliin and allicin, flavonoids, sapogenins and saponins, selenium compounds (fructosamines), proteins (amino acids, glutamyl peptides), glucides, enzymes (alliinase, peroxidase, myrosinase) reported their effectiveness in controlling fish diseases, increasing the cultured performance as well as immune response of farmed fish (Syahidah et al., 2015). An extensive phytochemical study of the methanolic extract of *Euphorbia hirta* identified the presence of secondary metabolites such as phenols, saponins, tannins, volatile oils (Sheikhlar et al., 2017). It was believed that these compounds contributed to the medicinal potential of the *E. hirta* extract included in the diets of African catfish when challenged with *Aeromonas hydrophila*. In another study, Venkatramalingam et al. (2007) reported that feeding post larvae of *Penaeus monodon* with herbal appetizer, *Zingiber officinalis* enriched *Artemia franciscana*, resulted in a considerable higher weight gain and particular growth rate.

On the other hand, Kaleeswaran et al. (2011a, b) showed that *Cynodon dactylon* ethanolic extract, containing tannins, quinones and phenols displayed immunostimulatory properties in *C. catla*. The chemical composition of plants may vary according to seasons, habitats, parts of the plant and geographical locations (Khan et al., 2010; Mikage and Mouri, 1999). However, limited literature provides information on the variation of the activity of extracts obtained from different batches of plants.

1.2.1.2 Plant and plant products administration to enhance fish health

Plants-derived antioxidants, i.e. tannins, lignans, stilbenes, coumarins, quinones, xanthones, phenolic acids, flavonols, catechins and anthocyanins could postpone or inhibit the initiation of degenerative diseases due to their redox properties, which enable them to work as hydrogen donors, reducing agents, hydroxyl radicals or superoxide radical scavengers (Marwah et al., 2007). Thus, plant supplementation in fish diet may result in the improvement of immune factors, hence, indirectly enhancing fish resistance to different stresses (Chakraborty and Hancz, 2011). Substantial variations on the physiological and biochemical conditions were found in fish continuously exposed to chemical, biological and physical disturbances stressors from the intensive culture systems. An alteration in biological condition beyond the ordinary resting state that challenges homeostasis is expected to cause stress and thus threats the fish health (Syahidah et al., 2015). A large variety of chemical compounds with antioxidative effects found in plants could help organisms to cope with oxidative stress resulting from free radical damage, and consequently, improve the general physiological condition of fish (Ali et al., 2008; Chakraborty and Hancz, 2011). For instances, Metwally (2009) reported that feeding *Tilapia nilotica* (*Oreochromis niloticus*) with diets containing various sources of garlic, *Allium sativum* could significantly lower glucose concentration in blood serum, while the activity of antioxidant enzymes, i.e. glutathione peroxidase, superoxide dismutase (SOD) and catalase (CAT) was increased. This was in accordance with the report of Li et al. (2008), which showed an increase of CAT and SOD activities and a decrease of malonaldehyde (which is a secondary product of fatty acid oxidation) in fish fed with feed supplemented with allicin. Shahsavani et al. (2010) showed that the supplementation with 10 mg/kg feed of allicin in common carp effectively reduced lead accumulation in the liver, kidney, brain, bone, and blood. This effect might be explained by the metal-chelating ability of allicin (Chakraborty and Hancz, 2011). Stress resistance and

immunological parameters of *Cyprinus carpio* were reported in a study on the effects of *Astragalus membranaceus*, *Portulaca oleracea*, *Sophora flavescens* and *Andrographis paniculata* (Wu et al., 2007). The results indicated the role of herbal extracts as antistress and inducer of serum lysozyme, SOD and nitric oxide synthases (NOS) activity, as well as increaser of levels of total serum protein, globulin and albumin of fish. The supplementation with 1.0–2.0% (w/w in feed) anthraquinone extract from rhubarb (*Rheum officinale*) in *C. carpio* var. Jian for 10 weeks enabled to moderate the negative impact of crowding stress, as shown by lowered levels of blood cortisol, glucose and hepatic malondialdehyde and higher hepatic activities of catalase and superoxide dismutase (Xie et al., 2008).

Regarding antimicrobial activities of plant extracts, various investigations have denoted the enormous antimicrobial potential of plants as alternatives drugs in aquaculture (Zheng et al., 2009). Plants showed their excellent antimicrobial properties on the protection against various tested microorganisms no matter which types of extraction they were subjected to (essential oil, hot or cold water or any solvents). Syahidah et al. (2012) pointed out that *Piper betle* methanolic extract possessed the highest antibacterial capacity among five plants examined against *Aeromonas hydrophilla*, *Pseudomonas* sp. and *Streptococcus agalactiae* throughout *in vitro* study on antimicrobial activity of aqueous and methanolic extracts of Malaysian local plants. A screening performed by Zilberg et al. (2010) on dried leaf and leaf extract from rosemary (*Rosmarinus officinalis*) exhibited optimistic results in preventing a common tilapia pathogen, *Streptococcus iniae*. Similarly, chamomile extract demonstrated its antibacterial property against *Streptococcus agalactiae* with a minimal inhibitory concentration (MIC) at 6.25 mg/mL (Abdelhadi et al., 2012). In addition, several aquatic pathogens, e.g. *Mycobacterium* sp., *Staphylococcus* sp., *Enterococcus* sp., *Pseudomonas* sp. and *Micrococcus* sp. *were* effectively impeded by cinnamon's (*Cinnamomum* sp.) extract, whereas its essential oils possess antimicrobial, antifungal, antiviral, insecticidal and antioxidant activities due to the presence of eugenol, cinnamic acid and cinnamaldehyde in its extract (Alsaid et al., 2010). Plant extracts were evidenced to inhibit and control infectious microbes in culture systems. Abdel-Tawwab et al. (2010) examined the effect of green tea (*Camellia sinensis*) supplementation at different levels (0.0, 0.125, 0.25, 0.50, 1.0, or 2.0 g/kg diet) on the survival of Nile tilapia (*O. niloticus*) challenged by pathogenic *Aeromonas hydrophila* after 12 weeks feeding. The results indicated that green tea could be supplemented in fish diets up to an optimum level of 0.50 g/kg diet for fish performance,

good health and preventing tilapia *Aeromoniosis*. This result was in agreement with another one where an ethanolic *Psidium guajava* extract incorporated in diets resulted in a mortality reduction and resistance against *A. hydrophila* in tilapia (Pachanawan et al., 2008).

1.2.2 Adverse effects of herbal applications in aquaculture

The application of pharmaceutic herbals in aquaculture has received interest for their promoting influences on growth and immune functions. Moreover, the administration of herbs in several animal models including humans is usually correlated to few or no side effects (Briskin, 2000). As regards this aspect in fish, similar evidences are underlined although there are still few studies on this topic. Abutbul et al. (2004) showed that feeding tilapia (*Oreochromis* sp.) with *Rosmarinus officinalis* had no negative effects on fish survival, appearance, and behaviour. No perceptible toxic impacts and mortality were detected in Nile tilapia (*O. niloticus*) fed with extracts of *Andrographis paniculata* (Rattanachaikunsopon and Phumkhachorn, 2009), *Cratoxylum formosum* (Rattanachaikunsopon and Phumkhachorn, 2010a), *P. guajava* (Pachanawan et al., 2008), or *Cinnamomum verum* essential oil (Rattanachaikunsopon and Phumkhachorn, 2010b). Further perception and appropriate approaches are necessary to assess whether plant extracts are harmful to fish at high concentrations or if administered for long duration, as well as to screen how the herbal bioactive constituents are metabolized and/or accumulated in fish tissues.

1.2.3. Legislation on herbal products in aquaculture

Information regarding the legislation about the use of herbal products in aquaculture in Vietnam is not available. Based on the European regulation No 1831/2003 (EC, 2003), several plant products are mentioned in a list of feed additives, which is continuously updated by the EU. Within this list, herbal products are classified into category 2 –additives in feedingstuffs, functional group b – flavouring compounds, and subclassification – natural products botanically defined. This list also supplies information with respect to the animal species (or sometimes for fish) and the dosages of each plant administered.

Regarding the use of plants as therapeutic agents in fish, the Commission regulation (EC) No 710/2009 (European Commission, 2009) about 'organic aquaculture' approves the use of plants and plant extracts with no anesthetic impacts as well as herbal compounds at a homeopathic dilution as veterinary therapy for fish. The usage of allopathic therapeutics is required to be limited to a maximum period of two treatments or a single treatment per year in case the production cycle takes less than 1 year. If these treatments exceeded the limits for allopathic treatment, fish cannot display the organic label. Besides, plants may be used for cleaning and decontamination in organic fish farms. Besides, plants may be employed for cleaning and disinfection in fish farms dedicated to organic production.

In the United States, the use of plant products in aquaculture, reviewed by Bulfon et al. (2015), is controlled by the US FDA, which is the legal organizations allowing the use of drugs and chemicals in fish farming. Amongst plants, garlic and onion were considered as new animal drugs of low regulatory priority after their approvement by the FDA. Their administration is authorized in salmonids at all stages of life for treating the symptoms of helminth and lice infestations (Bulfon et al., 2015).

1.2.4. Integrated studies on plant extracts in the AquaBioActive project

In our project, the bibliography review was initially performed with the aim to describe antimicrobial, antioxidant and immunostimulant activities useful in aquaculture of medicinal plants and their products. For this purpose, a comprehensive review of the literature was done by College of Natural Sciences, Can Tho university. Different types of databases of scientific literature have been used, (scopus, googlescholar, etc.), to screen for the most relevant literature dealing with bio-active compounds of plants from wild and cultivated origins. Based on these data and on the survey study, a list of 20 plants (Table 1A, Section 3) has been selected for *in vitro* testing of antimicrobial andantioxidative activities (presented in this thesis), and immunostimulant activities in aquaculture (by PhD student Truong Quynh Nhu, UNamur). This screening was performed using ethanol extracts prepared from the 20 identified plants.

Regarding the work of Namur university, twenty plants possessing potential immunostimulatory activities were assessed in *in vitro* using the striped catfish peripheral blood

mononuclear cells (PBMCs) and head kidney leukocytes (HKLs) models. Then, five plant extracts were validated for their effects on the blood indices, humoral immune responses and disease resistance in striped catfish after oral administration. In addition, the effects of fish supplementation with *Phyllanthus amarus* and *Psidium guajava* extracts, used individually or in mixtures, on striped catfish health were studied. In addition, the protein expression profile was also investigated to provide a better understanding of the metabolic pathways related to immune response, antioxidation and lipid metabolism in fish liver after oral administration of plant extracts. Results showed the ability of several plant extracts in activating humoral immune responses (lysozyme, complement, and total immunoglobulin) in a dose dependent manner in striped catfish PBMCs and HKLs after 24 h. Several extracts also induced a strong upregulation of 4 cytokines (il1β, ifnγ 2a and 2b, and a2 mhc class II) according to the concentration, time points and kind of leukocytes. Ethanolic extracts of *Phyllanthus amarus*, *Psidium guajava*, *Mimosa pudica*, *Azadirachta indica* and *Euphorbia hirta* stimulated the striped catfish innate immune response (lysozyme and complement) and adaptive immune response (total immunoglobulins) as well as increased the capacity of striped catfish to fight against *Edwardsiella ictaluri* pathogen. The study also revealed that fish supplementation with *P. amarus* and *P. guajava* extracts acted in the regulation of immune responses, and significantly reduced fish mortality. Moreover, oral administration of *P. amarus* and *P. guajava* extracts demonstrated an upregulation of several proteins involved in immune response in striped catfish liver. Moreover, positive synergistic effects on liver proteome profile related to immune system processes were observed after administration of a mixture of these two plant extracts.

1.3. Antioxidant and antimicrobial activity of plant extracts

1.3.1. Mechanism of antioxidant activity and methods to measure it

1.3.1.1 Mechanism of antioxidant activity

Antioxidants are determined as systems or compounds which can delay, retard or inhibit oxidation by intercepting production of free radicals or by disrupting propagation of free radicals following one (or more) specific mechanisms: (i) by scavenging species that initiate peroxidation (Adibhatla and Hatcher, 2006), (ii) by chelating metal ions (specially Fe^{2+}), which could catalyze oxidative processes, leading to production of hydroxyl radicals through Fenton reactions, as well as decomposition of hydroperoxides resulting in a stable radical (Brewer, 2011; Stohs and Bagchi, 1995), (iii) by quenching $^{\bullet}O_2^-$ preventing formation of peroxides (Stohs and Bagchi, 1995), (iv)

by breaking the auto-oxidative chain reaction, and/or (v) by decreasing localized O_2 concentrations (Burton and Ingold, 1981). Thanks to their characteristic structure containing aromatic or phenolic rings, these antioxidants are able to donate a hydrogen atom to free radicals generated during oxidation, and, consequently, become a radical themselves, and thus interrupting the free radical chain reaction (reviewed by Yehye et al. (2015)). It was shown that antioxidants act using two pathways, either the hydrogen atom transfer in the principal case of lipid peroxidation, either the electron transfer in deactivating a free radical (Minisci, 2013). The hydrogen atom transfer (HAT) is occurring for example when antioxidants inhibit the fatty acid oxidation (Figure 4). Once a free radical $R^{\bullet}$ is formed (step (1)), a chain reaction is occurring in steps 2 and 3, generating numerous fatty acid hydroperoxides (ROOH) from the reaction of a fatty acid (R-H) with a radical. According to step (4), the important role of the antioxidant ArOH is to interrupt the chain reaction of oxidation. To show effectiveness, the $ArO^{\bullet}$ is required to be a stable free radical, in order that it reacts gradually with the substrate, RH, but speedily with $ROO^{\bullet}$, in accordance with the term "chain-breaking antioxidant".

Another probable mechanism of antioxidant activity is the deactivation of a free radical by electron transfer (Wright et al., 2001). Firstly, the radical cation is formed followed by fast and reversible deprotonation in the solution (Figure 5).

$$RH \longrightarrow R^{\bullet} + H^{\bullet} \quad \text{(initiation)} \quad (1)$$

$$R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet} \quad \text{(addition of } O_2\text{)} \quad (2)$$

$$RO_2^{\bullet} + RH \longrightarrow ROOH + R^{\bullet} \quad \text{(H-atom exchange)} \quad (3)$$

$$RO_2^{\bullet} + ArOH \longrightarrow ROOH + ArO^{\bullet} \quad (4)$$

Figure 4. Oxidation reaction and H-atom transfer mechanism of an antioxidant (ArOH) (Minisci, 2013)

$RO_2^{\bullet} + ArOH \longrightarrow RO_2^{-} + ArOH^{\bullet+}$ (electron transfer)

$ArOH^{\bullet+} + H_2O \rightleftharpoons ArO^{\bullet} + H_3O^{+}$ (deprotonation equilibrium)

$RO_2^{-} + H_3O^{+} \rightleftharpoons ROOH + H_2O$ (hydroperoxide formation)

Figure 5. Electron transfer mechanism of an antioxidant (ArOH) (Minisci, 2013)

1.3.1.2 In vitro methods to determine antioxidant activity

The application of various methodologies is essential in evaluating the antioxidant activity of compounds as well as plant extracts. Table 1 shows some *in vitro* tests used to evaluate the antioxidant activity of plant extracts and phytochemicals. Various analytical methods of evaluation of the antioxidant capacity divide into distinctive categories: spectrometric techniques and electrochemical techniques. These techniques could offer an accomplished profile of the antioxidant content of foodstuffs.

Table 1. Examples of *in vitro* methods used for the assessment of antioxidant activity

Antioxidant activity assay	Principle of the method	End-product signal	References
Spectrometry			
2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging	Reaction between an antioxidant and an organic radical	Colorimetry	Brand-Williams (1995)
2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)	Reaction between an antioxidant and an organic cation radical	Colorimetry	Re et al. (1999)
Ferric reducing antioxidant power (FRAP)	Reaction between an antioxidant and a Fe(III) complex	Colorimetry	Benzie and Strain (1996)
Potassium ferricyanide reducing power (PFRAP)	Potassium ferricyanide reduced by antioxidants and ensuing reaction of potassium ferrocyanide with Fe^{3+}	Colorimetry	Oyaizu (1986)
Cupric reducing antioxidant power (CUPRAC)	Cu (II) reduced to Cu (I) by antioxidants	Colorimetry	Apak et al. (2004)
Ferric thiocyanate (FTC)	Hydroperoxides produced by linoleic acid oxidation decompose to secondary oxidation product, which react with Fe (II) to Fe (III), then to ferric thiocyanate	Colorimetry	Chapman and McFarlane (1943)
Oxygen radical absorption capacity (ORAC)	Reaction between antioxidant and peroxyl radicals, generated by AAPH (2,2'-azobis-2-amidino-propane)	Loss of fluorescence of fluorescein	Cao et al. (1993)
Hydroxyl radical averting capacity (HORAC)	Antioxidant capacity to reduce OH radicals induced by a Co(II) based Fenton-like system	Loss of fluorescence of fluorescein	Ou et al. (2002)

Total peroxyl radical trapping antioxidant parameter (TRAP)	Antioxidant capacity to scavenge luminol-derived radicals, produced from AAPH disintegration	Chemiluminescence quenching	Wayner et al. (1985)
Fluorimetry	Emanation of light by a constituent that has absorbed light or other electromagnetic radiation of a different wavelength	Recording of fluorescence excitation/ emission spectra	Omaye et al. (1979)
Electrochemical Techniques			
Cyclic voltammetry	The potential of a working electrode is linearly different from a preliminary value to a final value and reverse, and the corresponding current intensity is noted	Measurement of the intensity of the cathodic/ anodic peak	Chevion et al. (2000)
Amperometry	The potential of the working electrode is established at a stable value with respect to a reference electrode	Measurement of the intensity of the current produced by the oxidation/reduction of an electroactive analyte	Milardović et al. (2006)
Biamperometry	Reaction of the antioxidant and the oxidized form of a reversible indicating redox couple	Measurement of the current flowing of two equal operational electrodes, at a small potential difference and dipped in a solution comprising the analyzed sample and a reversible redox couple	Tougas et al. (1985)
Cell-based antioxidant assays			
Inhibition of reactive oxygen species (ROS) assay	The ability of peroxyl radicals, reactive products of lipid oxidation, to induce the formation of a fluorescent oxidative stress indicator in the cell culture and	Using an oxidation-sensitive fluorescence probe that is absorbable by cultured cells	Wolfe and Liu (2007)

	measures the prevention of oxidation by antioxidants.		
Cell membrane lipid peroxidation assay	Lipid peroxidation is determined by measuring the amounts of malondialdehyde produced primarily. The reaction of one molecule of malonaldehyde with two molecules of TBA to form a red coloured malonaldehyde–TBA complex	Colorimetry	Dhindsa et al. (1981)
Endogenous antioxidant assay			
Reduced glutathione (GSH) estimation	Reaction of DTNB (5,5'-Dithiobis (axit 2-nitrobenzoic)) and GSH to generate 2-nitro-5-thiobenzoic acid and GSSG (glutathione disulfide)	Colorimetry	Ellman (1959)
Superoxide dismutase (SOD) method	SOD is assayed by its capacity to lower the rate of $O_2^{\bullet-}$ mediated reduction of ferricytochrome c.	Colorimetry	McCord and Fridovich (1969)
Catalase (CAT)	Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor	Colorimetry	Aebi (1984)

1.3.2. Mechanism of antimicrobial activities and methods to measure it

1.3.2.1.Mechanism of action of antimicrobial agents

In the past, antimicrobials were the most acknowledged forms of medicinal therapy. Based on the specific effects of antimicrobial agents, the mechanism of action of agents can be generally classified into these following functions: inhibition of the synthesis of cell wall or nucleic acid, inhibition of the function of ribosome or cell membrane, and inhibition of puric and pyrimidic DNA bases through inhibition of folate metabolism.

On the other hand, herbal plants have been employed as traditionary remedies for treating various diseases during most of the human history (Cowan, 1999). Most of plants involve several constituents regarding antimicrobial properties for defense against invader agents, especially microorganisms. According to Cowan (1999), the chemical structures of some antibacterial components collected from plants are presented in Figure 6.

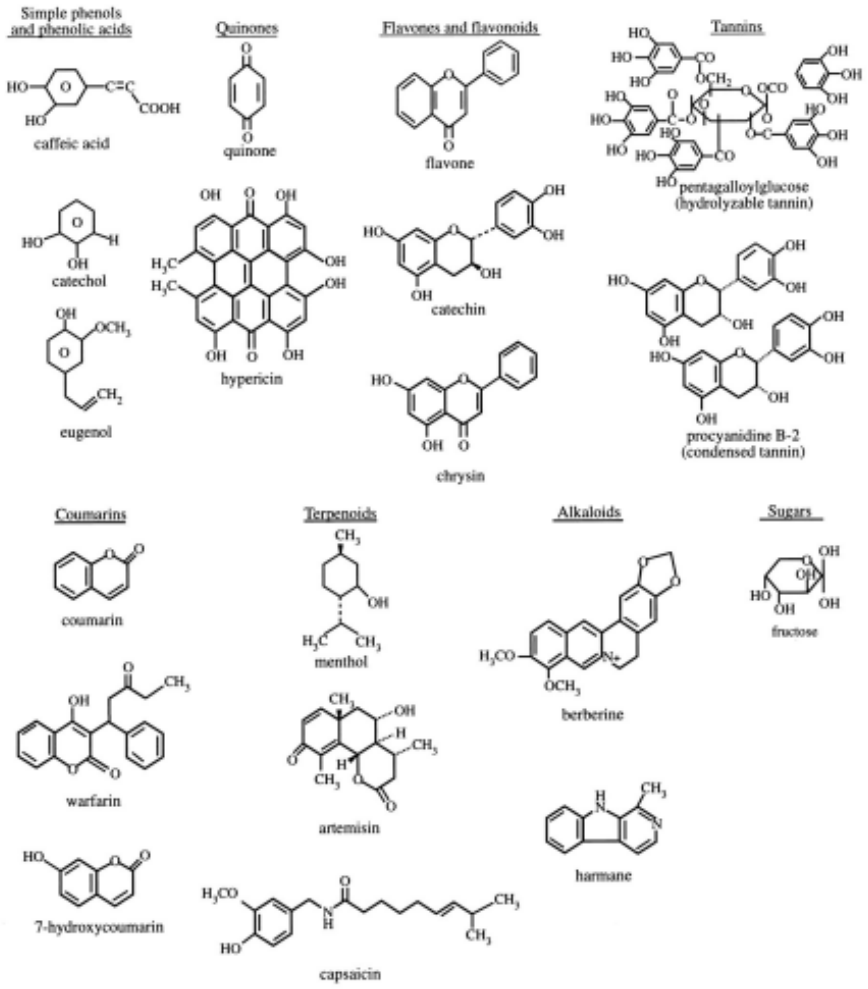


Figure 6. Chemical structures of antimicrobial compounds of plant origin (Cowan, 1999)

The major mechanisms of action of plant antimicrobials according to their chemical class are mentioned in Table 2.

Table 2. Main groups of plant compounds with antimicrobial activity (Cowan, 1999)

Class	Subclass	Examples	Mechanism	Minimum inhibitory concentration (MIC) against bacteria, from literature
Phenolics	Simple phenols	Catechol	Substrate deprivation	*Staphylococcus aureus* (1 mg/mL) (Peres et al., 1997)
		Epicatechin	Membrane disruption	*Escherichia coli* (7.5 mg/mL) (Demir, 2021)
	Phenolic acid	Gallic acid	Local rupture or pore formation in the cell membranes with consequent leakage of essential intracellular constituents.	*Pseudomonas aeruginosa* (500 µg/mL), *Listeria monocytogenes* (2000 µg/mL), *S. aureus* (1750 µg/mL), *E. coli* (1500 µg/mL) (Borges et al., 2013)
	Quinones	Hypericin	Adhesin binding, complex with cell wall, enzyme inactivation	*S. aureus* (78.12 µg/mL) (Bahmani et al., 2019)
	Flavonoids	Chrysin	Adhesin binding	*E. coli* (36.72 µg/mL) (Wu et al., 2013)
	Flavones	-	Complex with cell wall	*Mycobacterium tuberculosis* (50 µg/mL) (Sun et al., 2012)
		Abyssinone	Enzyme inactivation	*S. aureus* (50 µg/mL), *Bacillus subtilis* (50 µg/mL) (Taniguchi and Kubo, 1993)
			HIV reverse transcriptase inhibition	-
	Flavonols	Totarol		*S. aureus* (1.56 µg/mL) (Muroi and Kubo, 1996)
	Tannins	Ellagitannin	Protein binding Adhesin binding Enzyme inhibition Substrate deprivation Complex with cell wall Membrane disruption Metal-ion complexation	*S. aureus* (61.5 µg/mL) (Parashar et al., 2009), *E. coli* (123 µg/mL) (Barrajón-Catalán et al., 2010)

	Coumarins	Warfarin	Interaction with eucaryotic DNA (antiviral activity)	-
Terpenoids, essential oils	-	Thymol	Membrane disruption	*S. aureus* (0.662 mg/mL), *Salmonella enterica* (0.331 mg/mL) (Engel et al., 2017)
Alkaloids	-	Berberine	Intercalation into cell wall and/or DNA	*B. subtilis* (497 mg/L), *E. coli* (230 mg/L), *P. aeruginosa* (226 mg/L), *S. aureus* (212 mg/L) (Čerňáková and Košťálová, 2002)
		Piperine		*E. coli* (6.25 mg/mL), *S. aureus* (50 mg/mL), *P. aeruginosa* (100 mg/mL), *Klebsila pneumoniae* (25 mg/mL) (Aldaly, 2010)
Lectins and polypeptides	-	Mannose-specific agglutinin	Block of viral fusion or adsorption	-
		Falxatin	Disulfide bridge formation	-
Polyacetylenes	-	8s-heptadeca-2(Z),9(Z)-diene-4,6-diyne-1,8-diol		-

The various sites of the microbial cell which are considered as targets for the action of plant bioactive compounds are illustrated in Figure 7. These modes of action are associated with decomposition of cytoplasmic membrane, destabilization of the proton motive force, electron flow, active transport as well as coagulation of the cell content.

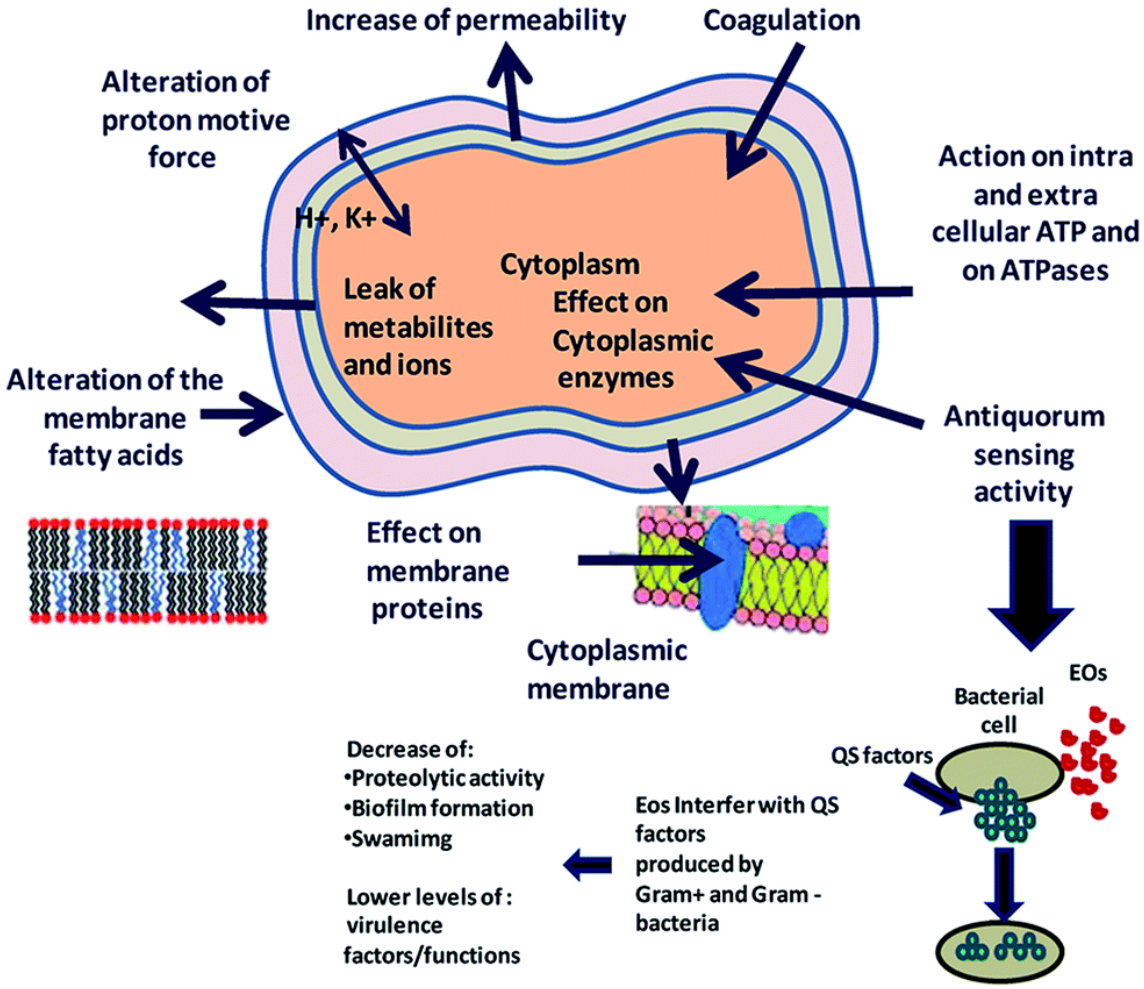


Figure 7. Sites in a bacterium cell where natural compounds are active (Bazaka et al., 2015)

Like most of antimicrobial agents, several herbal bioactive compounds exert their effects by acting at the level of the cellular membrane of microbes (Kamel, 2001). An *in vitro* study conducted by Kamel (2001) revealed that there are interrelations between the minimum inhibitory concentration (MIC_{50}), minimum bactericidal concentration (MBC_{50}) and the level of active compounds or the purity of the plant extract. In addition, the presence of plant extracts may cause a strong increase of the hydrophobicity of surface constituents of bacterial cells thereby influencing

the virulence properties of the microbes (Kamel 2001). This can be a significant mechanism in antimicrobial activity of some plant extracts.

1.3.2.2 Analytical methods for in vitro determination of antimicrobial activity

Various methods can be applied for evaluation of antimicrobial activity which are presented in Table 3. Many studies show that methods such as microdilution assay, well agar diffusion assay, paper disk diffusion assay are used for assessment of antibacterial activity of plant extracts and their constituents throughout determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Table 3. Methodology of assessment of antibacterial activity of plant extracts

Antimicrobial method	Target	Determination of end-product
Paper disk diffusion	To examine the antimicrobial activity	Appearance of bacterial growth inhibition zones (CLSI, 2012a)
Agar well diffusion	To evaluate the antimicrobial activity	Appearance of bacterial growth inhibition zones (Bassolé and Juliani, 2012)
Agar dilution	To determine MICs and MBCs	Colorimetry (CLSI, 2010)
Broth dilution	To determine MICs and MBCs	Colorimetry (CLSI, 2012b)
Time-kill analysis/survival curves	To determine the speed and period of antimicrobial activity	Lethality percentage obtained from bactericidal of 90% for 6 hours, corresponding to 99.9% of lethality for 24 hours (Pfaller et al., 2004)
Scanning electron microscopy	To survey the physical influence of antimicrobial activity	Observation of morphology of the bacterial cells with a scanning electronic microscope (Shan et al., 2007)

1.4. Lipid oxidation in fish

1.4.1. Lipid oxidation

In fish lipids, the amount of polyunsaturated fatty acids (PUFA) (with 14–22 carbon atoms) can be up to 40%. Among them, long chain PUFA such as eicosapentaenoic (EPA, C20:5n3) and docosahexaenoic (DHA, C22:6n3) acids are defined as "essential". However, PUFA long-chain fatty acids are known for their high sensitivity to oxidation. It has been demonstrated that the lipid oxidation of food in general and PUFA contained in fish in particular, is connected to the production of off-flavour compounds, deterioration of fish quality, loss of nutritional properties (e.g. loss of PUFAs) and formation of anti-nutritional molecules (Azhar and Nisa, 2006; German et al., 1985; Maqsood and Benjakul, 2011; Maqsood et al., 2012; Richards and Hultin, 2002).

Moreover, the susceptibility to lipid oxidation is more obvious in presence of heme pigments (myoglobin [Mb] and hemoglobin [Hb]) and when fish contain trace amounts of metallic ions (iron and copper) (Hsieh and Kinsella, 1989). Hb plays an important role as catalyst for lipid oxidation in fish. Hb can be a source of activated oxygen due to its autoxidation. Besides, heme iron released from protein can promote the lipid oxidation (Richards and Hultin, 2002). Other detrimental consequences caused by oxidation can be described such as vitamin destruction, discoloration, loss of essential fatty acids, organoleptic modification, and deterioration of nutritive value (Sherwin, 1978).

It is believed that heme pigments, Hb and Mb, are the most essential endogenous agents of oxidation in the lipids of fish muscle (Maqsood et al., 2012). Hb is characterized by a quaternary structure containing numerous multi-subunit globular proteins. Hb is constituted by four polypeptide chains which contains one heme group in each chain. A heme group comprises an iron (Fe) ion (charged atom) contained in a heterocyclic ring, named porphyrin (Figure 8a). This porphyrin ring includes four pyrrole molecules cyclically bound together (by methene bridges) with the iron ion attached in the centre (Figure 8a) (Steinberg, 2001). The iron ion can be present in the state of either Fe^{2+} (ferrous ion) or the Fe^{3+} (ferric ion). The conversion of ferrous Hb to ferric metHb is called autoxidation process (Figure 8b). Figure 8b describes the scheme of autoxidation and the formation of different radicals which can provoke lipid oxidation in fish muscle.

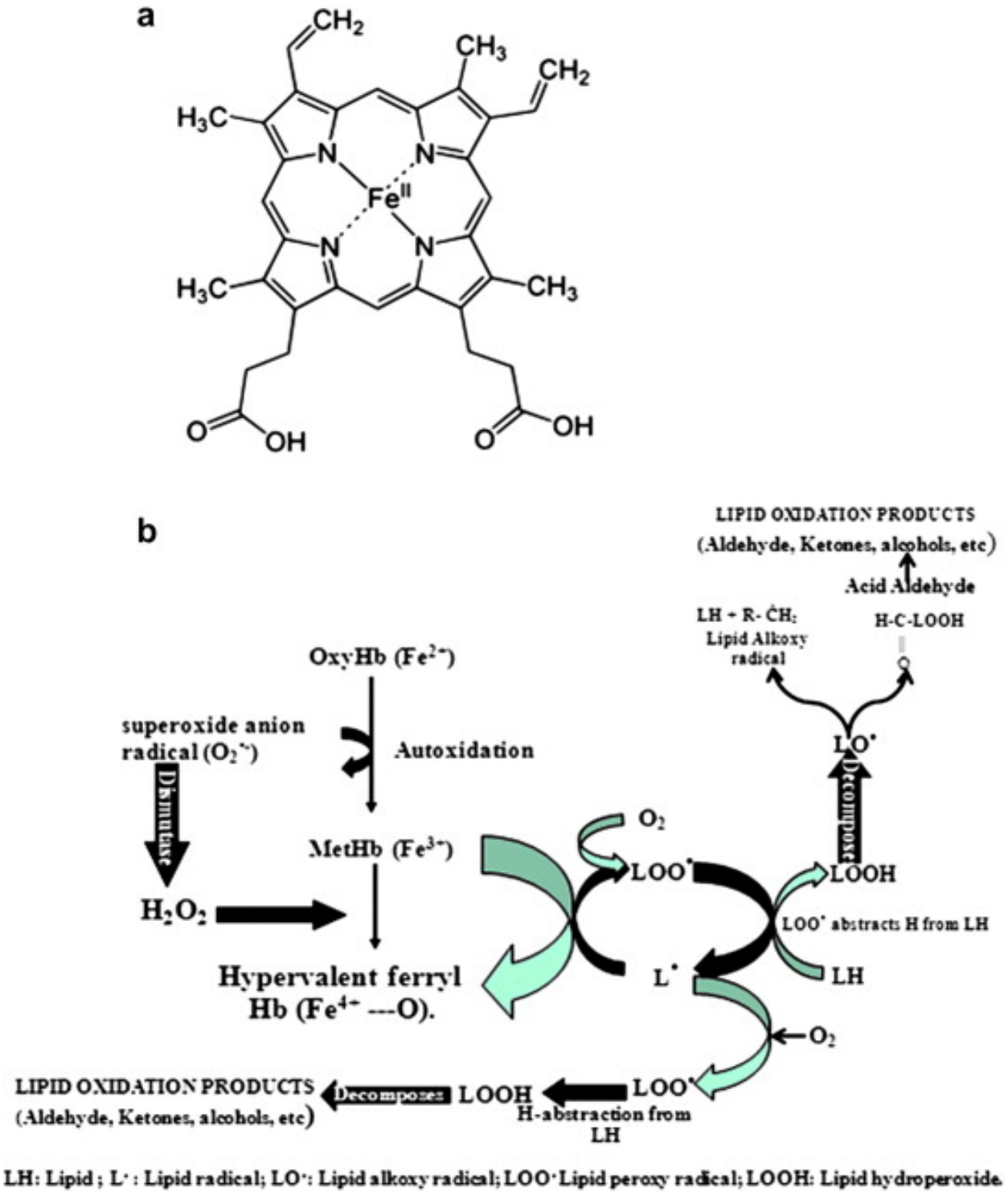


Figure 8. (a) Structure of hemoglobin and (b) schematic diagram of Hb autoxidation and its role in lipid oxidation (Maqsood et al., 2012)

In this process, the liberation of superoxide anion radical ($O_2^{\cdot-}$) or OOH• is depending on whether deoxy or oxy heme protein endures autoxidation (Richards and Hultin, 2002). $O_2^{\cdot-}$ or OOH• can readily be transferred to hydrogen peroxide (H_2O_2), which improves the capacity of heme proteins to enhance lipid oxidation. Hbs can degrade preformed lipid hydroperoxides, thus producing free radicals, an important mechanism postulated in the pro-oxidative action of Hbs (Erickson, 2002) (Figure 8b). It has been proposed that the promotion of lipid oxidation by Hb and Mb is due to a ferrylHb radical which can initiate the oxidation (Everse and Hsia, 1997). The formation of ferrylHb radical is coming from the reaction of metHb with either hydrogen peroxide or lipid hydroperoxides (Figure 8b).

1.4.2. Factors promoting lipid oxidation

Metal category such as iron, cobalt and copper can facilitate the exchange of electrons resulting to increased rates of free radical generation and promote the lipid oxidation (Ingold, 1962). The most popular path that metal ions arrive in food is by the way of the water used and in some case via salt and spices (Taylor, 1987). The configuration of the metal is as significant as the quantity of metal present (Taylor, 1987). A study of Pearson et al. (1977) on cooked uncured meat showed that the pro-oxidant activity of ferrous iron was stronger than ferric iron. The comparative efficiency of metals in oxygen absorption is reported in the subsequent decreasing order: iron (II), copper (II) and iron (III), when supplemented to fish homogenate (Mizushima et al., 1977). In addition, the catalytical effect of metal catalysts at low moisture content is reduced via hydration and in some instances via the generation of insoluble hydroxides; on the contrary, water enhances oxidation by way of its solvent activity (Labuza and Dugan, 1971). Various heme compounds are considered as factors which may accelerate lipid oxidation (Pearson et al., 1977). Both hemoproteins and non-heme iron can act as prooxidants when linked to purified lipids. Yong and Karel (1978) reported that inorganic iron and copper were great catalysts of mackerel flesh lipid oxidation, whereas Khayat and Schwall (1983) showed that heme iron is the main catalytic agent of lipid oxidation in mullet fish.

Iron released from the haem protein through the pathway of lipid oxidation mediated by hemoglobin (Hb) is capable of stimulating lipid oxidation, since it catalyzes the breakdown of preformed lipid hydroperoxides, thus initiating the production of alkoxyl radicals (Tappel, 1955). These radicals have the ability of substracting a hydrogen atom from polyunsaturated fatty acids

with subsequent propagation of lipid oxidation processes (Hargrove et al., 1996). The catalytical action of Hb is considered in relation to the quality loss of fish flesh during storage (Maqsood and Benjakul, 2011).

Hb from various fish is conceded to stimulate differently lipid oxidation in fish muscle. Lipid oxidation can be catalyzed by metHb based on a lipid hydroperoxide dependent mechanism while reduced or deoxyHbs functioned through a pH dependent mechanism (Richards and Hultin, 2000). Another pathway that deoxyHb can influence lipid oxidation reactions is via its ability to enhance the autoxidation rate of oxyHb (Rifkind et al., 1987). Hence, different forms of Hbs have various redox properties and affinity towards autoxidation and thereby can promote lipid oxidation differently.

1.4.3. Prevention of lipid oxidation

Antioxidant compounds could be categorized into three groups as following the description of Labuza and Dugan (1971):

(1) Free radical exterminators (donating hydrogen to the free radical and interrupting the chain reaction) such as for instances phenolic compounds, i.e. butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertbutyl hydroquinone (TBHQ) and tocopherol.

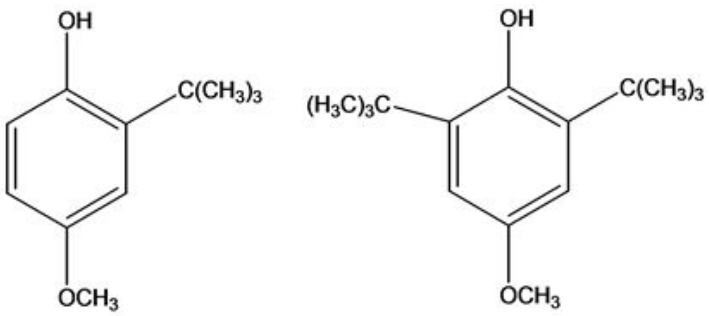
(2) Free radical inhibitors (controlling the formation of free radicals during the ionization) such as metallic complexing agents, for examples ethylenediaminetetraacetic acid (EDTA), citric acid and phosphates.

(3) Environmental factors (such as redox compounds i.e. cysteine and ascorbic acid), physical status and packaging matter.

Antioxidants as food additives, divided into natural and synthetic groups, are employed to postpone the initiation or slower the velocity of lipid oxidation reactions in food processing. Nevertheless, there has been increasing interest related to the possible health risks due to the use of food additives. BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), are known synthetic phenolic antioxidants (Figure 9), used in the food industry (reviewed by Shalaby and Shanab, 2013). In 1975, a researcher working on a project authorized by the Food and Drug Administration (FDA) regarding the safety and side effects of BHT/BHA perceived the development of cancerous tumors at an alarming proportion in experimented rats (reviewed by Shalaby and Shanab, 2013). It was found that carcinogenicity or tumorigenicity may be caused by

BHA and BHT due to their oxidative characteristics and/or metabolites. Further research has revealed that high dosages of these components can trigger substantial injury to the lungs, liver, and kidneys (Shalaby and Shanab, 2013). These authors reported that the blood coagulation system has been negatively affected after oral intake of this ingredient as well. Moreover, evidences are available demonstrating that metabolites of BHA and BHT are responsible of health and behavior changes. Koltover (2010) reviewed from an article which noticed that: "Repeated studies have shown that BHA and BHT increase the risk of cancer as well as its accumulation in body tissue, cause liver enlargement, and retard the rate of DNA synthesis and thus, cell development".

Despite the large number of scientific publications demonstrating the controversial efficiency of BHT and its analogs, in *in vitro* tests and in *in vivo* animal models, several studies showed positive effects of BHT, such as enhancing the intracellular levels of glutathione and related enzymes in rat (Ahmad et al., 1992), protecting against cancer due to its antioxidant activity (Botterweck et al., 2000) and having tumor reducing effects (Lanigan and Yamarik, 2002). BHT and BHA have been forbidden in Japan since 1958 and have use limitations in infant formulas in the UK (FSA, 2022). However, BHT and BHA are currently permitted in Canada, United States, Korea and certain countries within the European Union (Health Canada, 2021; Liu and Mabury, 2019; Nieva-Echevarría et al., 2015; Suh et al., 2005). They are classified as "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (FDA) and safe to use in cosmetics by the Cosmetic Ingredient Review (CIR) Expert Panel (Health Canada, 2021; Lanigan and Yamarik, 2002; Nieva-Echevarría et al., 2015; Tortosa et al., 2020), while according to the International Agency for Research on Cancer (IARC), BHT is not classifiable as to its carcinogenicity to humans (because insufficient data are available) and BHA as possibly carcinogenic to humans (class 2B).



BHA BHT

Figure 9. Structures of BHA (left) and BHT (right)

Plant extracts have been employed since thousands of years in various fields such as medical, pharmaceutical, sanitation, aromatherapy, phytotherapy, perfumery and cosmetic applications as well as food and beverage flavoring (Bakkali et al., 2008; Hammer et al., 1999). Up to now, they have been considered as natural preservatives or food additives based on their potential antibacterial, antifungal and antioxidant activities for raw and processed food storage (Benkeblia, 2004; Chouliara et al., 2007). Numerous studies showed that plant extracts were not only effective in lessening lipid oxidation but also in decreasing production of detrimental compounds for instance, heterocyclic amines (Gibis, 2007; Rounds et al., 2012; Smith et al., 2008; Viegas et al., 2012; Zeng et al., 2014). Thus, it is useful to use plants or products derived from plants (e.g., extracts, essential oils) to keep all desirable qualities demanded by customers, as they can prevent or minimize oxidation and rancidity. For example, the use of plant extracts in edible films and coatings for seafood packaging has been shown to be efficient treatments to extend the shelf life of food (Erkan et al., 2011; Falguera et al., 2011). Plant extracts possess both antioxidant and antimicrobial activities whereas edible films and coatings create obstacle against gases, water and microorganisms (Falguera et al., 2011; Mahmoud et al., 2004; Yanishlieva et al., 2006). The substances derived from plant extracts such as carotenoids, retinoids, tocopherols, ascorbic acid, phenolic acids, and polyphenols are known for their antimicrobial activity (Saxena et al., 2013). Similarly, the antioxidant efficiency of plant extracts is due to the presence of terpenoid and phenolic components (Bakkali et al., 2008). Besides, the biological activity of extracts from the same plants can vary since their chemical composition may be affected by many factors such as climate, season and geography, harvest period, plant maturity and extraction technique (Lahlou, 2004).

1.5. Spoilage in fish muscle

1.5.1. Fish spoilage bacteria

1.5.1.1 Initial microflora

Microorganisms are not contained in the muscle of living and healthy fish, but they are present in skin, gills, and in the intestinal tract (Liston, 1980; Shewan, 1962). When fish dies, microorganisms start to attack flesh. The initial microbiota in fish flesh is characterized by low abundance and high diversity of microorganisms. The total viable counts of fish flesh is generally

within 3-4 log cfu/g, while its diversity is relatively high. Numerous genera of the initial microbiota belong to the most common Gram-negative such as *Pseudomonas*, *Shewanella*, *Psychrobacter*, *Pseudoalteromonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, and *Vibrio*, *Photobacterium*, and *Aeromonas*, whereas the most popular Gram-positive genera of the indigenous microbiota are lactic acid bacteria (LAB), *Micrococcus*, *Corynebacterium*, *Vagococcus*, *Bacillus*, and *Clostridium* (Gennari et al., 1999; Gram, 2009; Gram and Huss, 1996; Gram et al., 1990; Parlapani et al., 2015; Svanevik and Lunestad, 2011). *Enterobacteriaceae*, *staphylococci*, *Listeria*, and other microorganisms can be detected in the early bacterial population (Huss et al., 2000). The composition of the initial microbiota is obviously influenced by geographic conditions (temperate or tropical) and the origin of water (marine or fresh waters) the fish are living in (Ashie et al., 1996; Gram, 2009; Gram and Huss, 1996).

Fish flesh is a favorable matrix for microbial development due to its high content of non-protein, low molecular weight, nitrogen compounds which are promptly metabolized by bacteria. These substances comprise free amino acids, creatine, nucleotides, urea, and trimethylamine N-oxide (TMA-O). The combination of high post-mortem pH (about 6) and the small amount of carbohydrates can facilitate the speedy growth of Gram-negative, pH-sensitive psychrophilic bacteria inherently existing in fish, i.e *Pseudomonas* and *Shewanella* (Françoise, 2010).

1.5.1.2 Microbial spoilage

In addition, the main reason of fish spoilage, resulting in distasteful and intolerable off-flavors, is the microbial growth and metabolism which can generate amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulfites, alcohols, aldehydes and ketones (Dalgaard et al., 2006; Emborg et al., 2005; Gram and Dalgaard, 2002). Gram-negative and fermentative bacteria (such as *Vibrionaceae*) development results in the spoilage of unpreserved fish, while chilled fish can be spoiled by psychrotolerant Gram-negative bacteria (e.g *Pseudomonas* spp. and *Shewanella* spp.) (Gram and Huss, 2000).

Despite the high diversity of bacterial genera initially in the initial fish microbiota, the processing and preservation conditions, as well as other factors, result in the selection of a small number of microorganisms that while they grow, surpass the others, and lastly dominate the product, and form the spoilage microbiota. Hence, spoilage microflora composition is depending on the fish origin, processing environment, storage temperature, and atmosphere or on any interaction with other microorganisms.

1.5.2. Fish preservation

There was a wide range of preservation techniques utilized to prevent spoilage, especially caused by microorganisms, and to extend the shelf life of fish after postmortem such as icing, chilling, freezing, chemical preservation, salting and smoking, fermentation and canning. Nevertheless, in the industry today, low temperature storage and chemical techniques are the most predominant methods to control the microbial and biochemical changes in freshly caught fish during distribution and marketing (Ghaly et al., 2010).

1.5.2.1 Low temperature storage

The low temperature storage is an effective technique for the preservation of several fish, but they do not enhance the product quality. This method reduces the physical and biochemical reactions and microbial metabolism leading to fish spoilage during the time storage (Ghaly et al., 2010).

Iced storage

The advantages of using ice as a cooling medium during fish storage include giving a quick cooling capacity, a cleaning effect during melting, harmlessness, and portability (Gokoglu and Yerlikaya, 2015). Hossain et al. (2005) reported a shelf life of 20 days for Pangasius fillets stored under ice in an insulated box. Viji et al. (2014) evaluated the shelf life of suchi catfish steaks under chilled (4ºC) and iced (0ºC) storage conditions with a ratio of fish to ice of 1:1 and the melted ice was replaced daily to conserve the ratio to achieve a temperature 1-2ºC. The results showed that although the chilled and iced steaks were rejected on the 14 and 17 days respectively by sensory analysis, all the biochemical quality parameters including pH, total volatile base nitrogen (TVB-N), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were within the acceptable limit of human consumption even after the rejection.

Freezing storage

Preservation fish for longer periods can be obtained by freezing. Freezing is the process of removing heat from product to lower product temperature to -18°C or below. This method is efficient to minimize microbial contamination; however, the enzymatic activity can continue at a slow rate in frozen fish. Many parameters can affect the survival of spoilage bacteria during

freezing storage such as microorganisms and fish species, initial fish quality, methods of catch and the handling and storage processes aboard the fishing vessel (Ghaly et al., 2010). Akter et al. (2014) investigated the effects of freezing storage method at -20ºC on the shelf life of Pangasius catfish fillets. This study concluded that sensory quality of fish fillets was found in acceptable conditions for 120 days of frozen storage before it becomes inedible.

1.5.2.2 Packaging technologies combined with low temperature storage

Oxygen scavenger packing technology

Oxygen scavengers can eliminate oxygen contained in the packaging headspace and in the product or permeating through the packaging material during storage. Therefore, packaging containing oxygen scavenger is advantageous in extending the shelf life of fresh fish products. According to Mohan et al. (2008), oxygen scavenger was efficient in decreasing oxygen concentration inside the package by 99.58% within 24h. By using this technique, the use of a vacuum packing machine can be avoided. Furthermore, the shelf life of Pangasius fillets was extended up to 20 days, maintaining its chemical, microbiological and sensory qualities, while the control samples packaged in air and stored at a temperature between 0ºC and 2ºC were found acceptable only up to 10 days.

Modified atmosphere packaging

The modified atmosphere packaging (MAP) can be combined with cold storage to considerably extend the freshness and shelf life of fish products. The effect of four packaging conditions of *Pangasius hypophthalmus* fillets including air packaged, vacuum packaged, MAP 1(50% CO_2, 50% N_2) and MAP 2 (50% CO_2, 50% O_2) on microbiological spoilage growth was evaluated during storage at 4ºC (Noseda et al., 2012). The result showed that the shelf life of the fillets packaged in air, vacuum, MAP 1 and MAP 2 was 7, 10, 12 and 14 days respectively. The combination of 50% CO_2 with 50% O_2 additionally inhibited the microbiological growth mainly lactic acid bacteria such as *Carnobacterium maltaromaticum* and *Carnobacterium divergens*. Therefore, this combination dramatically prolonged the shelf life of fish fillets compared to air and vacuum packaged fillets. In addition, this study also illustrated that several volatile compounds such as ethanol, 2,3-butanediol, diacetyl, acetoin, ethyl acetate, acetic acid and hydrogen sulfide, methylmercaptan, carbon disulfide and dimethyl disulfide were found in the headspace of Pangasius fillets.

1.5.2.3 The use of chemical preservation

Many organic acids such as lactic acid, acetic acid, gallic acid and citric acid are widely used in fish preservation because of their availability, low commercial price, and wide range of permitted concentrations for their use. Organic acids can be directly added to fish samples or included in aqueous solutions where fish fillets can be soaked for a certain time before storage. These compounds show some antimicrobial properties so that the shelf life of fish fillets is enhanced (García-Soto et al., 2014; Sanjuás-Rey et al., 2012; Sanjuás-Rey et al., 2011). Moreover, gallic acid has four potential acidic protons having pKa values of 4.0 (carboxylic acid) (Slabbert, 1977; Ji et al., 2006). Gallic acid can easily release the H-atom in aqueous medium. Thus, it leads to the reduction of pH values (Kalita et al., 2012).

Duy and Ha (2014) investigated the alone and combined effect of acetic acid and hot water treatment on total bacteria and *E. coli* of contaminated striped catfish fillets. The results indicated that a lower bacterial growth was observed in striped catfish muscle treated with hot water at 75 ºC during 15s and acetic acid at concentrations 2% during 120s by comparison with control samples. After 7 days storage at 4ºC, the decrease in *E. coli* and bacterial total levels of treated samples was 3.65 log cfu/g and 5.65 log cfu/g in comparison with the control fish. This study suggested that the combination of acetic acid and hot water treatment can be applied to decrease *E. coli* and total bacteria to assure the safety of catfish fillets, especially for exportation. Another research evaluated the effect of gallic acid combined with gelatin coating on the quality changes of refrigerated striped catfish paste by determining microbiological, texture, peroxide value and sensory parameters for 10 days (Thuy et al., 2015). During the time of storage, the increase in PV (from 0.15 to 2.32 meq kg-1) of Tra fillets coated a solution of 2% gelatin with gallic acid at the concentration of 2% was dramatically lower than control fillets (from 0.25 to 4.07 meq kg-1). The obtained results indicate that gelatin in the form of coating enriched with gallic acid could more effectively maintain the good quality and could extend the shelf life of striped catfish fillets by prevent the lipid oxidation during the refrigerated storage. Gallic acid, a small phenolic compound with 3-hydroxyl groups, plays roles as a cross-linking agent and antioxidant. The gallic acid also has a plasticizing effect on the zein-based film, therefore, it increases film elasticity. Gallic acid was used to provide antioxidant properties and to crosslink the peptide molecules in the casted

gelatin film. Gelatin film might function as a barrier to oxygen permeability on the fish meat surface and protect the product against lipid oxidation (Limpisophon and Schleining, 2017).

1.5.2.4 The use of chitosan as a natural preservative combined with freezing

Chitosan, the deacetylated form of chitin, has been widely applied to the preservation of seafood products because of its harmless feature, antimicrobial, antifungal activities, biodegradability and film forming property (Fan et al., 2009; Li et al., 2012a). Thuy and Thu (2011) compared the preservative capability of chitosan solution and polyphosphates to striped catfish fillets under frozen storage. The research results showed that using chitosan concentration of 0.5% for 25 minutes decreased the change of quality of fish fillets such as weight loss, protein content and lipid content, and improved sensory quality after 6 months of storage at -20°C. Moreover, chitosan showed a higher antimicrobial activity than polyphosphates. Jeyakumari et al. (2016) compared the shelf life and quality of fish products made with chitosan-corn flour and products prepared without chitosan. The results shown that sensory acceptability, texture and color attributes were higher for products incorporated with chitosan. In addition, chitosan is pre-dissolved in 1% acetic acid, which may result in the reduction of the pH of fish product which could contribute to the inhibition of bacterial growth. The shelf life of the product incorporated with chitosan (0.75 %) is extended by 7 days compared with the control products during chilled storage.

1.5.2.5 Smoking process

Smoking of fish products is one of the most ancient preservation technologies. The wood smoke contains amounts of flavorings, antibacterial and antioxidant substances such as phenol and carbonyl compounds, phenolic compounds (guaiacol, 4-methylphenol and 2,6-dimethoxyphenol). Therefore, the smoking method can increase the shelf life and contribute to the distinctive flavor, odor and color of the products (Kostyra and Baryłko-Pikielna, 2006). Luc et al. (2013) reported that the smoking process of Pangasius fish fillets with a smoking temperature of 34.4°C and a smoking time of 8h55 minutes showed the strongest effect in inhibiting total aerobic bacteria and improving sensory value. In another study, the combination of 10% salt and 10% garlic on smoked catfish yielded the best results as showed by the more useful nutrient property, lower moisture, higher fat, ash and protein. The study concluded that dipping in a concentration of salt and garlic before smoking improves overall quality and shelf life of striped catfish (Begum et al., 2012).

1.6. The use of plant extract to protect fish flesh from spoilage and lipid oxidation

Plant extracts were considered as alternatives to synthetic chemicals and preservatives (Sultanbawa et al., 2011), regarding the chemical and microbiological safety of food from animal origin, as well as their organoleptic quality (Holley and Patel, 2005). Literature extensively demonstrated that treatments with natural constituents were efficient preservation methods for fish products. Fan et al. (2008) investigated the effect of tea polyphenol (TP) dip treatment on the quality changes of silver carp during ice storage. In the research, changes in total viable counts (TVC), as well as chemical and sensory quality of fish samples were determined. The results showed that dip treatment with 0.2% TP could more effectively retard the spoilage due to bacterial growth and extend the shelf life of silver carp by 7 days compared with the control group during ice storage. The significant reduction in TVC of treated samples can be related to the inhibitory effect of TP on spoilage bacteria. In another study (Maqsood and Benjakul, 2010), the synergistic effect of tannic acid and MAP (Modified Atmosphere Packaging) on the quality of striped catfish slices during refrigerated storage was studied through the changes of microbiological, chemical and sensory parameters. The fish samples were divided into three groups including A0 (air stored fish without tannic acid treatment), M0 (MAP stored without tannic acid treatment) and M1 (MAP stored with tannic acid treatment). After 15 days of storage, PV (peroxide value) and TBARS (thiobarbituric acid reactive species) values of the fish samples treated with tannic acid at concentration of 200 mg/kg and preserved under MAP condition (60% N_2, 35% CO_2, 5% O_2) were found to be significantly lower than in the samples kept without tannic acid. Based on the microbiological acceptability limit (10^7 cfu/g), the authors concluded that the shelf life of A0, M0, M1 was estimated 3, 12 and 15 days, respectively. Therefore, the use of tannic acid in combination with MAP was able to retard the lipid oxidation and microbial growth and to extend the shelf life of striped catfish during refrigerated storage (Maqsood and Benjakul, 2010). Additionally, the increases of TVB-N and TBARS values of rainbow trout stored at 4 ± 1°C could be retarded by dipping of fish in turmeric extract (1.5%) and shallot extract (1.5%) (Pezeshk et al., 2011). The use of rosemary extract (0.2%) and tea polyphenols (0.2%) was highly efficient in extending the shelf life of crucian carp by 6 – 8 days compared with the control fish during chilled storage (Li et al., 2012b). Abdollahzadeh et al. (2014) evaluated the anti-listeria activity of thyme essential oil

(EO) in minced fish during refrigerated storage. The addition of thyme EO at 0.8% or 1.2% to minced fish meat exhibited a strong anti-listeria activity during storage. The addition of EO at 1.2% showed a higher effect against *L. monocytogenes* than the addition at 0.8% during refrigerated storage. The strong anti-listeria activity of thyme oil could be attributed to a high percentage of phenolic compounds. Another report demonstrated the efficiency of green tea polyphenols at the concentration of 200 ppm on the quality changes of common kilka (*Clupeonella cultriventris caspia*) during ice storage. This study indicated that the PV and TBA values of antioxidant treated sample was significantly lower than those of the control for the same storage period (Ojagh et al., 2005). According to Jeyakumari et al. (2017), pangasius (*Pangasianodon hypophthalmus*) fish chunks dipped with 20% spice extract (ginger or mint) could efficiently postpone the chemical deterioration, maintained, or improved the sensory quality and prolonged the shelf life during ice storage. A chilled storage of striped catfish fillets coated with plant oil combined with alginate gels was conducted by Rao et al. (2017). The results showed that 1% thyme oil incorporated into alginate gels were comparatively better as displayed by low total viable counts, low psychrotrophic counts, low PV, enhanced sensory attributes, and decreased rate of loss of texture compared to the control throughout a shelf life of 13 days in chilled storage of pangasius fillets. In a recent study, Greeshma et al. (2019) showed that a dip treatment of *Pangasianodon hypophthalmus* fillets in a solution of *Moringa oleifera* (Lam) leaf extract at the concentrations of 5 and 10% could improve the shelf life of fillets in terms of their sensory properties, biochemistry, and microorganism count until 6 days in comparison with control fish under vacuum package during cold storage at 2 ± 1°C.

2. THE USE OF DRUGS, CHEMICALS, PLANTS AND PLANT EXTRACT PRODUCTS IN GROW-OUT FARMS OF STRIPED CATFISH (*PANGASIANODON HYPOPHTHALMUS*) IN THE MEKONG DELTA, VIETNAM

Striped catfish (*Pangasianodon hypophthalmus*) is a dominant species, which has been intensively farmed in the Mekong Delta and exported to global seafood markets. Bacterial diseases have occured frequently causing a negative impact on fish production and economic profit of this culture system. Besides the popular use of drugs, chemicals and other compounds for water quality management, feed utilization enhancement and disease therapy, synthetic chemical disinfectants, plants, plant extract products and probiotics were also used in striped catfish farming for various purposes. Nevertheless, information on the plant and plant extract use in striped catfish aquaculture is scarce. The purposes of this study were to investigate the chemical use with a focus on plant extract product application in striped catfish farming providing background information for further in-depth studies.

2. THE USE OF DRUGS, CHEMICALS, PLANTS AND PLANT EXTRACT PRODUCTS IN GROW-OUT FARMS OF STRIPED CATFISH (*PANGASIANODON HYPOPHTHALMUS*) IN THE MEKONG DELTA, VIETNAM

Nguyen Le Anh Dao, Nguyen Quoc Thinh, Vo Nam Son, Huynh Van Hien, Nguyen Thanh Phuong, Do Thi Thanh Huong, Bui Thi Bich Hang, Tran Minh Phu, Joëlle Quetin-Leclercq, Patrick Kestemont, Marie-Louise Scippo

College of Aquaculture and Fisheries, Can Tho University, Vietnam

This manuscript is a part of a paper "The use of drugs, chemicals, herbs, and herbal extract products in grow-out farms of snakehead (*Channa striata*) and Pangasius catfish (*Pangasianodon hypophthalmus*) in the Mekong Delta, Vietnam". Vietnam Journal of Agricultural Sciences, 5(1), 1336-1344. https://doi.org/10.31817/vjas.2022.5.1.03

Abstract

This study aimed to investigate the current use of drugs, chemicals, plants and plant extract products in grow-out farms of striped catfish (*Pangasianodon hypophthalmus*) in the Mekong Delta, Vietnam. The survey was conducted with a total of 60 grow-out farms in two main striped catfish culture provinces including An Giang and Dong Thap. The results showed that bacterial diseases were commonly reported by farmers (1 to 12 episodes per crop). Farmers used 12 types of single antibiotics and a mixture of two antibiotics to treat diseases. However, farmers still used enrofloxacin and ciprofloxacin, which are banned antibiotic according to Vietnamese authorities' regulations. Various commercial products of plants and plant extracts were used by farmers, with the claims of purpose use in enhancing liver function, controlling ecto-parasites, bacterial diseases, and improving water quality. Quality and effectiveness of these products were still a question of many farmers. Some farmers used traditional plants as they are used in traditional human medicine without knowledge about doses for fish. Thus, there is a need for in-depth studies about the use of plants and plant extract products for on-farm treatment which could provide knowledge on plant use in striped catfish aquaculture.

Keywords: Antimicrobial, plant extracts, Mekong delta, striped catfish.

1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*) is one of the dominant species, which has been intensively farmed in the Mekong Delta and exported to global seafood markets. The production of striped catfish has risen up rapidly from 93 thousand tons in 2000 to 1.56 million tons in 2020 (Directorate of Fisheries, 2020). However, with the intensive culture practice, the frequent occurrences of diseases have been reported in striped catfish culture, e.g. Bacillary necrosis of Pangasius (BNP), caused by the bacteria *Edwardsiella ictaluri*, and Motile Aeromonad Septicaemia (MAS) caused by *Aeromonas hydrophila* and related motile *Aeromonads* (Phan et al., 2009; Phu et al., 2016).

The increase of bacterial diseases and other pathogens causes high mortality affecting fish production and economic losses. The use of chemicals and other compounds for water quality management, feed utilization improvement and disease treatment has been popular in aquaculture (Nguyen et al., 2014; Phu et al., 2016; Ström et al., 2019). Previous surveys have shown that 24 types of antimicrobials were used to control bacterial diseases in striped catfish, in 2016 (Phu et al., 2016) and 5 types were in 2019 (Ström et al., 2019). In addition, synthetic chemical disinfectants, plants, plant extracts and probiotics were also used in striped catfish farming (Phu et al., 2016; Rico et al., 2013). Information on plant use in striped catfish farming and aquaculture, in general, was however limited.

Even if the use of plants or plant extract products has not been widely studied at the farm level, accepting some examples have been reported. Garlic was used to control bacterial disease in aquaculture due to its antimicrobial property (Van, 2012) or *Yucca schidigera* extract added in diet enhanced shrimp growth and survival rate (Hoa, 2012). Other plant extracts were explored for their antimicrobial and antioxidant properties, potentially applied in aquaculture (Bussmann et al., 2010; Ocheng et al., 2014; Tekwu et al., 2012). However, information on the plant and plant extract products used in striped catfish (*Pangasianodon hypophthamus*) aquaculture has not been specifically studied yet. This study aims to investigate the chemical use with a focus on plant extract product application in striped catfish farming providing background information for further in-depth studies.

2. Methodology

The study was conducted from January to April 2017 by interviewing 60 grow-out striped catfish farmers in the main striped catfish production area including An Giang and Dong Thap provinces of the Mekong delta, Vietnam (Figure 1). A total of 60 farmers was interviewed using semi-structured questionnaire. The interviewed farms were randomly selected from the list of farmers provided by the provincial Department of Agriculture and Rural development. The number of 60 farmers included 30 farms in Dong Thap and 30 farms from An Giang. To determine the representative number of farms, we used this formula (Israel, 1992):

$$n = \frac{N}{1 + N(e)^2}$$

where n is the sample size, N (154 farms from Dong Thap and An Giang) is the population size, and e is the level of precision (0.1). The information in the questionnaire included technical information (e.g. grow-out pond, input pond, stocking density), year of practice, production, feed conversion ratio (FCR), training, disease occurrences (including types of disease and chemical use). The information on the use of plant and plant extract products focused on types of plant, dosage and mode of application.

The information from the questionnaires was checked, cleaned and computed. Results were expressed in descriptive statistics, e.g. frequency of occurrence, mean values, and standard deviation using Microsoft Excel 2019.

Figure 1. Map of the Mekong Delta and locations of the survey (●)

3. Results and discussion

3.1 General information of the striped catfish grow-out farms and practices

Striped catfish farmers had various experiences in fish farming (Table 1). The striped catfish aquaculture started since 2000 (De Silva & Phuong, 2011).

Table 1. General information of the striped catfish grow-out farms and practices reported by farmers

	Striped catfish farms (n = 60)
Year of farming practice (years)	10.6 ± 6.4 (1-30); 10
Training (%)	75.0
Area of grow-out pond (m^2)	5,767 ± 3,430 (1,000-2,0000); 5,000

Stocking density (fish/m^2)	56.0 ± 24.0
	(17-114); 49
Feed conversion rate (FCR)	1.60 ± 0.20
	(1.4-2.4); 2.0
Harvest size (g/fish)	974 ± 338
	(435-2300); 850
Antibiotic test before harvest (%)	86.7
Productivity (tons/ha/crop)	422 ± 162
	(167-917); 375

Data expressed as mean ± SD (min-max), median

The survey revealed that there are 75% of farmers who were trained by the provincial Agricultural Extension Centers and/or instructed by extension workers and company technicians. The productivity of striped catfish was higher than other cultured fish species (such as red tilapia, snakehead fish, etc.), making up an extremely high total production of 50% (GSO, 2021; VASEP, 2021). The regular antibiotic test conducted by the seafood processing plants prior to harvest and trade in striped catfish can be explained as this species is mainly for global markets or export. According to VMARD (2012), enrofloxacin was classified as a banned antibiotic in aquaculture use. Later, both ofloxacin and levofloxacin (class of flouroquinolones) were also listed in the forbidden antibiotic use in Vietnamese aquaculture (VMARD, 2016). Chloramphenicol, nitrofurans, enrofloxacin and ciprofloxacin were listed in the banned antibiotics traced in striped catfish fillets under the control of the Vietnamese National Agro-Forestry-Fisheries Quality Assurance Department (NAFIQAD) (VMARD, 2015).

3.2 Disease symptoms reported by striped catfish farmers

Most farmers reported that Bacillary Necrosis of Pangasius and Motile Aeromonad Septicaemia were the two most common bacterial diseases in the grow-out stage of striped catfish (Table 2). These diseases have not been controlled yet as they were reported in the previous survey in 2016 (Phu et al., 2016). The diseases occur often during the flooding period with the frequency of 1 to 12 episodes per crop. Thus, the control of bacterial disease in striped catfish should be taken into account for a better fish production. Pale gill and liver syndrome and parasitic infection occurred frequently, similarly to the previous report of Phu et al. (2016). Yellow fillet syndrome

in striped catfish seemed to occur more frequently compared to the previous findings in 2011 (Phu et al., 2016) with less than 5% of farmers reported. Other diseases symptoms in striped catfish e.g. liver syndrome, fungal infection, body hemorrhages and abnormality were not recorded by farmers. The higher frequency of diseases in striped catfish found in the current survey if compared to previous ones (especially for BNP or MAS) indicates that farmers have not managed fish health effectively yet. Hence, fish health management practices in fish farming needs to be addressed by different stakeholders, who could contribute to the sustainable development of striped catfish aquaculture.

Table 2. **Main disease symptoms in striped catfish farming in comparison with the previous study (% of surveyed farms)**

Disease symptoms	This study (n = 60)	Previous survey (Phu et al., 2016) (n=26)
Bacillary Necrosis of Pangasius (BNP)	91.7	92.3
Motile Aeromonad Septicaemia (MAS)	90	80.8
Pale gill and liver syndrome	60	19.2
Parasitic infection	43.3	15.4
Yellow fillet syndrome	30	11.5

3.3 Antibiotic uses

The number of antibiotics used in striped catfish included 12 types of single antibiotic and 1 mixture of two antibiotics, which are much less than reported cases in 2016 (Phu et al., 2016) (Table 3). A more recent survey performed by Ström et al. (2019), there were five types of antibiotics used by catfish farmers including enrofloxacin. The higher number of antibiotics used in this study can be explained by the larger number of interviews (60 farmers), whereas only 13 farmers were interviewed in An Giang provinces in the survey of Ström et al. (2019). However, enrofloxacin and ciprofloxacin, which are banned antibiotics according to VMARD (2016), have been used by striped catfish farmers in this survey. According to Mai (2012), 28 types of antibiotics were used in aquaculture in Vietnam, while in USA for example, only four types of antibiotics are

approved for their use in aquaculture. The farmers reported that the uses of antibiotics are mainly based on their own experiences, while no antimicrobial susceptibility test were conducted. Therefore, it would be recommended to propose to fish farmers a service of testing antimicrobial susceptibility to help them to use antibiotics more efficiently.

Table 3. **Antibiotic use in striped catfish farming (% of surveyed farms)**

Groups of antibiotics	Antibiotic	This study (n=60)	Previous studies	
			Phu et al. (2016) (n=26)	Ström et al. (2019) (n=13)
Betalactam	Amoxicillin	40	23.1	50
	Ampicillin	1.7	3.8	-
	Cephalexin	13.3	19.2	-
	Cefotaxime	3.3	NR	-
Polymyxin	Colistin	10	3.8	-
Quinolone	Ciprofloxacin	8.3	3.8	-
	Enrofloxacin	28.3	73.1	25
	Levofloxacin	16.7	3.8	-
Aminoglycoside	Gentamicin	11.7	NR	-
Tetracycline	Doxycycline	35	34.6	17
	Oxytetracycline	20	7.7	-
Phenicol	Florfenicol	36.7	57.7	-
Mixture	Sulfonamide+ trimethoprim	28.3	46.2	63

NR: Not reported by farmers; -: Not reported in the publication

About banned antibiotics (Enrofloxacin, Ciprofloxacin), it appeared from the result of the survey that there is a low percentage of those antibiotics which are used in striped catfish farms. This shows that the regulation is not completely applied. The authority in charge of controls is the Vietnamese National Agro-Forestry-Fisheries Quality Assurance Department (NAFIQAD), which is tasked to implement a national residue control plan to help meet food safety requirements for export markets.

3.4 Chemical, probiotic and nutritional products

Many types of chemicals were used to disinfect water, control ecto-parasites and to improve water quality (Table 4). Iodine and Benzalkonium chloride have been widely used to

disinfect water in catfish ponds, whereas coper sulfate and chlorine were used periodically (twice a month) to control the ectoparasites in striped catfish. The practice was similar to previous findings (Phu et al., 2016), meaning that the way to control water quality has not improved since 2011, and was performed only using chemical disinfection and water exchange. Praziquantel and ivermectin were used to control internal parasites in striped catfish. Most of farmers used probiotic and feed additives containing vitamins and minerals, even if their effectiveness was not assured, as reported by farmers.

Table 4. **The use of chemicals, probiotics and nutritious products in striped catfish farming (% of surveyed farms)**

Chemicals, probiotics, and nutritious products	Striped catfish farms (n = 60)
Chemicals	
Iodine	53.3
Coper sulfate	30.0
Benzalkonium chloride	41.7
$KMnO_4$	30.0
Lime	73.3
Chlorine powder	53.3
Glutaraldehyde	8.30
Salt	70.0
Internal parasite control	
Praziquantel	23.3
Ivermectin	41.7
Nutritious products (mixture minerals and vitamins)	78.3
Probiotics	63.3

Note: Probiotic is a commercial product containing various bacteria and is used via feed.

3.5 Plants and plant extract products

Plants and plant extract products were commonly used by striped catfish farmers, with 45% of farmers using commercial products and 8.3% of farmers using natural plants or direct use (Table 5). Natural plants, *Cleome chelidonii*, *Combretum quadrangulare* and *Allium sativum* (garlic), are commonly used to enhance liver function, control ecto-parasites and to treat bacterial diseases. The natural plants normally are dried and boiled in water before being applied into ponds or mixed

into feed. Some plants are ground and used freshly, such as *Allium sativum*, *Areca catechu*, and *Curcuma longa* (curcuma). Farmers can apply plants every two weeks for prophylactic purpose. Nine types of plant extracts produced by commercial companies were popularly used by striped catfish farmers. These products are labelled to enhance liver function, control ecto-parasites and bacterial diseases and improve water quality. Farmers used plant products as following the instruction of producers. However, the effectiveness of these products was unknown as the traditional plant medicines were used empirically by farmers. In addition, the quality of the plant extract products, which can contain different types of compounds, was not validated.

Table 5. The use of plants, plant extract products in striped catfish farming (% of surveyed farms)

Items and description	Surveyed farms (n = 60)	
	Plants	Plant extract products
To enhance liver function		
+ *Eclipta alba*	-	1.67
+ *Phyllanthus urinaria*	-	1.67
+ *Cynara cardunculus* (Artichoke). *Phyllanthus emblica* (Amalaki). *Terminalia arjuna* (Arjuna)	-	3.33
+ *Cleome chelidonii*	3.33	1.67
+ *Cynara cardunculus* (Artichoke)	-	1.67
To control ecto-parasites		
+ *Combretum quadrangulare*	1.67	10.0
To control bacterial diseases		
+ *Allium sativum* (garlic)	6.67	8.33
+ Alkaloid, flavones, gallic acid, terpenoid, and neolignan	-	1.67
To improve water quality		
Yucca schidigera extract	-	63.3

Plant extracts have been reported to contain alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils (Chakraborty and Hancz, 2011; Citarasu, 2010) which help to reduce stress, improve growth and supply essential compounds. Nguyen et al. (2020), in a study of 20 plant extracts, reported that *Phyllanthus amarus* extract had the highest *in vitro* antioxidant properties, followed by *Piper betle, Psidium guajava, Euphorbia hirta and Mimosa pudica*, while for antimicrobial activities, *P. amarus* extract also showed the

highest activities against two different strains of *Aeromonas hydrophila*. Nhu et al. (2019a) revealed that plant extract-based diets differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*). *Perilla frutescens* showed the highest antifungal activity on snakehead pathogenic fungi among five herbal extracts collected in the Mekong delta (Dang et al., 2020). Earlier studies have presented that skin mucosal immune responses could be promoted in rohu (*Labeo rohita*), Caspian roach (*Rutilus rutilus*), and common carp (*Cyprinus carpio*), respectively when the fish were fed with diets containing extracts of ginger (*Zingiber officinale*) (Sukumaran et al., 2016), garlic (*Allium sativum*) (Ghehdarijani et al., 2016) and date palm fruit (*Phoenix dactylifera*) (Hoseinifar et al., 2015). Many scientific publications documented that the crude ethanol extract of *Psidium guajava* is able to enhance immune responses and defense mechanisms in striped catfish (Nhu et al., 2020; Nhu et al., 2019b), rohu (Fawole et al., 2016; Giri et al., 2015) and tilapia (Gobi et al., 2016). In this study, it was observed that a large number of commercial products without verification of quality and bioactive compounds content are available and popular on the markets. As regarding the production and marketing chain for these herbal products, the plant products were distributed from companies to agents. Farmers can buy these products from the agents, on their own initiative, or following advices from the agents. Farmers could not know about the quality of these products and were unsure about their effectiveness during the application. Some farmers, following their own experiences, have used plants as they are used in traditional human medicine and did not really know the correct dose for use in aquaculture.

Thus, it would be recommended to perform an in-depth study on the efficiency of the use of plant extracts as on-farm treatment which can be applied widely in the industry of striped catfish culture. The use of plant extracts could reduce costs of treatment and be a more environmentally friendly treatment as they tend to be more biodegradable than synthetic molecules and less likely to produce drug resistance in fish due to the high diversity of plant extract molecules (Blumenthal et al., 2000; Logambal et al., 2000).

Conclusions

The survey revealed that Bacillary Necrosis of Pangasius and Motile Aeromonad Septicaemia are still main pathogens that striped catfish farmers have to deal with, in the fish health management. Farmers use antibiotics to control bacterial diseases. Twelve types of single

antibiotic and 1 mixture of two antibiotics are used in striped catfish aquaculture. However, enrofloxacin and ciprofloxacin which were banned antibiotics according to VMARD, are still used by striped catfish farmers. Thus, it is urgent to proper the use of antibiotics. For the use of plants and plant extract products, the varieties of commercial products are available for farmers, which claim different types of purpose uses. However, the quality and the effectiveness of these products are questionable. Some farmers are using the traditional plants e.g. *Eclipta alba*, *Phyllanthus urinaria*, *Cleome chelidonii*, *Areca catechu*, *Allium sativum*, etc. basing on their experiences as traditional medicines for human and do not really know about the applied doses. Thus, it would be recommended to further study the efficiency of plants under on-farm treatment to prove the use in the industry of striped catfish culture.

Acknowledgements

This work was supported by the Académie de Recherche et d'Enseignement supérieur - Commission de la Coopération au Développement (ARES-CCD) through AquaBioActive (Natural bio-active plant products for environmentally friendly aquaculture production in the Mekong Delta) project.

References

Blumenthal, M., A. Goldberg, and J. Brinckmann, 2000. Herbal medicine. Expanded commission E monographs, ed. M. Blumental, A. Goldberg, and J. Brinckmann. Newton, MA: Integrative Medicine Communications. 519.

Bussmann, R.W., G. Malca-García, A. Glenn, D. Sharon, G. Chait, D. Díaz, K. Pourmand, B. Jonat, S. Somogy, and G. Guardado, 2010. Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. Journal of ethnopharmacology. 132, 101-108.

Chakraborty, S.B. and C. Hancz, 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. Reviews in Aquaculture. 3, 103-119.

Citarasu, T., 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International. 18, 403-414.

Dang, T.M.T., T.T.H. Tran, and T.B.H. Bui, 2020. Antifungal activity of extracted herbs on snakehead pathogenic fungal. Can Tho University Journal of Science. 56, 129-136.

De Silva, S.S. and N.T. Phuong, 2011. Striped catfish farming in the Mekong Delta, Vietnam: a tumultuous path to a global success. Reviews in Aquaculture. 3, 45-73.

Fawole, F.J., N.P. Sahu, A.K. Pal, and A. Ravindran, 2016. Haemato-immunological response of *Labeo rohita* (Hamilton) fingerlings fed leaf extracts and challenged by *Aeromonas hydrophila.* Aquaculture Research. 47, 3788-3799.

Directorate of Fisheries, 2020. Reduction of striped catfish export in 2019. Available from: https://tongcucthuysan.gov.vn/vi-vn/th%C6%B0%C6%A1ng-m%E1%BA%A1i-th%E1%BB%A7y-s%E1%BA%A3n/xu%E1%BA%A5t-nh%E1%BA%ADp-kh%E1%BA%A9u/doc-tin/013997/2019-12-05/du-bao-kim-ngach-xuat-khau-ca-tra-nam-2019-giam-manh

Ghehdarijani, M.S., A. Hajimoradloo, R. Ghorbani, and Z. Roohi, 2016. The effects of garlic-supplemented diets on skin mucosal immune responses, stress resistance and growth performance of the Caspian roach (*Rutilus rutilus*) fry. Fish & shellfish immunology. 49, 79-83.

Giri, S.S., S.S. Sen, C. Chi, H.J. Kim, S. Yun, S.C. Park, and V. Sukumaran, 2015. Effect of guava leaves on the growth performance and cytokine gene expression of *Labeo rohita* and its susceptibility to *Aeromonas hydrophila* infection. Fish & Shellfish Immunology. 46, 217-224.

Gobi, N., C. Ramya, B. Vaseeharan, B. Malaikozhundan, S. Vijayakumar, K. Murugan, and G. Benelli, 2016. *Oreochromis mossambicus* diet supplementation with *Psidium guajava* leaf extracts enhance growth, immune, antioxidant response and resistance to *Aeromonas hydrophila.* Fish & shellfish immunology. 58, 572-583.

GSO, 2021. General statistics office. Aquaculture production; Available from: https://www.gso.gov.vn/px-web-2/?pxid=V0655&theme=N%C3%B4ng%2C%20l%C3%A2m%20nghi%E1%BB%87p%20v%C3%A0%20th%E1%BB%A7y%20s%E1%BA%A3n

Hoa, N.P., 2012. The use of Yucca extract in aquaculture, in Principals and technique in aquaculture, T.Q. Phu, Editor., Agriculture Publisher: HCMC. p. 170.

Hoseinifar, S.H., M. Khalili, R. Rufchaei, M. Raeisi, M. Attar, H. Cordero, and M.Á. Esteban, 2015. Effects of date palm fruit extracts on skin mucosal immunity, immune related genes expression and growth performance of common carp (*Cyprinus carpio*) fry. Fish & shellfish immunology. 47, 706-711.

Logambal, S., S. Venkatalakshmi, and R. Dinakaran Michael, 2000. Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). Hydrobiologia. 430, 113-120.

Mai, V.T., 2012. Use of veterinary medicines in Vietnamese aquaculture: current status. FAO Fisheries and Aquaculture Technical Paper. 91-98.

Nguyen, L.A.D., M.P. Tran, C. Douny, J. Quetin-Leclercq, T.B.H. Bui, T.B. Le, Q.N. Truong, T.B.H. Bui, T.T.H. Do, T.P. Nguyen, P. Kestemont, and M.-L. Scippo, 2020. Screening and comparative study of *in vitro* antioxidant and antimicrobial activities of ethanolic extracts of selected Vietnamese plants. International Journal of Food Properties. 23, 481-496.

Nguyen, Q.T., M.P. Tran, S.N. Huynh, S. Quennery, T.T.H. Do, T.P. Nguyen, P. Kestemont, and M.L. Scippo, 2014. Situation of chemicals used in rice-fish, stripped catfish cultured in pond and red tilapia cultured in cage in Mekong Delta. Sci J can Tho Uni. 2, 278-283.

Nhu, T.Q., N.P. Dam, B.T.B. Hang, B.T.B. Hue, M.-L. Scippo, N.T. Phuong, J. Quetin-Leclercq, and P. Kestemont, 2020. Immunomodulatory potential of extracts, fractions and pure compounds from *Phyllanthus amarus* and *Psidium guajava* on striped catfish (*Pangasianodon hypophthalmus*) head kidney leukocytes. Fish & Shellfish Immunology.

Nhu, T.Q., B.T.B. Hang, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019a. Plant extract-based diets differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*). Fish & shellfish immunology. 92, 913-924.

Nhu, T.Q., B.T.B. Hang, A. Vinikas, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019b. Screening of immuno-modulatory potential of different herbal plant extracts using striped catfish (*Pangasianodon hypophthalmus*) leukocyte-based *in vitro* tests. Fish & shellfish immunology. 93, 296-307.

Ocheng, F., F. Bwanga, M. Joloba, A.-K. Borg-Karlson, A. Gustafsson, and C. Obua, 2014. Antibacterial activities of extracts from Ugandan medicinal plants used for oral care. Journal of ethnopharmacology. 155, 852-855.

Phan, L.T., T.M. Bui, T.T. Nguyen, G.J. Gooley, B.A. Ingram, H.V. Nguyen, P.T. Nguyen, and S.S. De Silva, 2009. Current status of farming practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta, Vietnam. Aquaculture. 296, 227-236.

Phu, T.M., N.T. Phuong, T.T. Dung, D.M. Hai, V.N. Son, A. Rico, J.H. Clausen, H. Madsen, F. Murray, and A. Dalsgaard, 2016. An evaluation of fish health-management practices and occupational health hazards associated with Pangasius catfish (*Pangasianodon hypophthalmus*) aquaculture in the M ekong D elta, V ietnam. Aquaculture Research. 47, 2778-2794.

Rico, A., T.M. Phu, K. Satapornvanit, J. Min, A. Shahabuddin, P.J. Henriksson, F.J. Murray, D.C. Little, A. Dalsgaard, and P.J. Van den Brink, 2013. Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. Aquaculture. 412, 231-243.

Ström, G.H., H. Björklund, A.C. Barnes, C.T. Da, N.H.Y. Nhi, T.T. Lan, U. Magnusson, A. Norman Haldén, and S. Boqvist, 2019. Antibiotic use by small-scale farmers for freshwater aquaculture in the upper Mekong Delta, Vietnam. Journal of Aquatic Animal Health. 31, 290-298.

Sukumaran, V., S.C. Park, and S.S. Giri, 2016. Role of dietary ginger *Zingiber officinale* in improving growth performances and immune functions of *Labeo rohita* fingerlings. Fish & shellfish immunology. 57, 362-370.

Tekwu, E.M., A.C. Pieme, and V.P. Beng, 2012. Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance. Journal of ethnopharmacology. 142, 265-273.

Van, K.V., 2012. The use of garlic in treatment of bacterial disease, in Principles and technique in aquaculture, T.Q. Phu, Editor., Agriculture Publisher: HCMC. p. 170.

VASEP, 2021. Vietnam Association of Seafood Exporters and Producer. Characteristics of the sector; Available from: https://vasep.com.vn/san-pham-xuat-khau/ca-tra/tong-quan-nganh-ca-tra

VMARD, 2012. Vietnamese Ministry of Agriculture and Rural Development. https://vanban.chinhphu.vn/default.aspx?pageid=27160&docid=156490

VMARD, 2015. Vietnamese Ministry of Agriculture and Rural Development. https://www.mard.gov.vn/Pages/tang-cuong-kiem-soat-du-luong-khang-sinh-trong-nuoi-trong-thuy-san-28436.aspx

VMARD, 2016. Vietnamese Ministry of Agriculture and Rural Development. http://vanban.chinhphu.vn/portal/page/portal/chinhphu/hethongvanban?class_id=1&mode=detail&document_id=186403

3. SCREENING AND COMPARATIVE STUDY OF *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ETHANOLIC EXTRACTS OF SELECTED VIETNAMESE PLANTS

The previous study regarding the survey on grow-out farms of striped catfish indicated that Bacillary Necrosis of Pangasius and Motile Aeromonad Septicaemia, the major pathogens that striped catfish farmers have to deal with, were caused by *Edwardsiella ictaluri* and *Aeromonas hydrophila*, respectively. Among twelve types of antibiotics used in bacterial diseases management by striped catfish farmers, two (enrofloxacin and ciprofloxacin) were banned antibiotics according to Vietnamese Ministry of Agriculture and Rural Development. This pointed the need to improve the use of antibiotics as well as the Vietnamese regulation. Regarding the use of plants and plant extract products, quality and the efficiency of these products are still not demonstrated. Several farmers used the traditional plants following their experiences as conventional medicines for human without knowing which dose to apply for fish farming. Hence, further study would be recommended for the review of the effectiveness of plants under on-farm treatment. In this study, 20 locally available plants empirically used by fish farmers in the Mekong Delta regions and three commercial products have been selected for screening about their antioxidant and antimicrobial activities (in particular against *A. hydrophila* and *E. ictaluri*). The plants were selected based partly on results of chapter 2 and results of literature search performed in the first step of the AquaBioActive project.

This study will allow an initial selection of plants with potential application in aquaculture.

3. SCREENING AND COMPARATIVE STUDY OF *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ETHANOLIC EXTRACTS OF SELECTED VIETNAMESE PLANTS

Adpated from:

Nguyen Le Anh Dao, Tran Minh Phu, Caroline Douny, Joëlle Quetin-Leclercq, Bui Thi Buu Hue, Le Thi Bach, Truong Quynh Nhu, Bui Thi Bich Hang, Do Thi Thanh Huong, Nguyen Thanh Phuong, Patrick Kestemont, Marie-Louise Scippo

International Journal of Food Properties, 23:1, 481-496.https://doi.org/10.1080/10942912.2020.1737541

ABSTRACT

This study aimed to screen the *in vitro* antioxidant and antimicrobial activities of ethanolic extracts from 20 plants and three herbal commercial products empirically used for aquaculture improvement in Vietnam. The results of 2,2-diphenyl-1-pycrylhydrazyl (DPPH) radical scavenging assays showed that *Phyllanthus amarus* extract was the strongest antioxidant, followed by four extracts in the subsequent order: *Piper betle* > *Psidium guajava* > *Euphorbia hirta* > *Mimosa pudica*. These five plant extracts were very active in a DPPH radical scavenging assay with concentrations needed to scavenge half of the DPPH (IC_{50}) below 30 µg/mL. Seven plant extracts showed an IC_{50} ranging from 31.9 to 59.7 µg/mL, while eleven extracts showed an IC_{50} above 70 µg/mL. A positive association was found between phenolic content (expressed as gallic acid equivalents) and antioxidant activity of the plant extracts. Concerning *in vitro* antimicrobial activities*, P. amarus* extract showed the highest activity against two different strains of *Aeromonas hydrophila* as demonstrated by its low minimal inhibitory concentration (MIC) of 156 and 625 µg/mL, respectively; whereas, *P. betle* displayed a moderate activity against *Edwardsiella ictaluri* with a MIC value of 625 µg/mL. Tannins were observed as significant factors contributing to antioxidant and antimicrobial properties of the plant extracts tested.

Keywords: *Phyllanthus amarus*, *Aeromonas hydrophila*, *Edwardsiella ictaluri,* tannins, Vietnam

INTRODUCTION

Medicinal therapies using plant extracts expanded popularly in the late 1990s (Cowan, 1999). Beside the Chinese herbs, during the last decades, Vietnamese medicinal plants have also received interest as novel sources of alternative medication. It is estimated that approximately 2500 species of the Southeast Asian tropical exotic herbals have been used in folk medicine for their biological/therapeutic properties (Banskota et al., 2003; Chi, 1997), such as diuretic (Doan et al., 1992), antioxidant (Thuong et al., 2006), cytotoxic (Namhui and Bae, 2010, Nguyen et al., 2005), and antimicrobial (Hue Ngan et al., 2008). Moreover, a variety of herbs and plants have been receiving enormous interest as alternatives to synthetic additives or preservatives in the food industry in general and in aquaculture products in particular (Madsen and Bertelsen, 1995).

Aquaculture in Vietnam has been growing remarkably in recent years, producing 3.84 million tons aquaculture products in 2017 (GSO, 2018). Mekong Delta is the main fish production area in the Southern part of Vietnam with a contribution of 70% to the national aquaculture production. Striped catfish (*Pangasianodon hypophthalmus*) is the dominating cultured species for export with an annual production of 1.25 million tons (VASEP, 2019). However, *Aeromonas hydrophila* and *Edwardsiella ictaluri* are pathogenic bacteria causing major diseases in the striped catfish industry, including "bacillary necrosis of pangasius" (BNP) (Crumlish et al., 2002) or motile aeromonad septicemia (MAS) (Subagja et al., 1999), which is often related to stressed or immunocompromised hosts (Roberts, 1993).

Chemicals including antimicrobials have been extensively applied to control pathogens and water quality management (Phu et al., 2016, Rico et al., 2013). The imprudent use of antimicrobials to control bacterial disease in fish farming may lead to development of antibiotic resistant bacteria, residues of antimicrobials in fish products, environmental impacts, etc. The access to export markets is certainly warned by the risk of non-compliance to international food safety regulations and quality standards. The final outcome could be a decline of profits made by the aquaculture sector, which is one of the most important activities in Vietnam (especially in the Mekong Delta).

Nowadays, more environment friendly prophylactic and protective solutions are claimed and natural bio-active products are examined for enhancing the immune system and health status of cultivated animals (Nhu et al., 2019a), (Nhu et al., 2019b). However, in spite of a great variety of wild plants allocated in the various eco-regions of Vietnam and the concern of aquaculture farmers in using alternatives to antibiotics, the use of natural products in aquaculture is not yet popular in the country.

In this study, 20 plants, which are locally available, inexpensive and have been empirically used by fish farmers in the Mekong Delta regions, have been selected for a literature review about their antioxidant and antimicrobial activities (in particular against *A. hydrophila* and *E. ictaluri*). Ethanolic extracts of these 20 selected plants have been tested *in vitro* testing for these activities (Table 1A). Besides these 20 plants, three commercial products have been added (Table 1B), coming from Vietnamese companies, which are supposed to be efficient in treating white feces syndrome, enteritis and hepatic disease in shrimp, as well as in regenerating liver tissue in fish.

MATERIALS AND METHODS

Chemicals and media

DPPH (2,2-diphenyl-1-pycrylhydrazyl), (±)-α-tocopherol, Folin-Ciocalteu's reagent, gallic acid, resazurin and gentamicin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Brain Heart Infusion media (BHI), isosensitest media, buffer pepton water (BPW) and plate count agar (PCA) were obtained from Oxoid (Basingstoke, UK). All solvents and reagents used in the analysis were of analytical grade.

Plant extracts preparation

Twenty plant species (Table 1A) were collected from various areas in Mekong Delta, Vietnam. The plants were authenticated by the Department of Biology, College of Natural Science, Can Tho University and compared to literature. All collected parts of plants were then washed to remove mud and dust; the rotten and damaged parts were also discarded. Samples were air dried

in shade for some days and then put in an oven at about 60°C until well-dried. After that, they were ground into a fine powder with a blender and stored in sealed containers in a dry and cool place. In this study, ethanol was chosen as an extraction solvent since it is the most common organic solvent used by herbal medicine manufacturers to obtain crude extracts of phytochemicals from plant materials and to produce safe therapeutic products for consumers (Low Dog, 2009). The dried-powder (100 g) was soaked in ethanol 96% (800 mL) for at least 24 hours at room temperature with frequent agitation. The solvent-containing extracts were then decanted and filtered. The ground samples were further extracted 4 times with ethanol 96%. The filtrates from each extraction were combined and the solvent was evaporated under reduced pressure using a rotary evaporator to give crude ethanolic extracts.

Three commercial products (two in powders, one in liquid) named A, B, C were purchased from purchasers willing to stay anonymous. However, they gave us as a personal communication the plant composition of their product (Table 1B). For both commercial powder products, a 1 g dry sample was continuously shaken in 30 mL ethanol (96%) for 24 hours. After filtration, the filtrates obtained were evaporated to dryness as explained above. The liquid commercial product was lyophilized. All ethanolic extracts were finally lyophilized until dryness to remove any trace of water and stored in a dry place at room temperature before use.

Table 1A. List of 20 selected plants used for the screening of their antioxidant and antimicrobial activities

Identification number *	Botanical name	Common name	Ecology	Distributed in Vietnam	Level of threats in the wild	Status of species	Part used	Reference
CTU 1731	*Ageratum conyzoides* L.	Billygoat-weed, chick weed	Grow and develop on different soils; from lowland to highland 1800m	Widely grown in Vietnam	Low	Invasive	The whole plant	(Ho, 2003a; Chi, 2003; Ban, 2005)
CTU1922	*Allium sativum* L.	Garlic	Spice herb	Widely grown in Vietnam	No	Native	Bulb	(Ho, 2003a; Chi, 2003; Ban, 2005)
CTU 1716	*Alternanthera sessilis* (L.) DC.	Sessile joyweed and dw arf copperleaf	Uncultivated growth in the garden, paths at edge of ricefield, edge of canal	Widely grown in Vietnam (at height 1000m)	Low	Native	Aerial parts (leaves + twigs)	(Ho, 2003a; Chi, 2003; Ban, 2005)
CTU1720	*Annona reticulata* L.	Custard apple; bullock's heart; raamphal plant	Aquatic growth, edge of canal (alum tolerant tree)	Widely grown in Mekong Delta	Low	Native	Leaves	(Chi, 2003; Ho, 2003c; Ban, 2005)

CTU1608	*Azadirachta indica* A.Juss.	Neem	Grow in the forest, leaves harvested as vegetable	Forest: Ninh Thuận, An Giang, Kiên Giang; cultivated in the South of Vietnam	Low	Native	Leaves, flower and stem bark	(Ho, 2003a; Chi, 2003; Ban, 2005)
CTU17161	*Cayratia trifolia* (L.) Mabb. & J.Wen	Fox grape	Scattered growth in secondary forest, shrub, garden	Widely grown in Vietnam	Low	Native	The whole plant	(Ho, 2003b; Chi, 2003; Ban, 2005)
CTU1823	*Centella asiatica* (L.) Urb.	Asiatic pennywort, Indian pennywort	Grow in clusters at wild land, paths at edge of ricefield, roadside	Widely grown in Vietnam	Low	Native	The whole plant	(Ho, 2003b; Chi, 2003; Ban, 2005)
CTU1836	*Eclipta alba* (L.) Hassk.	False daisy	Grow in paths at edge of ricefield, roadside, from lowland to highland 1800m	Widely grown in Vietnam	Low	Native	The whole plant	(Ho, 2003a; Chi, 2003; Ban, 2005)
CTU1874	*Euphorbia hirta* L.	Asthma-plant	Photophilic herb, grow in wild land, paths at edge of ricefield	Widely grown in Vietnam	Low	Native	The whole plant	(Ho, 2003b; Chi, 2003; Ban, 2005)

CTU17174	*Houttuynia cordata* Thunb.	Fish mint	Grow in clusters at wild land, paths at edge of ricefield, edge of canal	Uncultivated and widely grown in Vietnam	Low	Native	Leaves + stem	(Ho, 2003c; Chi, 2004; Ban, 2005)
CTU17113	*Mimosa pudica* L.	Sensitive plant, sleepy plant, shy plant	Grow in roadside, wild land, sea wall	Widely grown in Vietnam (exotic species)	Medium	Introduced*	The whole plant	(Ho, 2003c; Chi, 2004; Ban, 2005)
CTU1871	*Momordica charantia* L.	Bitter melon, bitter squash	Uncultivated growth in secondary forest or cultivated in farms	Widely grown in Vietnam (as vegetable)	Low	Native	Leaves, stem	(Ho, 2003c; Chi, 2004; Ban, 2005)
CTU1896	*Ocimum basilicum* L.	Basil	Photophilic herb	Widely grown in Vietnam (as spice herb)	No	Native	Aerial parts (leaves + twigs)	(Ho, 2003b; Chi, 2004; Ban, 2005)
CTU1899	*Perilla frutescens* (L.) Britton	Perilla mint	Photophilic herb	Widely grown in Vietnam (as spice herb)	No	Native	Leaves	(Ho, 2003b; Chi, 2004; Ban, 2005)

CTU1778	*Phyllanthus amarus* Schumach. & Thonn.	Chamber bitter, gripe-weed, shattersto ne, stone-breaker or leaf-flower	Uncultivated grow in paths of grass, paths at edge of ricefield or mountain field, roadside	Widely grown in Vietnam	Low	Native	Aerial parts (leaves + twigs)	(Ho, 2003b; Chi, 2004; Ban, 2005)
CTU1623	*Piper betle* L.	Betel	Planted in home gardens	Widely grown in Vietnam	No	Native	Leaves	(Ho, 2003c; Chi, 2004; Ban, 2005)
CTU17137	*Portulaca oleracea* L.	Purslane	Scattered growth or in clusters at wild land, edges of ricefield	Widely grown in Vietnam	Low	Native	The whole plant	(Ho, 2003a; Chi, 2004; Ban, 2005)
CTU17125	*Psidium guajava* L.	Apple guava	Planted in farms	Widely grown in Vietnam	Low	Introduced*	Leaves	(Ho, 2003b; Chi, 2004; Ban, 2005)
CTU1644	*Wedelia chinensis* (Osbeck) Merr.	-	Uncultivated grown in paths of grass	Widely grown in the Central and North of Vietnam	Low	Native	Leaves	(Ho, 2003a; Chi, 2004; Ban, 2005)

CTU1898	*Zingiber officinale* Roscoe	Ginger	Uncultivated grown in forest		Widely grown in Vietnam	Low invasion	Native	Rhizome	(Ho, 2003a; Chi, 2004; Ban, 2005)

* Species identification was done in/by Can Tho University (CTU), Vietnam.
Introduced*: this introduced species was perennially planted in Mekong Delta, Vietnam.

Table 1B. List of 3 commercial products used for the screening of their antioxidant and antimicrobial activities

Product name	Composition*	Form
A	*Blumea balsamifera* DC.	Solid
B	*Cynara cardunculus* L. *Phyllanthus emblica* L. *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.	Liquid
C	*Phyllanthus urinaria* L. *Eclipta alba* (L.) Hassk.	Solid

*
Personal communication from the purchasers who asked to remain anonymous

Total phenolic compounds determination

The amount of total phenolic compounds (TPC) was determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). This assay was performed in triplicate for all plant extracts, dissolved in methanol. A standard curve was established using gallic acid as standard, in the range of 0-10 µg/mL (in methanol). The amount of total phenolic compounds in plant extracts was calculated as gallic acid equivalents (GAE) in mg of GAE per 100 mg (%) of freeze-dried plant material. Limit of detection (LOD) and limit of quantitation (LOQ) were 0.51 and 1.69 µg GAE/mL, respectively.

Antioxidant capacity

Antioxidant capacity of plant extracts was measured through DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging assay (Thiangthum et al., 2012). The DPPH method is very rapid, simple, sensitive, reproducible and does not require special instrumentation. The sensitivity of the method is determined by the strong absorption of $DPPH^{\bullet}$. In our study, it was very convenient for the screening of numerous of samples of different polarity because of its high throughput. The DPPH method is considered to have a mixed mechanism (i.e. both electron transfer-based and hydrogen atom transfer-based). In addition, Pyrzynska and Pękal (2013) confirmed that the DPPH method is independent of the substrate polarity. This assay was performed in triplicate for each plant extract sample and α-tocopherol was used as a positive control. Plant extracts dissolved in methanol were tested for their DPPH scavenging activity in concentrations ranging from 1 to 125 µg/mL. Plant extract concentrations were plotted against percentages of remaining DPPH after 30 minutes reaction. A sigmoidal curve was then fitted, allowing the determination of the $IC_{50,}$ i.e. the plant extract concentration (in µg/mL) needed to scavenge 50% of the DPPH initially introduced.

In vitro investigation of antimicrobial activities of plant extracts

Resazurin method was performed as described by Sarker et al. (2007) with some adaptations for minimum inhibitory concentration (MIC) determination of plant extracts against *A. hydrophila* and *E. ictaluri*. Stock solutions of the plant extracts were prepared at a concentration of 10 mg/mL in sterilized normal saline containing 25% dimethyl sulfoxide (DMSO) (when testing

A. hydrophila) or 12.5% DMSO (when testing *E. ictaluri).* The resazurin and the antibiotic gentamicin solutions were prepared in sterile distilled water at the concentration of 10 mg/mL and 200 µg/mL, respectively. Two strains of *Aeromonas hydrophila* (1 and 2) were isolated from red tilapia (*Oreochromis* sp.*)*, whereas, *Edwarselia ictaluri* was isolated from striped catfish (*P. hypophthalmus*) in Mekong Delta, Vietnam. These strains were subcultured in BHI broth, incubated for 24 h at 37°C (*A. hydrophila*) and for 48 h at 30°C (*E. ictaluri*), and then pelleted in 20 mL of sterile normal saline solution. The approximate number of bacteria were estimated by the optical density at 600 nm, reaching a value of $4x10^8$ and $1x10^8$ cfu/mL for *A. hydrophila* and *E. ictaluri*, respectively according to the turbidity of McFarland Standards (e.g., Dalynn Biologicals, cat no. TM50-TM60). Further dilutions were performed to use final concentrations of $2x10^4$ in double strength isosensitest broth and $0.5x10^4$ cfu/mL in BHI broth for *A. hydrophila* and *E. ictaluri*, respectively. One mg of resazurin dissolved in water was then added into 10 mL of the liquid inoculum.

The MIC determination was performed in a 96-well plate, using concentrations of plant extracts ranging from 5 µg/mL to 2500 µg/mL and 100 µL of working inoculum solution per well. The plates were prepared in triplicate and gentamicin was used as positive control in each plate. After incubation at 37°C for 18 h (*A. hydrophila*) or 48 h (*E. ictaluri*), plates were visually observed to determine the lowest concentration at which color changed from purple to pink, which was taken as the MIC value (Drummond and Waigh, 2000).

Tannins quantification and removal from plant extracts

The extracts of *P. amarus* and *E. hirta* were prepared at 10 mg/mL in methanol. Tannins were quantified according to the European pharmacopeia method and the results were expressed in pyrogallol equivalents (g/100g extract) (European Pharmacopoeia Commission, 2018). LOD and LOQ are 1.5 and 5 µg/mL, respectively. Tannins were excluded from methanolic plant extracts by using a polyamide column (Macherey – Nagel, Germany), as described by Houghton and Raman (1998). Ten grams of polyamide were soaked in 100 mL of water during one night. A column was packed with the gel. Ten milligrams of plant extract dissolved in 1 mL of methanol were loaded onto the column. A volume of 200 mL of methanol was gradually poured into the

column for the elution. An empty dried flask was prepared to collect the eluates, which were evaporated to dryness. Tannins were retained on the column while the dried residue contained the extract free from tannins.

Statistical analysis

Data of total phenolic content and IC_{50} values were expressed as mean ± standard deviation by Microsoft Excel software. Analysis of variance (one-way ANOVA) was performed by using SPSS 16.0 (SPSS Inc, Chicago, II, USA).

RESULTS AND DISCUSSION

Total phenolic content and antioxidant activity of 23 plant extracts

The content of phenolic compounds determined in the 23 plant extracts samples ranged between 0.5 and 18.8 mg gallic acid equivalent/100 mg plant extract (Table 2). Among the first five plant extracts mentioned in Table 2, considered as having a "high" antioxidant activity (see below), *P. amarus* showed the significantly ($p<0.05$) highest total phenolic content. Interestingly, the commercial products contained lower levels of phenolic compounds than these 5 plant extracts. The antioxidant activity measured using the DPPH assay was expressed as an IC_{50}, corresponding to the concentration of plant extract needed to scavenge half of the DPPH. According to Thiangthum et al. (2012), the antioxidant activity of samples can be classified as high ($IC_{50}<30$ µg/mL), intermediate ($30< IC_{50}<50$ µg/mL), low ($50< IC_{50}<70$ µg/mL) or absent ($IC_{50}>70$ µg/mL). In our assay, the IC_{50} of the (±)-α-tocopherol, the reference antioxidant, was 12 µg/mL. Eleven out of the 23 extracts displayed an antioxidant activity, with concentrations able to inhibit half of the maximum response (IC_{50}) ranging from 5.83 to 49.5 µg/mL (Table 2). *P. amarus* extract showed the apparent strongest radical scavenging effect ($IC_{50} = 5.83$ µg/mL) but no significant difference ($p>0.05$) was observed with the antioxidant activities of *P. betle* and *P. guajava* extracts. The remaining 2 samples showing a high antioxidant activity (i.e. $IC_{50}< 30$ µg/mL) were *E. hirta* and *M. pudica*. A group of six samples, including *Z. officinale*, commercial product B, *E. alba*, commercial product A, *A. reticulata*, *H. cordata*, showed an intermediate antioxidant capacity (i.e 30 µg/mL $< IC_{50} < 50$ µg/mL).

Table 2. Total phenolic content and results from the DPPH radical scavenging assay (IC_{50}) of 23 plant extracts (the plants are shown by decreasing antioxidant capacity, i.e. increasing IC_{50}), and comparison with data from the literature

Scientific name	This study		Literature studies		
	Phenolic content (%)*	IC_{50} (µg/mL)	Phenolic content (%)*	IC_{50} (µg/mL)	Reference
Phyllanthus amarus	$18.8^{j} \pm 0.75$	$5.83^{a} \pm 0.50$	17	11	Kumaran and Karunakaran (2007), Thiangthum et al. (2012)
Piper betle	$16.4^{i} \pm 0.76$	$8.32^{a} \pm 0.90$	15.4	11	Atiya et al. (2018)
Psidium guajava	$14.5^{h} \pm 0.79$	$8.55^{a} \pm 0.53$	15.6	1.56	Ekaluo et al. (2015)
Euphorbia hirta	$10.3^{g} \pm 0.91$	$17.8^{b} \pm 1.28$	29.1	2.81	Teeli et al. (2018)
Mimosa pudica	$7.46^{f} \pm 0.29$	$18.0^{b} \pm 0.96$	4.3	127	Das et al. (2014)
Zingiber officinale	$6.92^{f} \pm 0.31$	$31.9^{c} \pm 2.48$	1	46.5	Mojani et al. (2014)
Commercial product B	$0.53^{a} \pm 0.04$	$33.7^{cd} \pm 0.34$	-	-	-
Eclipta alba	$4.57^{d} \pm 0.25$	$36.6^{cd} \pm 0.86$	13.2	42	Le et al. (2018)
Commercial product A	$5.65^{e} \pm 0.20$	$38.1^{d} \pm 2.55$	-	-	-
Annona reticulata	$5.01^{de} \pm 0.24$	$45.1^{e} \pm 4.58$	13.6	51	Subba and Aryal (2016)
Houttuynia cordata	$5.45^{e} \pm 0.28$	$49.5^{e} \pm 5.83$	12.6	73	Wang et al. (2006)
Cayratia trifolia	$3.31^{c} \pm 0.22$	$59.7^{f} \pm 5.11$	7.3	74	Rabeta and Lin (2015), Perumal et al. (2012)
Perilla frutescens	$3.61^{c} \pm 0.21$	$73.7^{g} \pm 2.09$	11.6	7.97	Lin et al. (2010)

Azadirachta indica	$4.33^{d} \pm 0.39$	$79.2^{h} \pm 3.93$	10.1	60.6	Narendhirakannan et al. (2012)
Commercial product C	$2.04^{b} \pm 0.17$	$85.9^{i} \pm 1.95$	-	-	-
Ageratum conyzoides	$2.05^{b} \pm 0.07$	$118^{j} \pm 1.92$	0.85	214	Neelabh et al. (2017)
Portulaca oleracea	ND	> 125	0.43	2950	Alam et al. (2014)
Allium sativum	ND	> 125	0.005	600	Chekki et al. (2014)
Ocimum basilicum	$2.25^{b} \pm 0.26$	> 125	0.07	350	Aydemir and Becerik (2011)
Centella asiatica	$2.27^{b} \pm 0.09$	> 125	2.4	45	Dewi and Maryani (2015)
Wedelia chinensis	$1.66^{b} \pm 0.02$	> 125	-	45	Gurusamy and Saranya (2010)
Momordica charantia	ND	> 125	1.02	307	Rezaeizadeh et al. (2011)
Alternanthera sessilis	$1.73^{b} \pm 0.11$	> 125	3.7	946	Ho et al. (2012), Othman et al. (2016)

* mg gallic acid equivalent /100 mg dry plant extract; ND = Not determined; "-": no information available.
High antioxidant activity: $IC_{50} < 30$ µg/mL, Intermediate antioxidant activity: $30 < IC_{50} < 50$ µg/mL, No activity: $IC_{50} > 70$ µg/mL.
Values are mean ± SD (n = 3). Mean values within a column with the same letter are not significantly different ($p > 0.05$).

For the 23 selected plant extracts, the antioxidant activity was related with their phenolic content with a correlation coefficient R^2=0.9137 (Fig. 1). The antioxidant properties of plant extracts might be influenced by the number of specific chemical groups from phenolic compounds, such as hydroxyl or methoxy groups, keto or free carboxylic groups, as well as by other antioxidant secondary metabolites, such as vitamins, volatile oils and carotenoids (Pratt and Hudson, 1990). In the present study, the highest antioxidant activity was found for the *P. amarus* extract. This result agrees with the previous study of Thiangthum et al. (2012) reporting that *P. amarus* extracts showed high antioxidant activity as shown by low IC_{50} values in a DPPH assay, ranging from 10 to 16 µg/mL. Regi Raphael et al. (2002) also reported that the extract of *P. amarus* was found as a remarkable antioxidant throughout its inhibition capacity in scavenging free radicals *in vitro*, while other studies confirmed that the high content of phenolic compounds in this plant was positively correlated with its radical scavenging potential (Guha et al., 2010). The differences between our results and literature data in Table 2 can come from the dependence of IC_{50} on the DPPH concentration which vary between studies. In addition, other factors may contribute to dissimilarity of the results such as habitats, geographical locations, seasons and parts of the plant used (Khan et al., 2010; Mikage and Mouri, 1999).

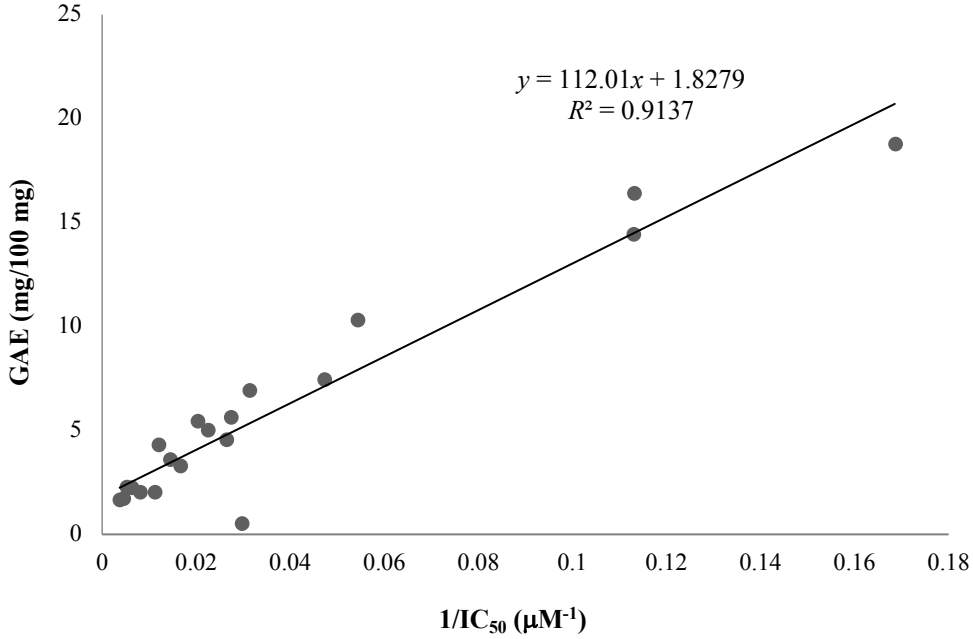


Figure 1. Correlation between phenolic compounds contents (expressed as mg GAE/100 mg extract) and antioxidant activity measured in the DPPH assay (expressed as $1/IC_{50}$, μM^{-1}) of the 23 selected extracts

Many investigations were conducted concerning the chemical components of this *Phyllanthus* species and bioactive constituents. According to Igwe et al. (2007), extracts from leaves of *P. amarus* (which were the part of the plant used in this study) contained high level of saponins and tannins: 24 and 17%, respectively. In the study of Sharma et al. (1993), the leaves of *P. amarus* were shown to contain a high quantity of lignans (phyllanthin (0.7%) and hypophyllanthin (0.3%)) in comparison with other parts of the plant. These active compounds might be responsible for the major part of the antioxidant activity.

The good relationship found in this study between total phenolic contents and DPPH measurements is also in agreement with a study of Dudonne et al. (2009) who showed a high positive correlation between the free radical scavenging and total phenolic content of 30 plant extracts. For both total phenolic content and IC_{50} found in the DPPH assay, table 2 compares the results of this study and data found in the literature. For most of the tested plants, the results are roughly similar except for two plants giving opposite results. In this study, the ethanolic extract from the whole *Mimosa pudica* displayed a high antioxidant activity (IC_{50} of 21 µg/mL) while Das et al. (2014) reported an IC_{50} of 127 µg/mL for leaves methanolic extract of the same plant, which would mean no antioxidant capacity (Table 2). On the contrary, for *Perilla frutescens,* the ethanolic extract from its leaves showed a low antioxidant capacity in this study (IC_{50} of 70 µg/mL), but a high one in Lin et al. (2010) (IC_{50} of 8 µg/mL).

Surprisingly, the antioxidant capacity determined in two (A and B) out of the three tested commercial products was shown to be intermediate only and was lower than the one of the 5 plant extracts mentioned here above, while the third commercial product (C) was qualified as non active. Plants used as the main ingredients in each product are herbs that have been acknowledged since ancient times for their valuable and therapeutic efficiency. Extracts of *Blumea balsamifera* (contained in the commercial product A), *Cynara cardunculus, Phyllanthus emblica, Terminalia arjuna* (contained in the commercial product B), or *Phyllanthus urinaria* and *Eclipta alba* (contained in the commercial product C) revealed different activities in the DPPH assay with IC_{50} values of 72 µg/mL (Shyur et al., 2005), 23 µg/mL (Soumaya et al., 2013), 11 µg/mL (Liu et al., 2008), 8 µg/mL (Shahriar et al., 2012), 17 µg/mL (Eldeen et al., 2011), and 42 µg/mL (Le et al., 2018), respectively. However, it is well known that many factors of manufacture (such as the

materials, the extraction procedure of plant extracts, the ingredients, etc.) can affect the product's activity. Moreover, Bruno and Munro (1991) determined that the formulation of commercial products frequently include constituents other than the active ingredient(s), which can be stabilizers, carriers or diluent agents (Rodgers and Furones, 2009).

Antimicrobial activities of plant extracts against A. hydrophila and E. ictaluri

The *in vitro* antimicrobial activities of the 23 samples against two *A. hydrophila* strains isolated from red tilapia (*Oreochromis* sp.*)* in Vietnam are presented in Table 3, which also presents the antibacterial activities of the selected plants reported in the literature. According to Kuete (2010), antimicrobial activities can be classified as weak or absent if MIC are above 1250 or 2500 µg/mL, respectively. For MIC between 156 and 625 µg/mL, the antimicrobial activity was qualified as moderate. Four plant extracts (*P. amarus*, *P. betle*, *P. guajava* and *E. hirta*) and one commercial product (A) showed moderate antimicrobial activities against the first strain of *A. hydrophila* (1) (Table 3), with MICs values ranging between 156 and 625 µg/mL, while the rest of the samples showed weak or no antibacterial activity (MIC ≥ 1250 or 2500 µg/mL, respectively). The growth of the first strain of *A. hydrophila* (1) was also inhibited by *P. amarus*, *P. betle* and commercial product A, but at higher concentrations of plant extracts (MIC = 625 µg/mL). *P. guajava* and *E. hirta*, which inhibited the growth of the first strain of *A. hydrophila* (1) at a concentration of 312 and 625 µg/mL respectively, needed a concentration of 1250 µg/mL to inhibit the second strain (2) (Table 3). The results showed that both strains of *A. hydrophila* display the same pattern of sensitivity towards tested plant extracts, but the first strain was more sensitive than the second one. The MIC of the gentamycin antibiotic, used as positive control, was 6.25 µg/mL for both strains (Table 3).

The antibacterial activities of the 23 samples against *Edwardsiella ictaluri* strain are summarized in Table 3. Results show that most plant extracts do not possess antimicrobial activities (MIC≥2500 µg/mL). *Piper betle* and commercial product A showed a moderate activity, with MIC values of 625 µg/mL. However, the strain of *E. ictaluri* used in this work was not very sensitive to gentamicin (MIC=12.5 µg/mL). In another study, where 64 different strains of *E. ictaluri* were tested for their sensitivity to antibiotics, gentamicin showed MIC of 2 µg/mL for 50% of the tested strains and 1 µg/mL (25%) or 4 µg/mL (25%) for the remaining strains (Dung et al., 2008).

Limited information is available about previous studies concerning the antimicrobial capacity of *P. amarus* and *P. betle* against *Aeromonas* spp. and *Edwardsiella* spp. For example, a methanolic extract of *P. amarus* showed an antibacterial activity against *A. hydrophila* with a MIC of 128 µg/mL according to De Britto et al. (2011), while, for the same bacteria, a MIC of 25 µg/mL was found by Caruso et al. (2017) for the ethanolic extract from leaves of *Piper betle*. The capacity of *P. amarus* to inhibit bacterial growth showed a concentration-dependent antibacterial activity particularly against gram-negative microbes (Saranraj and Sivasakthivelan, 2012; Ushie et al., 2013) while Kaveti et al. (2011) showed that ethanolic extracts of *P. betle* leaves were active against several strains of gram positive and negative bacteria. Chopade and Sayyad (2015) reported that *P. amarus* extracts contain considerable amount of lignans (phyllanthin and hypophyllanthin) and tannins. Gbadamosi (2015) mentioned that phyllanthin, a major component of *P. amarus* did not possessed antimicrobial activity, whereas tannins were able to form irreversible complexes with proline rich proteins of bacteria, leading to the inhibition of cell protein synthesis (Olowosulu and Ibrahim, 2006). Hydroxychavicol is one of the major active compounds found in *P. betle* leaves, which has been extensively reported for its antimicrobial activity (Ramji et al., 2002; Sharma et al., 2009). Previous study of Singh et al. (2018) showed that the antibacterial activity of hydroxychavicol involved reactive oxygen species (ROS) generation and DNA damage leading to cell death.

The findings of this study regarding a significant antibacterial activity of two plant extracts (*P. amarus* and *P. betle*) against two strains of the pathogenic bacteria *A. hydrophila* could be useful for the initial selection of natural alternative to antibiotics as well as to prevent bacterial growth in fish products during storage.

Table 3. *In vitro* antibacterial activity of 23 plant extracts against two strains of *A. hydrophila* (1 and 2) and *E. ictaluri,* and other antibacterial activities from literature studies

Scientific name	MIC (µg/mL)			MIC against other bacteria, from literature
	***A. hydrophila* 1**	***A. hydrophila* 2**	***E. ictaluri***	
Phyllanthus amarus	156	625	>2500	*Staphylococcus aureus* (100 µg/mL), *Streptococcus pneumoniae* (400 mg/mL), *Shigella* spp. (25 µg/mL), *E. coli* (50 µg/mL) (Mazumder et al., 2006)
Piper betle	156	625	625	*S. aureus* (1000 µg/mL), *Propionibacterium acnes* (4000 µg/mL) (Taukoorah et al., 2016)
Psidium guajava	312	1250	>2500	*S. mutans* (250 µg/mL), *S. mitis* (250 µg/mL), *S. oralis* (250 µg/mL) (Braga et al., 2014)
Commercial product A	312	625	625	-
Euphorbia hirta	625	1250	>2500	*S. aureus* (25 mg/mL), *Candida albicans* (12.5 mg/mL) (Gupta et al., 2018)
Mimosa pudica	1250	2500	>2500	*Escherichia coli (250* mg/mL*), S. aureus* (250 mg/mL), *Bacillus subtilis* (200 mg/mL) (Tomar et al., 2014),
Eclipta alba	1250	1250	>2500	*E. coli* (12.5 mg/mL), *S. aureus* (3.125 mg/mL), *B. subtilis* (6.25 mg/mL), *B. cereus* (1.56 mg/mL) (Borkataky et al., 2013)
Commercial product B	>2500	>2500	>2500	-
Zingiber officinale	2500	2500	>2500	*Pseudomonas aeruginosa* (40 mg/mL), *E. coli* (40 mg/mL), *S. aureus* (20 mg/mL) (Aghazadeh et al., 2016)
Annona reticulata	2500	2500	2500	*E. coli* (30 µg/mL), *S. aureus* (40 µg/mL), *B. subtilis* (10 µg/mL) (Jamkhande et al., 2016)
Houttuynia cordata	2500	2500	>2500	*Bacillus dysenteriae* (0.08 mg/mL) (Zhou et al., 2006)
Cayratia trifolia	2500		>2500	-
Perilla frutescens	>2500		>2500	-
Azadirachta indica	>2500		>2500	*E. coli* (0.781 mg.mL), *K. pneumonia* (1.562 mg/mL), *E. faecalis* (3.125 mg/mL), *S. aureus* (1.562 mg/mL), *P.*

				aeruginosa (1.562 mg/mL) (Narendhirakannan et al., 2012)
Commercial product C	2500		>2500	-
Ageratum conyzoides	>2500		>2500	*E. coli* (100 µg/mL), *S. aureus* (200 µg/mL) (Prajapati et al., 2014)
Portulaca oleracea	>2500		>2500	*S. aureus* (12.5 mg/mL), *Streptococcus pyogenes* (12.5 mg/mL), *P. aeruginosa* (50 mg/mL), *E. coli* (50 mg/mL) (Agyare et al., 2015)
Allium sativum	2500		>2500	*E. coli* (150 µg/mL), *Klebsiella pneumonia* (150 µg/mL), *B. subtilis* (100 µg/mL) (Meriga et al., 2012)
Ocimum basilicum	>2500		>2500	Bacillus cereus (62.5 µg/mL), *B. subtilis* (125 µg/mL), *S. aureus* (62.5 mg/mL), *E. coli* (125 µg/mL), *Salmonella typhi* (500 µg/mL) (Hossain et al., 2010)
Centella asiatica	2500		>2500	*S. aureus* (8 mg/mL) (Taemchuay et al., 2009)
Wedelia chinensis	>2500		>2500	*B. cereus* (3.13 mg/mL), *B. subtilis* (6.25 mg/mL), *S. aureus* (6.25 mg/mL), *E. coli* (25 mg/mL) (Darah et al., 2013)
Momordica charantia L.	>2500		>2500	*Enterococcus faecalis* (1.25 mg/mL), *E. coli* (5 mg/mL), *K. pneumonia* (5 mg/mL) (Svobodova et al., 2017)
Alternanthera sessilis	>2500		>2500	-
Gentamicin	6.25	6.25	12.5	

Significant activity: MIC < 100 µg/mL
Moderate activity: 100 < MIC ≤ 625 µg/mL
Weak activity: MIC ≥ 625 µg/mL

Impact of tannin removal on the antimicrobial and antioxidant activities of P. amarus and E. hirta ethanolic extracts

The medicinal usefulness of the *P. amarus* and *E. hirta* have been the subject of numerous chemical and microbiology studies. Guha et al. (2010) demonstrated that the extract of *P. amarus* is active in inhibiting lipid peroxidation and in scavenging hydroxyl and superoxide radicals. Moreover, Sheikhlar et al. (2017) investigated the antibacterial activity of several extracts of *E. hirta, Citrus limon* and *Trigonella foenum-graecum*. Results showed that the methanolic extract of *E. hirta* exhibited the strongest antimicrobial activity with the lowest MIC (70 µg/mL) against *A. hydrophila* and their potential to be a beneficial dietary supplement for enhancing the resistance of *Clarius gariepinus* to *A. hydrophila* contamination. Moreover, *E. hirta* extract induced the best immune response at the highest tested concentration (100 µg/mL) in leukocyte-based *in vitro* test (Nhu et al., 2019b). That is why *P. amarus* and *E. hirta* were chosen as the representative plants for further investigations. Previous screenings of bioactive compounds in *P. amarus* and *E. hirta* revealed that tannins may be responsible for their antioxidant and antimicrobial properties (Bensky et al., 1993; Yoshida et al., 1988).

Tannins constitute a group of secondary metabolites which are widely distributed among vegetal species (Haslam, 1989). They are found in approximately 80% of wooden and 15% of herbal dicotyledonous species. Tannins are known to be responsible for general antimicrobial and antioxidant activities (Chung et al., 1998; Gu et al., 2008). Up to now, investigations on the effects of tannin removals from crude plant extracts are limited. Additionally, the prediction of which class of compounds (alkaloids, anthraquinones, flavonoids, saponins and tannins) possess antioxidant and antimicrobial activities is a challenge (Cowan, 1999; Cushnie and Lamb, 2005; Karou et al., 2006). Hence, the comparison of the activities of two selected extracts before and after tannins removal on a polyamide column was performed to clarify whether tannins are the main contributors to their antioxidant and antimicrobial activities.

The results showed that, after tannin removal, both *P. amarus* and *E. hirta* extracts were less active against both strains of *A. hydrophila* (Table 4), while their inhibiting activity against *E. ictaluri* remained as weak as before tannin removal (Table 4). As expected, for both plant extracts, the total phenolic content decreased after tannin removal while their IC_{50} in the DPPH assay increased, meaning less antioxidant activity (Table 5). About half of the initial phenolic content

remains for *E. hirta* extracts and only the seventh for *P. amarus*, showing that a high amount of tannins was present in these extracts. This apparent higher proportion of tannins in *P. amarus* was confirmed after quantification of tannins in both extracts according to the European pharmacopeia method, which gave 5.6 % for *P. amarus* and 1.79 % for *E. hirta* (expressed in pyrogallol). Hydrolysable tannins appear to be the main polyphenol constituents found in *P. amarus*, with geraniin being the most abundant (Foo, 1993; Foo, 1995; Foo and Wong, 1992). This could explain the fact that *P. amarus* displayed high antioxidant and antimicrobial activities. This is in agreement with Catteau et al. (2015), reporting that tannins were at least in part responsible for the activities of several plant methanolic extract effects, but other compounds may also explain a part of the activity.

Patel et al.(2011) reported that high content of tannins could be isolated from *P. amarus* which are associated with some health importance and antimicrobial activity. According to Bensky et al. (1993), hydrolysable tannins are among the main secondary metabolites, which have been discovered to prevent viral DNA polymerase and reverse transcriptase in HIV infection, and to act on angiotensin-converting enzymes in diabetes complication. Moreover, antimicrobial activity of *E. hirta* was attributed to tannins and other bioactive components. Yoshida et al. (1988) and Yoshida et al. (1990a, 1990b) reported the isolation of hydrolysable dimeric ellagitannins (euphorbin A, B, C and E) from the leaves of the plant.

Table 4. Antimicrobial activity of *Phyllanthus amarus* and *Euphorbia hirta* before and after tannins removal

	MIC (µg/mL)			
	Before removing tannins		**After removing tannins**	
	Aeromonas hydrophila 1			
Phyllanthus amarus	156	Moderate	625	Moderate
Euphorbia hirta	625	Moderate	2500	Weak
	Aeromonas hydrophila 2			
Phyllanthus amarus	625	Moderate	>1250	Weak
Euphorbia hirta	1250	Weak	2500	Weak
	Edwardsiella ictaluri			
Phyllanthus amarus	>2500	Weak	>2500	Weak
Euphorbia hirta	>2500	Weak	>2500	Weak

Table 5. Antioxidant activities of *Phyllanthus amarus* and *Euphorbia hirta* before and after tannins removal

Name	**Before removing tannins**		**After removing tannins**	
	DPPH assay IC_{50} (µg/mL)	**Phenolic content (%*)**	**DPPH assay IC_{50} (µg/mL)**	**Phenolic content (%*)**
Phyllanthus amarus	5.83 ± 0.50	18.8 ± 0.75	83.6 ± 5.38	2.68 ± 0.20
Euphorbia hirta	17.8 ± 1.28	10.3 ± 0.91	38.3 ± 2.40	5.02 ± 0.54

Values are mean ± SD (n = 3); * Expressed as mg GAE per 100 mg plant extract

CONCLUSION

In our study, twenty three ethanolic extracts from 20 plant and 3 commercial products obtained in Mekong Delta (Vietnam) were screened for their *in vitro* antioxidant and antimicrobial activities. The results from DPPH free radical scavenging assays revealed that the *P. amarus* extract showed the highest antioxidant activity. Four other plant extracts showed a high antioxidant activity in the following descending order: *P. betle* > *P. guajava* > *E. hirta* > *M. pudica*. There was a positive correlation between total phenolic content and antioxidant activity of the 23 extracts. Beside its high antioxidant activity, *P. amarus* extract also showed antibacterial activity against two different strains of *A. hydrophila*. Tannins were shown to be mainly responsible of these activities, as after tannins removal from *P. amarus* and *E. hirta* extracts, both antioxidant and antimicrobial activities decreased. Both *P. amarus* and *E. hirta* appeared to be promising active plants for further studies. The extracts of these two plants could be valuable natural antibiotic alternatives or natural additives to improve seafood and aquaculture product preservation.

Acknowledgements

This work was financed by the Académie de Recherche et d'Enseignement supérieur - Commission de la Coopération au Développement (ARES-CCD) through AquaBioActive project. The authors are grateful to Ir. Guy Degand and François Brose, who worked in the Laboratory of Food Analysis (University of Liège), for their scientific assistance and valuable support in this project. The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Aghazadeh, M., A.Z. Bialvaei, M. Aghazadeh, F. Kabiri, N. Saliani, M. Yousefi, H. Eslami, and H.S. Kafil, 2016. Survey of the antibiofilm and antimicrobial effects of *Zingiber officinale* (*in vitro* study). Jundishapur journal of microbiology. 9, 1-6.

Agyare, C., E. Baiden, J.A. Apenteng, Y.D. Boakye, and L. Adu-Amoah, 2015. Anti-infective and Anti-inflammatory Properties of *Portulaca oleracea* L. Donnish Journal of Medicinal Plant Research. 2, 1-6.

Alam, M., A.S. Juraimi, M. Rafii, A. Abdul Hamid, F. Aslani, M. Hasan, M. Zainudin, M. Asraf, and M. Uddin, 2014. Evaluation of antioxidant compounds, antioxidant activities, and mineral composition of 13 collected purslane (*Portulaca oleracea* L.) accessions. BioMed Research International. 2014, 1-10.

Atiya, A., B.N. Sinha, and U. Ranjan Lal, 2018. New chemical constituents from the *Piper betle* Linn.(Piperaceae). Natural product research. 32, 1080-1087.

Aydemir, T. and S. Becerik, 2011. Phenolic content and antioxidant activity of different extracts from *Ocimum basilicum*, *Apium graveolens* and *Lepidium sativum* seeds. Journal of food biochemistry. 35, 62-79.

Ban, N.T., 2005. Checklist of plant species of Vietnam. Vol. 3. Ha Noi: Agriculture Publishing House. 1248.

Banskota, A.H., Y. Tezuka, Q. Tran, and S. Kadota, 2003. Chemical constituents and biological activities of Vietnamese medicinal plants. Current topics in medicinal chemistry. 3, 227-248.

Bensky, D., A. Gamble, and T. Kaptchuk, 1993. Chinese Herbal Medicine Materia Medica. Seattle, USA: Eastland Press.

Borkataky, M., B. Kakoty, and L. Saikia, 2013. Proximate analysis and antimicrobial activity of *Eclipta alba* (L.) Hassk.—a traditionally used herb. International Journal of Pharmacy and Pharmaceutical Sciences. 5, 149-154.

Braga, T.V., R.G.R. das Dores, C.S. Ramos, F.C.G. Evangelista, L.M. da Silva Tinoco, F. de Pilla Varotti, M. Carvalho, and A. de Paula Sabino, 2014. Antioxidant, antibacterial and antitumor activity of ethanolic extract of the *Psidium guajava* leaves. American journal of plant sciences. 5, 3492-3500.

Bruno, D. and A. Munro, 1991. The calculation of various treatment dose rates in fish farming. Aquaculture Information Series. Aberdeen, Scotland: Scottish Office Agriculture and Fisheries Department, Marine Laboratory. 10.

Caruso, D., A.M. Lusiastuti, T. Taukhid, J.C. Avarre, M. Yuhana, and S. Sarter, 2017. Ethnobotanical Uses and Antimicrobial Properties of Plants in Small-Scale Tropical Fish

Farms: The Case of Indonesian Fish Farmers in Java (Indonesia). Journal of the World Aquaculture Society. 48, 83-92.

Catteau, L., F. Van Bambeke, and J. Quetin-Leclercq, 2015. Preliminary evidences of the direct and indirect antimicrobial activity of 12 plants used in traditional medicine in Africa. Phytochemistry reviews. 14, 975-991.

Chekki, R.Z., A. Snoussi, I. Hamrouni, and N. Bouzouita, 2014. Chemical composition, antibacterial and antioxidant activities of Tunisian garlic (*Allium sativum*) essential oil and ethanol extract. Mediterranean Journal of Chemistry. 3, 947-956.

Chi, V.V., 1997. Dictionary of medicinal plants in Vietnam, Medical Publishing House: Ha Noi. p. 210-215.

Chi, V.V., 2003. Dictionary of common plants in Vietnam, Science and Technic Publishing House: Ha Noi.

Chi, V.V., 2004. Dictionary of common plants in Vietnam, Science and Technic Publishing House: Ha Noi.

Chung, K.-T., T.Y. Wong, C.-I. Wei, Y.-W. Huang, and Y. Lin, 1998. Tannins and human health: a review. Critical reviews in food science and nutrition. 38, 421-464.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical microbiology reviews. 12, 564-582.

Chopade, A.R., and F.J. Sayyad, 2015. Pain modulation by lignans (phyllanthin and hypophyllanthin) and tannin (corilagin) rich extracts of *Phyllanthus amarus* in carrageenan-induced thermal and mechanical chronic muscle hyperalgesia. Phytotherapy Research. 29, 1202-1210.

Crumlish, M., T. Dung, J. Turnbull, N. Ngoc, and H. Ferguson, 2002. Identification of Edwardsiella ictaluri from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. Journal of fish diseases. 25, 733-736.

Cushnie, T.T. and A.J. Lamb, 2005. Antimicrobial activity of flavonoids. International journal of antimicrobial agents. 26, 343-356.

Darah, I., S. Lim, and K. Nithianantham, 2013. Effects of methanol extract of *Wedelia chinensis* osbeck (asteraceae) leaves against pathogenic bacteria with emphasise on *Bacillus cereus.* Indian journal of pharmaceutical sciences. 75, 533-539.

Das, K., M. Yasin, N.U. Mahbub, M.S. Islam, and N. Mahbuba, 2014. Evaluation of antioxidant and cytotoxic activity of methanolic extract of *Mimosa pudica* leaves. The Pharma Innovation. 3, 32.

De Britto, A.J., D.H.S. Gracelin, and S.R. Sebastian, 2011. Antibacterial activity of a few medicinal plants against *Xanthomonas campestris* and *Aeromonas hydrophila.* Journal of Biopesticides. 4, 57.

Dewi, R.T. and F. Maryani, 2015. Antioxidant and α-glucosidase inhibitory compounds of *Centella asiatica.* Procedia Chemistry. 17, 147-152.

Doan, D.D., N. Nguyen, H. Doan, T. Nguyen, T. Phan, M. Grabe, R. Johansson, G. Lindgren, and N. Stjernström, 1992. Studies on the individual and combined diuretic effects of four Vietnamese traditional herbal remedies (*Zea mays*, *Imperata cylindrica*, *Plantago major* and *Orthosiphon stamineus*). Journal of ethnopharmacology. 36, 225-231.

Drummond, A.J. and R.D. Waigh, 2000. The development of microbiological methods for phytochemical screening. Recent research developments in phytochemistry. 4, 143-152.

Dudonne, S., X. Vitrac, P. Coutiere, M. Woillez, and J.-M. Merillon, 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. Journal of agricultural and food chemistry. 57, 1768-1774.

Dung, T.T., F. Haesebrouck, N.A. Tuan, P. Sorgeloos, M. Baele, and A. Decostere, 2008. Antimicrobial susceptibility pattern of *Edwardsiella ictaluri* isolates from natural outbreaks of bacillary necrosis of *Pangasianodon hypophthalmus* in Vietnam. Microbial drug resistance. 14, 311-316.

Ekaluo, U., E. Ikpeme, E. Ekerette, and C. Chukwu, 2015. *In vitro* antioxidant and free radical activity of some Nigerian medicinal plants: bitter leaf (*Vernonia amygdalina* L.) and guava (*Psidium guajava* Del.). Research Journal of Medicinal Plant. 9, 215-226.

Eldeen, I., E. Seow, R. Abdullah, and S. Sulaiman, 2011. *In vitro* antibacterial, antioxidant, total phenolic contents and anti-HIV-1 reverse transcriptase activities of extracts of seven Phyllanthus sp. South African Journal of Botany. 77, 75-79.

European Pharmacopoeia Commission, 2018. Tannins in herbal drugs (method 2.8.14), in European Pharmacopoeia. European Directorate for the Quality of Medicines (EDQM): Strasbourg. p. 275-276.

Foo, L.Y. and H. Wong, 1992. *Phyllanthusiin* D, an unusual hydrolysable tannin from *Phyllanthus amarus.* Phytochemistry. 31, 711-713.

Foo, L.Y., 1993. Amariin, a di-dehydrohexahydroxydiphenoyl hydrolysable tannin from *Phyllanthus amarus.* Phytochemistry. 33, 487-491.

Foo, L.Y., 1995. Amariinic acid and related ellagitannins from *Phyllanthus amarus.* Phytochemistry. 39, 217-224.

Gbadamosi, I.T., 2015. Antibacterial attributes of extracts of *Phyllantus amarus* and *Phyllantus niruri* on *Escherichia coli* the causal organism of urinary tract infection. Journal of Pharmacognosy and Phytotherapy. 7, 80-86.

GSO, 2018. General Statistics Office of Vietnam, Production of fishery. http://www.gso.gov.vn/default_en.aspx?tabid=778.

Gu, H.-F., C.-M. Li, Y.-j. Xu, W.-f. Hu, M.-h. Chen, and Q.-h. Wan, 2008. Structural features and antioxidant activity of tannin from persimmon pulp. Food Research International. 41, 208-217.

Guha, G., V. Rajkumar, R.A. Kumar, and L. Mathew, 2010. Aqueous extract of *Phyllanthus amarus* inhibits chromium (VI)-induced toxicity in MDA-MB-435S cells. Food and chemical toxicology. 48, 396-401.

Gupta, D., M. Kumar, and V. Gupta, 2018. An *in vitro* investigation of antimicrobial efficacy of *Euphorbia hirta* and *Murraya koenigii* against selected pathogenic microorganisms. Asian journal of pharmaceutical and clinical research. 11, 359-363.

Gurusamy, K. and P. Saranya, 2010. *In vitro* antioxidant potential of ethanolic contents of *Eclipta alba* and *Wedelia chinensis.* Journal of Pharmacy Research. 3, 2825-2827.

Haslam, E., 1989. Plant polyphenols: vegetable tannins revisited. Cambridge, UK: Cambridge University Press.

Ho, P.H., 2003a. An illustrated flora of Vietnam. Vol. 3. Ho Chi Minh City: Tre Publishing House. 1020.

Ho, P.H., 2003b. An illustrated flora of Vietnam. Vol. 2. Ho Chi Minh City: Tre Publishing House. 951.

Ho, P.H., 2003c. An illustrated flora of Vietnam. Vol. 1. Ho Chi Minh City: Tre Publishing House. 991.

Ho, Y.-L., S.-S. Huang, J.-S. Deng, Y.-H. Lin, Y.-S. Chang, and G.-J. Huang, 2012. *In vitro* antioxidant properties and total phenolic contents of wetland medicinal plants in Taiwan. Botanical studies. 53,

Hossain, M.A., M. Kabir, S. Salehuddin, S.M. Rahman, A. Das, S.K. Singha, M.K. Alam, and A. Rahman, 2010. Antibacterial properties of essential oils and methanol extracts of sweet basil *Ocimum basilicum* occurring in Bangladesh. Pharmaceutical biology. 48, 504-511.

Houghton, P. and A. Raman, 1998. Methods for extraction and sample clean-up, in Laboratory handbook for the fractionation of natural extracts. Chapman and Hall: London, UK. p. 199.

Hue Ngan, D., H.T.C. Hoai, L. Mai Huong, P.E. Hansen, and O. Vang, 2008. Bioactivities and chemical constituents of a Vietnamese medicinal plant Che Vang, *Jasminum subtriplinerve* Blume (Oleaceae). Natural product research. 22, 942-949.

Igwe, C.U., L.A. Nwaogu, and C.O. Ujuwondu, 2007. Assessment of the hepatic effects, phytochemical and proximate compositions of *Phyllanthus amarus.* African Journal of Biotechnology. 6, 728-731.

Jamkhande, P.G., A.S. Wattamwar, A.D. Kankudte, P.S. Tidke, and M.G. Kalaskar, 2016. Assessment of *Annona reticulata* Linn. leaves fractions for invitro antioxidative effect and antimicrobial potential against standard human pathogenic strains. Alexandria Journal of Medicine. 52, 19-25.

Karou, D., A. Savadogo, A. Canini, S. Yameogo, C. Montesano, J. Simpore, V. Colizzi, and A.S. Traore, 2006. Antibacterial activity of alkaloids from *Sida acuta.* African journal of biotechnology. 5, 195-200.

Kaveti, B., L. Tan, K.T. Sarnnia, and M. Baig, 2011. Antibacterial activity of *Piper betle* leaves. International Journal of Pharmacy Teaching and Practices. 2, 129-132.

Khan, S., F. Al-Qurainy, M. Ram, S. Ahmad, and M.Z. Abdin, 2010. Phyllanthin biosynthesis in *Phyllanthus amarus*: Schum and Thonn growing at different altitudes. Journal of Medicinal Plants Research. 4, 041-048.

Kuete, V., 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta medica. 76, 1479-1491.

Kumaran, A. and R.J. Karunakaran, 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Science and Technology. 40, 344-352.

Le, Q.U., H.L. Lay and M.C. Wu, 2018. Antioxidant activities and HepG2 cells growth inhibitory capacity of whole plant ethanol extracts (*Eclipta alba* Hassk and *Mesona procumbens* Hemsl). Journal of food biochemistry. 42, e12454.

Lin, E.-S., H.-J. Chou, P.-L. Kuo, and Y.-C. Huang, 2010. Antioxidant and antiproliferative activities of methanolic extracts of *Perilla frutescens.* Journal of Medicinal Plants Research. 4, 477-483.

Liu, X., M. Zhao, J. Wang, B. Yang, and Y. Jiang, 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. Journal of food composition and Analysis. 21, 219-228.

Low Dog, T. 2009. Smart Talk on Supplements and Botanicals: Flor de Jamaica-Herbal Diuretics-Hawthorn. Alternative and Complimentary Therapies. 15, 214-215.

Madsen, H.L. and G. Bertelsen, 1995. Spices as antioxidants. Trends in food science & technology. 6, 271-277.

Mazumder, A., A. Mahato, and R. Mazumder, 2006. Antimicrobial potentiality of *Phyllanthus amarus* against drug resistant pathogens. Natural product research. 20, 323-326.

Meriga, B., R. Mopuri, and T. MuraliKrishna, 2012. Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum*. Asian Pacific journal of tropical medicine. 5, 391-395.

Mikage, M. and C. Mouri, 1999. Pharmacognostical studies of berberis plants (Berberidaceae) from Nepal (1): altitudinal, interspecific, and partial variations of berberine content in the bark. Natural medicines. 53, 249-254.

Mojani, M., A. Ghasemzadeh, A. Rahmat, S. Loh, and R. Ramasamy, 2014. Assessment of bioactive compounds, nutritional composition and antioxidant activity of Malaysian young ginger (*Zingiber officinale* Roscoe). International Food Research Journal. 21, 1931.

Namhui, Y. and K. Bae, 2010. Screening of Vietnamese medicinal plants for cytotoxic activity. Natural product sciences. 16, 1.

Narendhirakannan, R.T., J.G. Nirmala, A. Caroline, S. Lincy, M. Saj, and D. Durai, 2012. Evaluation of antibacterial, antioxidant and wound healing properties of seven traditional medicinal plants from India in experimental animals. Asian Pacific Journal of Tropical Biomedicine. 2, S1245-S1253.

Neelabh, C., A. Nahid and N. Kumar, 2017. Study on methanolic extract of *Ageratum conyzoides* for its ability to act as an antioxidant and to suppress the microbial growth. The Pharma Innovation. 6, 170.

Nguyen, A.T., J. Fontaine, H. Malonne, M. Vanhaelen, J. Dubois, T. Ky Pham, and P. Duez, 2005. Cytotoxicity of five plants used as anticancer remedies in Vietnamese traditional medicine, in Recent Progress in Medicinal Plants, V.K. Singh, Singh, Arunachalam V.K.; Govil, J.N., Editor., Studium-Press: New Delhi, India. p. 145-155.

Nhu, T.Q., B.T.B. Hang, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019a. Plant extract-based diets differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*). Fish & shellfish immunology. 92, 913-924.

Nhu, T.Q., B.T.B. Hang, A. Vinikas, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019b. Screening of immuno-modulatory potential of different herbal plant extracts using striped catfish (*Pangasianodon hypophthalmus*) leukocyte-based in vitro tests. Fish & shellfish immunology. 93, 296-307.

Othman, A., A. Ismail, F.A. Hassan, B.N.M. Yusof and A. Khatib, 2016. Comparative evaluation of nutritional compositions, antioxidant capacities, and phenolic compounds of red and green sessile joyweed (Alternanthera *sessilis*). Journal of Functional Foods. 21, 263-271.

Patel, J.R., P. Tripathi, V. Sharma, N.S. Chauhan, and V.K. Dixit, 2011. *Phyllanthus amarus*: ethnomedicinal uses, phytochemistry and pharmacology: a review. Journal of Ethnopharmacology. 138, 286-313.

Perumal, P.C., D. Sophia, C.A. Raj, P. Ragavendran, T. Starlin, and V.K. Gopalakrishnan, 2012. *In vitro* antioxidant activities and HPTLC analysis of ethanolic extract of *Cayratia trifolia* (L.). Asian Pacific Journal of tropical disease. 2, S952-S956.

Phu, T.M., N.T. Phuong, T.T. Dung, D.M. Hai, V.N. Son, A. Rico, J.H. Clausen, H. Madsen, F. Murray, and A. Dalsgaard, 2016. An evaluation of fish health-management practices and occupational health hazards associated with Pangasius catfish (*Pangasianodon hypophthalmus*) aquaculture in the M ekong D elta, Vietnam. Aquaculture Research. 47, 2778-2794.

Prajapati, R., S. Roy, S. Mishra, S. Raza, and L. Thakur, 2014. Formulation development, standardization and antimicrobial activity of *Ageratum conyzoides* extracts and their formulation. International Journal of Pharmacy and Pharmaceutical Sciences. 6, 369-374.

Pratt, D.E. and B.J. Hudson, 1990. Natural antioxidants not exploited commercially, in Food antioxidants. Springer. p. 171-191.

Pyrzynska, K., and A. Pękal, 2013. Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. Analytical Methods. 5, 4288-4295.

Rabeta, M. and S. Lin, 2015. Effects of different drying methods on the antioxidant activities of leaves and berries of *Cayratia trifolia.* Sains Malaysiana. 44, 275-280.

Ramji, N., N. Ramji, R. Iyer, and S. Chandrasekaran, 2002. Phenolic antibacterials from *Piper betle* in the prevention of halitosis. Journal of ethnopharmacology. 83, 149-152.

Regi Raphael, K., T. Ajith, S. Joseph, and R. Kuttan, 2002. Anti-mutagenic activity of *Phyllanthus amarus* Schum & Thonn *in vitro* as well as *in vivo.* Teratogenesis, carcinogenesis, and mutagenesis. 22, 285-291.

Rezaeizadeh, A., A. Zuki, M. Abdollahi, Y. Goh, M. Noordin, M. Hamid, and T. Azmi, 2011. Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia.* African Journal of Biotechnology. 10, 4932-4940.

Rico, A., T.M. Phu, K. Satapornvanit, J. Min, A. Shahabuddin, P.J. Henriksson, F.J. Murray, D.C. Little, A. Dalsgaard, and P.J. Van den Brink, 2013. Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. Aquaculture. 412, 231-243.

Roberts, R.J., 1993. Motile aeromonad septicemia, in Bacterial diseases of fish, V. Inglis, R.J. Roberts, and N.R. Bromage, Editors., John Wiley and Sons: New York. p. 143–156.

Rodgers, C. and M. Furones, 2009. Antimicrobial agents in aquaculture: practice, needs and issues. Options Méditerranéennes. 86, 41-59.

Saranraj, P. and P. Sivasakthivelan, 2012. Screening of antibacterial activity of the medicinal plant *Phyllanthus amarus* against urinary tract infection causing bacterial pathogens. Applied journal of hygiene. 1, 19-24.

Sarker, S.D., L. Nahar, and Y. Kumarasamy, 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 42, 321-324.

Shahriar, M., S. Akhter, M.I. Hossain, M.A. Haque, and M.A. Bhuiyan, 2012. Evaluation of *in vitro* antioxidant activity of bark extracts of *Terminalia arjuna.* Journal of Medicinal Plants Research. 6, 5286-5298.

Sharma, A., R.T. Singh and S.S. Handa, 1993. Estimation of phyllanthin and hypophyllanthin by high performance liquid chromatography in *Phyllanthus amarus.* Phytochemical analysis. 4, 226-229.

Sharma, S., I.A. Khan, I. Ali, F. Ali, M. Kumar, A. Kumar, R.K. Johri, S.T. Abdullah, S. Bani, A. Pandey, K.A. Suri, B.D. Gupta, N.K. Satti, P. Dutt, and G.N. Qazi, 2009. Evaluation of the antimicrobial, antioxidant, and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. Antimicrobial agents and chemotherapy. 53, 216-222.

Sheikhlar, A., G.Y. Meng, R. Alimon, N. Romano, and M. Ebrahimi, 2017. Dietary *Euphorbia hirta* extract improved the resistance of sharptooth catfish *Clarias gariepinus* to *Aeromonas hydrophila.* Journal of aquatic animal health. 29, 225-235.

Singh, D., S. Narayanamoorthy, S. Gamre, A.G. Majumdar, M. Goswami, U. Gami, S. Cherian, and Subramanian, M., 2018. Hydroxychavicol, a key ingredient of *Piper betle* induces bacterial cell death by DNA damage and inhibition of cell division. Free Radical Biology and Medicine. 120, 62-71.

Shyur, L.-F., J.-H. Tsung, J.-H. Chen, C.-Y. Chiu, and C.-P. Lo, 2005. Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. International Journal of Applied Science and Engineering. 3, 195-202.

Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 16, 144-158.

Soumaya, K., F. Chaouachi, R. Ksouri, and M. El Gazzah, 2013. Polyphenolic composition in different organs of Tunisia populations of *Cynara cardunculus* L. and their antioxidant activity. Journal of Food and Nutrition Research. 1, 1-6.

Subagja, J., J. Slembrouck, L.T. Hung, and M. Legendre, 1999. Larval rearing of an Asian catfish *Pangasius hypophthalmus* (Siluroidei, Pangasiidae): Analysis of precocious mortality and proposition of appropriate treatments. Aquatic Living Resources. 12, 37-44.

Subba, B. and P. Aryal, 2016. Study of Biological Activity and Chemical Constituent of *Annona reticulata.* Journal of Institute of Science and Technology. 21, 157-163.

Svobodova, B., L. Barros, R.C. Calhelha, S. Heleno, M.J. Alves, S. Walcott, M. Bittova, V. Kuban, and I.C. Ferreira, 2017. Bioactive properties and phenolic profile of *Momordica charantia* L. medicinal plant growing wild in Trinidad and Tobago. Industrial crops and products. 95, 365-373.

Taemchuay, D., T. Rukkwamsuk, T. Sakpuaram, and N. Ruangwises, 2009. Antibacterial activity of crude extracts of *Centella asiatica* against *Staphylococcus aureus* in bovine mastitis. Kasetsart Veterinarians. 19, 119-128.

Taukoorah, U., N. Lall, and F. Mahomoodally, 2016. *Piper betle* L. (betel quid) shows bacteriostatic, additive, and synergistic antimicrobial action when combined with conventional antibiotics. South African journal of botany. 105, 133-140.

Teeli, R.A., S.A. Ganie, M.S. Dar, W. Raja, and S.S. Yadav, 2018. Antioxidant activity of *Euphorbia hirta* L. leaf extracts. Research Journal of Pharmacy and Technology. 11, 199-202.

Thiangthum, S., B. Dejaegher, M. Goodarzi, C. Tistaert, A. Gordien, N.N. Hoai, M.C. Van, J. Quetin-Leclercq, L. Suntornsuk, and Y. Vander Heyden, 2012. Potentially antioxidant compounds indicated from *Mallotus* and *Phyllanthus* species fingerprints. Journal of Chromatography B. 910, 114-121.

Thuong, P.T., M.-K. Na, N.H. Dang, T.M. Hung, P.T. Ky, T.V. Thanh, N.H. Nam, N.D. Thuan, D.-E. Sok, and K.-H. Bae, 2006. Antioxidant activities of Vietnamese medicinal plants. Natural Product Sciences. 12, 29-37.

Tomar, R.S., V. Shrivastava, and S. Kaushik, 2014. *In vitro* efficacy of methanolic extract of *Mimosa pudica* against selected micro-organisms for its broad spectrum antimicrobial activity. International Journal of Current Microbiology and Applied Sciences. 3, 780-784.

Ushie, O., P. Neji, E. Etim, and G. Nsor, 2013. Phytochemical screening and antimicrobial activities of *Phyllanthus amarus* stem bark extracts. International Journal of Modern Biology and Medicines. 3, 101-112.

VASEP, 2019. Vietnam Association of Seafood Exporters and Producers. Fishery profile; Available from: http://vasep.com.vn/1192/OneContent/tong-quan-nganh.htm.

Wang, K.-H., R.-D. Lin, F.-L. Hsu, Y.-H. Huang, H.-C. Chang, C.-Y. Huang, and M.-H. Lee, 2006. Cosmetic applications of selected traditional Chinese herbal medicines. Journal of ethnopharmacology. 106, 353-359.

Yoshida, T., L. Chen, T. SHINGU, and T. Okuda, 1988. Tannins and Related Polyphenols of Euphorbiaceous Plants. IV.: Euphorbins A and B, Novel Dimeric Dehydroellagitannins from *Euphorbia hirta* L. Chemical and Pharmaceutical bulletin. 36, 2940-2949.

Yoshida, T., O. Namba, L. Chen, and T. Okuda, 1990a. Euphorbin E, a hydrolyzable tannin dimer of highly oxidized structure, from *Euphorbia hirta.* Chemical and Pharmaceutical Bulletin. 38, 1113-1115.

Yoshida, T., O. Namba, L. Chen, and T. Okuda, 1990b. Tannins and Related Polyphenols of Euphorbiaceous Plants. V.: Euphorbin C, an Equilibrated Dimeric Dehydroellagitannin Having a New Tetrameric Galloyl Group. Chemical and Pharmaceutical Bulletin. 38, 86-93.

Zhou, G., H. Yu, and Y. Yu, 2006. Study on bacteriostatic action in different parts *of Houttuynia cordata.* Hunan Forestry Science & Technology 33, 38-40.

4. EFFECTS OF *PHYLLANTHUS AMARUS* AND *EUPHORBIA HIRTA* DIP TREATMENTS ON THE PROTECTION OF STRIPED CATFISH (*PANGASIANODON HYPOPHTHALMUS*) FILLETS AGAINST SPOILAGE DURING ICE STORAGE

The previous study showed that *P. amarus* extract showed the highest antioxidant activity, followed by *P. betle*, *P. guajava*, *E. hirta* and *M. pudica*. Beside its high antioxidant activity, *P. amarus* extract also possessed antimicrobial activity against two different strains of *A. hydrophila* and one strain of *E. ictaluri*. In the Aquabioactive project, the combination of *in vitro* results of antioxidant and antimicrobial activity (this PhD work) as well as the findings of *in vitro* evaluation of immune system stimulation using fish Peripheral Blood Mononuclear cells (PBMC) and kidney cells (PhD student Truong Quynh Nhu, in CTU and UNamur) led to the choice of *P. amarus* and *E. hirta* as the representative plants for further investigations. Therefore, these two plant extracts were selected for dip treatment of fish fillets in this study, as tentative preservative agents. Based on the *in vitro* antimicrobial activity screening, MIC values of 156 µg/mL and 625 µg/mL were determined for *P. amarus* and *E. hirta* extracts, respectively, corresponding to 0.02 and 0.06% extract in the dip solutions. Moreover, other publications reported the efficiency of plant extracts dip treatments at the concentrations ranging from 0.04 to 0.2%. According to these information, we decided to perform the plant extract dip experiments using 0.02 and 0.04% of *P. amarus* extract, and 0.06 and 0.2% of *E. hirta* extract.

4. EFFECTS OF *PHYLLANTHUS AMARUS* AND *EUPHORBIA HIRTA* DIP TREATMENTS ON THE PROTECTION OF STRIPED CATFISH (*PANGASIANODON HYPOPHTHALMUS*) FILLETS AGAINST SPOILAGE DURING ICE STORAGE

Adapted from:
Nguyen Le Anh Dao, Tran Minh Phu, Caroline Douny, Joëlle Quetin-Leclercq, Bui Thi Buu Hue, Le Thi Bach, Truong Quynh Nhu, Bui Thi Bich Hang, Do Thi Thanh Huong, Nguyen Thanh Phuong, Patrick Kestemont & Marie-Louise Scippo

Journal of Aquatic Food Product Technology, 30:10, 1218-1234,
https://doi.org/10.1080/10498850.2021.1987606

Abstract

This study was conducted to evaluate the effects of herbal extracts on the quality of striped catfish (*Pangasianodon hypophthalmus*) fillets throughout storage period. Quality changes during ice storage of striped catfish fillets were studied after dip treatments in aqueous solutions of ethanolic extract of *Phyllanthus amarus* (0.02% and 0.04%, w/v) or *Euphorbia hirta* (0.06% and 0.2%, w/v). The control (dipped in tap water) and the treated fish samples were analyzed periodically for total viable counts (TVC), peroxide value, physicochemical parameters (pH, texture) and sensory properties. Results indicated that *Pseudomonas* spp. and *Listeria monocytogenes* were absent in the raw fish fillets, reflecting the safety of raw materials regarding specific spoilage and psychrotrophic pathogenic microorganisms. Dip treatments in water containing *P. amarus* or *E. hirta* extracts significantly reduced the primary lipid oxidation in fish samples. In conclusion, dip treatment in water containing 0.04% *P. amarus* or 0.06% *E. hirta* extract was effective to maintain a good sensory quality of the fish fillets and to prolong their shelf life up to 8 days under ice storage.

Keywords: Dip treatments, *Euphorbia hirta*, ice storage, *Pangasianodon hypophthalmus*, *Phyllanthus amarus*

1. Introduction

Striped catfish (*Pangasianodon hypophthalmus*), a freshwater fish species, is the main commercial cultured fish in the Mekong delta, Vietnam (Phan et al., 2009). Striped catfish production reached 1.42 million tons in 2019 (VASEP, 2019). It is mainly exported to more than 132 countries in the world, and about 10% is marketed in Vietnam.

However, as it is generally the case for fish, striped catfish fillet is an easily perishable product, mainly because of its high water activity. The spoilage of fish during storage is usually caused by the microbial growth and metabolic activities, protein degradation, lipid oxidation, and results in short shelf life and the decrease in flesh quality (Olatunde and Benjakul, 2018).

In modern seafood industry, quality loss of the end products could be prevented or delayed by using chemical preservatives to control microbiological, enzymatical or chemical changes, and prolong the shelf life of seafood. However, these synthetic chemicals can be at risk for public health due to possible adverse effects such as carcinogenicity, acute toxicity and teratogenicity (Embuscado, 2015; Faleiro, 2011; Yang et al., 2017). Consequently, a pressure exists on agrifood industry in general, and seafood industry in particular, to replace synthetic preservative chemicals, by natural alternatives with antioxidant and antimicrobial activities (Embuscado, 2015; Yashin et al., 2017). Among natural products, plant extracts are good candidates as potential successful means to extend the shelf life of seafood (Erkan et al., 2011; Falguera et al., 2011). In recent years, a lot of studies were conducted about herbals possessing antioxidants and antimicrobials capacity which are used to improve the quality and to maintain shelf life of perishable foods in general and of striped catfish in particular (Greeshma et al., 2019; Jeyakumari et al., 2017; Rao et al., 2017).

Phyllanthus and *Euphorbia* genera, belonging to the family of the Euphorbiaceae, are usually found in tropical and subtropical countries e.g. India, Malaysia, Thailand, China, Vietnam, and Nigeria. These plants were shown to display pharmacological properties (Kumar et al., 2010; Kumar Sarangi and Padhi, 2017; Nguyen et al., 2015) and have been extensively used in folk medicine for treatments of a broad spectrum of diseases for thousands of years. In Vietnam, *P. amarus* and *E. hirta* were studied for their use in aquaculture for prophylaxis and treatment of fish and shrimp diseases against *Aeromonas hydrophila* and *Edwardsiella ictaluri* (Direkbusarakom, 2004; Nhu et al., 2019a, 2019b; Nhu et al., 2020a, 2020b).

Phyllanthus amarus (Mitra and Jain, 1985) *(P. amarus)* is one of the herbs that show a wide spectrum of pharmacological effects including antioxidant, antimicrobial, anticancer, anti-inflammatory, antiviral and antidiabetic activities (Devi et al., 2017; Sen and Batra, 2013; Tan et al., 2013; Ushie et al., 2013). It contains various bioactive compounds e.g. lignans, flavonoids, hydrolysable tannins, triterpenes, alkaloids (Patel et al., 2011). The methanolic extract of *P. amarus* was found to contain high amounts of phenolic compounds. It was also shown to be able of free radical scavenging and to inhibit lipid peroxidation and consecutive cellular damages induced by chromium (VI) (Guha et al., 2010; Nguyen et al., 2017). In addition, the extract of *P. amarus* also showed significant antimicrobial activity against *Shigella* spp., *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa.* The antibacterial action was shown to be mainly due to the phyllanthin isolated from *P. amarus* (Mazumder et al., 2006; Oluboyo et al., 2016; Senjobi et al., 2017).

Similarly*, Euphorbia hirta* (*E. hirta*) displays biological activities such as antioxidant, antibacterial, antifungal, antihistaminic, antiasthmatic and anticancer (Ahmad et al., 2017; Al-Snafi, 2017). Perumal et al., (2012) reported that *E. hirta* hold potential antimicrobial effects against a wide spectrum of pathogenic microorganisms and therefore can be used as a safe, reliable, economical and natural antimicrobial source for therapeutics (Perumal et al., 2012). These findings may also be useful in food industry as the plant extracts could be used as preservatives to protect food from spoilage and food borne pathogens contamination. However, there have been few studies on preservative effect of *P. amarus* and *E. hirta* in fish flesh during ice storage, which is the research subject of the present paper.

2. Materials and methods

2.1. Preparation of *P. amarus* and *E. hirta* extracts

Two fresh plants were collected from various areas in Mekong Delta, Vietnam. The plants were authenticated by the Department of Biology, College of Natural Science, Can Tho University and compared to literature. Plant extracts were prepared as described by Bach et al. (2018). All collected parts of plants were then washed to remove mud and dust; the rotten and damaged parts were also discarded. Samples were air dried in shade for some days and then put in an oven at about 50°C until well-dried. After that, they were ground into a fine powder with a blender and stored in sealed containers in a dry and cool place. The dried powder (100 g) was soaked in ethanol

96% (800 mL) for at least 24 hours at room temperature with frequent agitation. The solvent-containing extracts were then decanted and filtered. The ground samples were further extracted 4 times with ethanol 96%. The filtrates from each extraction were combined and the solvent was evaporated under reduced pressure using a rotary evaporator to give crude ethanolic extracts. All extracted samples were lyophilized until dryness and stored at -20°C before use.

2.2. Experimental design

Striped catfish fillets (450 fish fillet weighting about 80 to 100 g) were obtained from a striped catfish processing company (Can Tho, Vietnam) at the stage of trimming after skinning, and divided into two batches of 225 fillets (one batch per treatment). No protective treatment was applied by the company to the fish fillets used in this study, and the fish fillets were used for the experiments the same day they were produced from the company. Striped catfish originated from the same pond, same harvest age and fairly same in weight before catching.

In *P. amarus* experiment, fish fillets (225 fillets) were given a dip treatment, during 30 minutes, at 4°C in tap water containing 0 (negative control), 0.02% and 0.04% (weight/volume) of *P. amarus* ethanolic extract. The ratio between fish fillet and aqueous solution of plant extract was 1:1 (w:v). After that, fish fillets were drained well for 5 minutes before to be packed in sterile polyethylene (PE) bags (5 fillets per bag). Fish fillets were then placed into insulated box (100 L) with fish and ice ratio of 1:1 (w:w). Ice was added and water in the box was removed every day of storage. The temperature of fish fillets was recorded at the time of sampling.

The *E. hirta* experiment was done in the same way as the *P. amarus* experiment, except that *E. hirta* ethanolic extract was used at concentrations of 0% (negative control), 0.06% and 0.2% in tap water.

Both experiments were replicated three times: 3 bags containing 5 fish fillets were prepared for each sampling time and each treatment. In total, in each experiment, 45 bags containing 5 fish fillets each were prepared.

2.3. Samplings

Sampling was done on the day of experimental setup (day 0), and after 4, 8, 12, 16 days of ice storage. At each sampling time and for each treatment, 3 bags were collected. The sampling at day 0 was done about after 1 hour of ice storage. From each bag, two fish fillets were used for sensory analysis and to measure the texture property, one fish fillet was used for a duplicate total

viable counts determination, and the two remaining fillets were minced together for duplicate measurement of pH and peroxide value (PV).

2.4. Proximate composition analyses

A proximate composition analysis (moisture, protein, ash and lipid content) was performed on 6 fish fillets taken from a same big batch of 450 fillets before any treatment, according to the procedures of the AOAC (2016).

2.5. Texture analysis

The texture profile analysis (TPA) indices of fillets were determined using a texture analyzer (Model TA.XTplus Texture Analyzer, Stable Micro Systems, Godalming, UK). The conditions of the texture analyzer were as follows: pretest speed, 1.0 mm/s; posttest speed, 10.0 mm/s; distance, 5.0 mm; trigger type, auto; and trigger force, 5g. The calculation of TPA values was obtained by graphing a curve using force and distance. Penetration values (peak force of the compression cycle) of fish fillets were measured by using a P/5S probe (5 mm spherical stainless, Stable Micro Systems).

2.6. Sensory property

The sensory quality of striped catfish fillets was evaluated using the quality index method (QIM) by a panel of seven trained members (Sveinsdottir et al., 2003). QIM is based on significant, well-defined changes of appearance attributes that occur in raw fish after storage, such as odor, texture, color, gaping and surface. A score from 0 to 3 demerit (index) points was given for 5 quality parameters according to the specific parameter descriptions (Appendix A). A value of 0 corresponded to very fresh fillets. The scores increased according to spoilage with a maximum score of 3 for each parameter. The 5 scores are summed to give an overall sensory score referred to as the Quality Index (QI) which can vary from 0 (very fresh) to a maximum 14 score (very bad) (Bao, 2006).

Sensory evaluation of cooked striped catfish fillets in term of taste was conducted according to Simeonidou et al. (1997). The taste of cooked fillet samples was scored using a nine-point scale. A sensory score of five was taken as the threshold of acceptability (Appendix B). The sample of fish fillet were not less than 50 g per person. Fish fillets were cooked in a steam oven at 100ºC for 10 minutes. The samples were coded before serving.

2.7. *Pseudomonas* spp. and *Listeria monocytogenes* determination

The determination of *Pseudomonas spp. and Listeria monocytogenes* was conducted on 6 striped catfish fillets before carried out any treatment at the microbiological laboratory of Intertek Vietnam Limited Company-Total Quality Assurance provider. The tests were performed according to testing methods of ISO 13720:2010 and ISO 11290-2:2017, respectively.

2.8. Total viable counts (TVC)

Striped catfish fillets were taken aseptically in a vertical flow hood and 1 g was transferred to a sterile tube and homogenized with 9 mL of sterile normal saline water for 60 s. From this first 10^{-1} dilution, other decimal dilutions were prepared. A portion (1 mL) of these dilutions was pipetted into sterile petri dishes and 15 mL PCA medium at 45ºC were added. TVC were determined by counting the number of colony-forming units after incubation at 30°C for 72 h. Petri dishes containing from 25 to 250 colonies were selected for the counting according to Nordic Committee on Food Analysis (2006).

2.9. Determination of pH

A 20 g sample of fish fillet was homogenized in 20 mL KCl 0.15 M. The pH was measured using a digital pH meter according to Hultmann et al. (2012).

2.10. Peroxide value (PV)

Peroxide values were determined according to the spectrophotometric ferric thiocyanate method of International IDF standards (1991). Five grams of fish sample were added to twenty mL of chloroform: methanol mixture (2:1) (v:v), shaken for 3 hours, and extracted solution was centrifuged at 4000 rpm at 25°C for 5 minutes. The lower phase was collected for determination of fat content and considered as the sample extract for the latter analysis. Fat content of each sample was determined at every sampling time.

For calibration, a set of solutions of increasing Fe^{3+} concentration in the range 0–4 µg/mL was prepared by successive dilutions of the working solution. The calibration curve was obtained by plotting absorbance (at 480 nm) with Fe^{3+} concentration.

To determine the peroxide value, the sample extract (1 mL) was mixed with 3.9 mL chloroform: methanol (2:1). Then, 50 µL of Fe^{2+} solution (0.018 M) was first added followed by 50 µL NH_4SCN 30%. The solution was stirred on a vortex mixer for 15 s. The absorbance of the sample was measured at 480 nm against a blank that contained all the reagents except the sample. Peroxide values, expressed as milliequivalents (meq) peroxide/kg fish fat, were calculated based

on the concentration of Fe^{3+} determined from regression line (y = ax + b) and the fat content of fish samples.

2.11. Statistical analysis

All data were expressed as mean ± standard deviation, calculated using Microsoft Excel software. The data of all parameters analyzed at each sampling time were subjected to analysis of variances (one-way ANOVA) using SPSS 16.0 (SPSS Inc, Chicago, II, USA).

3. Results and discussion

3.1. Proximate composition

Proximate analysis was determined in the initial fish fillets used in each experiment before any treatment with plant extracts. The proximate composition of striped catfish fillets is given in Table 1. The chemical composition of striped catfish fillets used in both experiments was characterized by high moisture (83.0%) and relatively low protein (14.9%) and lipid (0.44%) contents. In this study, the percentages of moisture and protein are similar with the results of Vietnamese striped catfish fillets reported by Orban et al. (2008) (moisture 83.6% and protein 13.6%) and Karl et al. (2010) (moisture 82.1% and protein 15.7%) whereas the lipid content is much lower than 1.84% in the study of Orban et al. (2008) and 1.4% in the research of Karl et al. (2010).

Table 1. Proximate composition of striped catfish fillets

Proximate composition (%)			
Moisture	**Ash**	**Lipid**	**Protein**
83.0	0.87	0.44	14.9
(82.7-83.2)	(0.74-1.09)	(0.14-0.81)	(14.5-15.3)

Data expressed as mean (min-max) (n = 6)

3.2. Temperature

The internal temperature of fillets collected during storage time in both experiments of dip treatment with *P. amarus* and *E. hirta* extracts were under 4°C (from 0.85 to 2.03°C, data not

shown). There was no significant difference between fish fillets temperature at each sampling day. Icing is one of the most prevalent techniques for fresh fish preservation (Viji et al., 2015), which could exert the increase of psychrophilic strains along with the expense of mesophilic population (Roberts et al., 2005). At the point of spoilage of iced fresh-water fish, *Pseudomonas* spp. is the dominant specific spoilage Gram-negative bacteria (Gram et al., 1989). This species had been known as the dominant bacteria identified at the end of the shelf-life of defrosted Vietnamese Pangasius products (Noseda et al., 2012). Among a few psychrotrophic pathogenic bacteria in striped catfish fillets which are of concern, *L. monocytogenes* is a recognized foodborne pathogen (Donnelly et al., 1992; RASFF, 2011) which can grow below 5^{o}C. Thus, checking the presence of *Pseudomonas* spp. and *L. monocytogenes* in the raw fish flesh is necessary although Roberts et al. (2005) suggested that maintaining chilled storage temperatures as low as ≤2^{o}C could inhibit the development of psychrophilic pathogens in fresh products.

3.3. pH

According to Huss (1988), a decrease of pH in fish muscle within the first day of death is due to the post-mortem anaerobic formation of lactic acid. During the later post-mortem changes, the production of basic compounds results in a pH more or less stable or slightly increased. However, other authors describe a post-mortem increase of the pH due to fish stress during harvesting or to the post-mortem accumulation of microbial metabolites like biogenic amines (Abbas et al., 2008; Gill, 1983).

Changes in the pH values of striped catfish fillets during ice storage observed in this study are shown in Table 2. pH values obtained in both dip treatment experiments with *P. amarus* and *E. hirta* extracts during the time storage were ranging from 6.38 to 6.71 and from 6.51 to 6.60, respectively. The pH values remained under 7, corresponding to a slightly increasing trend with no significant differences. These results are not surprising as, in both experiments, TVB-N levels in fish fillets remained below the maximum acceptable limit of 25–30 mg/100 g for TVB-N in ice stored *Pangasianodon hypophthalmus* (data not shown), showing that the products were not affected by the spoilage (Ashie et al., 1996; Abbas et al., 2008; Viji et al., 2015). These low pH values are in agreement with the study of Azam et al. (2005), showing fresh *Pangasius hypophthalmus* with pH ranging from 6.57 to 7.30 during 18 days stored on ice. In the study of Jeyakumari et al. (2017), pH values of Pangasius chunks treated with spice extracts were observed

in an interval of 6.25 and 6.65. Other studies reported variations of pH values between 6.2 and 6.6 for gilthead sea beam and from 6.34 to 6.69 for European sea bass muscle (Abbas et al., 2008). The differences of species, diet, season, level of stress during the catch and type of muscle have been reported to influence post-mortem pH in fish muscle (Huss, 1988).

Table 2. pH of striped catfish fillets during ice storage

Treatment group	pH				
	Day 0	**Day 4**	**Day 8**	**Day 12**	**Day 16**
P. amarus					
Control (0%)	6.58±0.11^{b}	6.67±0.05^{b}	6.68±0.04^{b}	6.71±0.02^{b}	6.66±0.02^{b}
0.02%	6.62±0.03^{b}	6.58±0.07^{b}	6.59±0.07^{ab}	6.70±0.07^{b}	6.55±0.02^{a}
0.04%	6.38±0.04^{a}	6.43±0.07^{a}	6.57±0.08^{a}	6.56±0.00^{a}	6.56±0.06^{a}
E. hirta					
Control (0%)	6.56±0.13^{a}	6.60±0.04^{b}	6.56±0.10^{a}	6.55±0.10^{a}	6.49±0.02^{a}
0.06%	6.55±0.04^{a}	6.52±0.04^{a}	6.55±0.06^{a}	6.56±0.04^{a}	6.59±0.15^{a}
0.2%	6.57±0.02^{a}	6.51±0.02^{a}	6.52±0.06^{a}	6.59±0.06^{a}	6.51±0.11^{a}

Values are mean ± SD (n = 6). Mean values within a column with the same letter are not significantly different ($p > 0.05$).

In this study, at the beginning of storage (day 0), the fish flesh dipped with 0.04% *P. amarus* showed a significant lower ($p < 0.05$) pH values compared to the control and 0.02% plant extract treated fish fillets (Table 2). Similar trend was observed from day 4 to day 12, where the lower pH values of 0.04% *P. amarus* treated samples might be due to the acidic nature of *P. amarus* extract. Low pH values obtained in this study were analogous to those reported in the dip treatment of *P. hypophthalmus* with 10% *Moringa oleifera* leaf aqueous extract (Greeshma et al., 2019). The author documented that the higher concentration was used, the lower pH values were obtained. The increase of pH values during storage were postulated to be associated with the production of volatile basic components, such as ammonia and trimethylamine from the decomposition of nitrogenous compounds by either the microbial enzymes (Ruiz-Capillas and Moral, 2001) or muscle endogenous protease (Liu et al., 2018).

In the *E. hirta* extract dip treatment group, pH values of the three groups did not show much variation during the entire period of storage (Table 2). No statistically significant differences

were found between pH values of the control fish fillets and of *E. hirta* extract treated fish fillets ($p > 0.05$), except after 4 days of storage, where the pH of the control filets was found to be significantly higher. In this study, the treatment of *P. amarus* 0.04% was seen to be able to reduce pH values (compared to control) better than *E. hirta* treatments.

3.4 Microbiological quality

The presence of *Pseudomonas* spp. and *Listeria monocytogenes* in raw materials of striped catfish fillets was tested, and results showed that both bacteria were not detected in 25 grams of fish samples (Table 3). *Pseudomonas* spp. are acknowledged as specific spoilage microorganisms of iced tropical fresh water fish, in general (Ghaly et al., 2010; Gram, 1993; ICMSF, 2005) and defrosted Vietnamese striped catfish products at the end of their shelf-life, in particular (Noseda et al., 2012). According to Bagge-Ravn et al. (2003), *Pseudomonas* spp. was demonstrated as environmentally opportunistic bacteria which can contaminate fish fillets due to the adherence of these species on contact surfaces. Besides, *L. monocytogenes* is a foodborne infection agent causing listeriosis in humans. It is capable of growth under refrigerated conditions (Fernandes et al., 1998) and may represent the main contamination risk of chilling fresh catfish fillets (Chen et al., 2010). Duffes (1999) evidenced that *L. monocytogenes* could be transmitted from the raw products to processing surfaces and equipment during filleting, leading to a potential source of contamination. In this study, striped catfish fillets were not contaminated by *Pseudomonas* spp. and *L. monocytogenes*. This can reflect a good hygienic status of the processing environment and good personal hygiene (Novoslavskij et al., 2016). Furthermore, several findings revealed that the potential use of plant extracts was considered as an effective type of active coating to protect fish fillets against the microorganism hazards during cold storage. Zhuang et al. (2019) reported that using ethanolic pomegranate peel extract (0.5 mg of phenolic compounds/mL) induced the reduction of *Pseudomonas* counts in bighead carp (*Aristichthys nobilis*) fillets during chilled storage (4^{o}C). Besides, the application on cod fillets of water-soluble oregano (*Origanum vulgare*) and cranberry (*Vaccinium macrocarpon*) phenolic compounds mixture (0.1 mg of phenolic compounds/mL) inhibited and inactivated *L. monocytogenes* (Lin et al., 2004).

Table 3. Determination of *Pseudomonas* spp. and *Listeria monocytogenes* in 25 grams of striped catfish fillets (n=6)

Raw materials	*Pseudomonas* spp.	*Listeria monocytogenes*
Striped catfish fillet	Not detected	Not detected

In Vietnam, TVC is one of the standardized parameters which is required for evaluation the quality of striped catfish fillets (Vietnam Standard TCVN8338: 2010). Values of TVC of striped catfish fillets during the 16-day ice storage are presented in Figure 1. The TVC values of all treatments showed an increasing trend during the 16 days storage time although the usual errors on bacterial enumeration were seen within the range of 0.3-0.5 $\log_{10}$ CFU/g, showing significant difference was not obviously found among the experimental treatments. The increasing of TVC in striped catfish during ice storage has been shown in various studies. Rao et al. (2017) reported that the total viable counts of striped catfish fillets coated with plant (clove, thyme) oil incorporated alginate gels increased from 4 $\log_{10}$ CFU/g to less than 5 $\log_{10}$ CFU/g during 15 days of chilled storage. In a study of striped catfish fillets dip treatment with *Moringa oleifera* leaf extract, TVC of the control and treated samples went up from 4.2 log10 CFU/g to above 6 $\log_{10}$ CFU/g during 15 days stored at 2±1^{o}C (Greeshma et al., 2019). Bacteria could be transported from different sources (water, air, human, etc.) and can be spread by wind to various sites. From a study in Ho Chi Minh City (South of Vietnam), the major airborne bacterial genera identified were *Bacillus siamensis*, *Staphylococcus aureus*, *Micrococcus varians*, and *Enterobacteriaceae* (Hai et al., 2019).

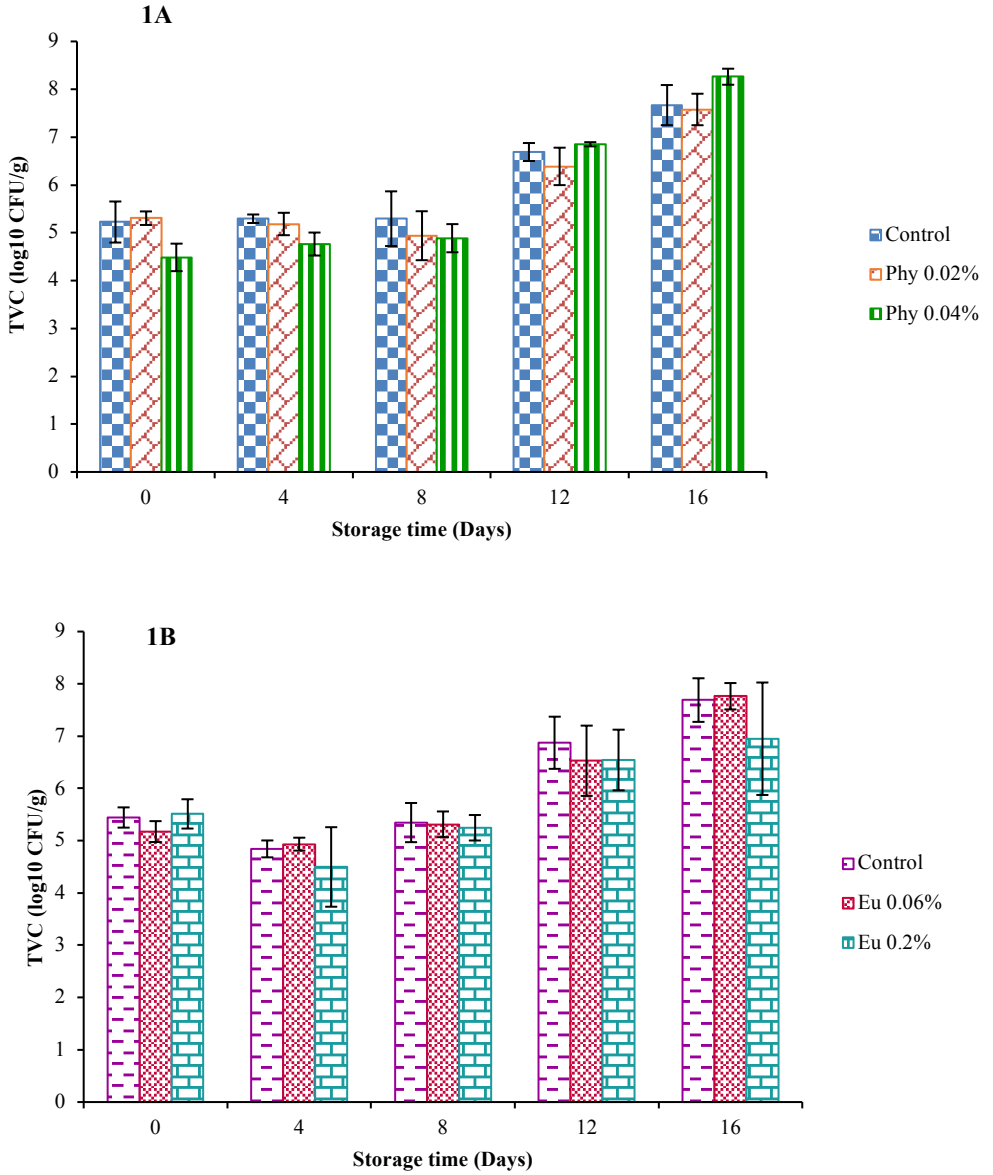


Figure 1. Total viable count of striped catfish fillets after *P. amarus* (Phy) (A), *E. hirta* (Eu) (B) dip treatment, during ice storage (mean ± SD, n=6)

In the *P. amarus* group, the TVC in untreated and 0.02% plant extract treated fish fillets showed an increasing trend which was not observed in the 0.04% plant extract treated fish fillets, at days 0 (i.e. after one hour of iced storage after dip treatment) and day 4 (Figure 1A). This increasing trend was not observed for the TVC in the *E. hirta* group (Figure 1B). These results could be due to an inhibiting effect of the 0.04% *P. amarus* ethanolic extract treatment on

microorganism development after the dip treatment whereas this was not observed in *E. hirta* extract treatment. These findings may indicate a good correlation with the results of the *in vitro* antimicrobial activity testing, where the ethanolic extract of *P. amarus* showed a higher activity against *Aeromonas hydrophila*, one of the most active specific spoilage bacteria in chilled fish, with MIC of 156 µg/mL compared to *E. hirta* extract with MIC of 625 µg/mL (Nguyen et al., 2020). On the other hand, pH is one of the main factors that might impact the antimicrobial growth. The reciprocal relationship between studied antimicrobial plant extracts and pH were indicated in the results of TVC. It can be shown that the lower pH values of 0.04% *P. amarus* treatment might restrain microbial growth as well as contribute to the prolonging of the fish fillets preservation. In the group of dip treatment with *E. hirta*, no difference was observed between TVC of control and treated during the whole ice storage time (Figure 1B). At day 16, fish treated with *E. hirta* 0.06% and 0.2% displayed TVC of 7.76 and 6.95 $\log_{10}$ CFU/g, respectively, while the TVC of the control fish was 7.69 $\log_{10}$ CFU/g.

The fish samples of all treatments achieved TVC values higher than 6 $\log_{10}$ CFU/g on the day 12 and reached more than 7 $\log_{10}$ CFU/g after 16 days of storage. Thus, the microbiological shelf-life was about 8 days for the striped catfish fillets during ice storage according to the microbiological acceptability limit value (< 6 $\log_{10}$ CFU/g) for raw fish of Vietnam Ministry of Public Health (2012). When comparing with the TVC acceptable level of 7 $\log_{10}$ CFU/g proposed by the International Commission on Microbiological Specifications for Foods (ICMSF 1986), the shelf life of fish fillets stored in ice would be 12 days.

3.5 Peroxide value

The peroxide value (PV) allows to determine the fatty acid hydroperoxides, which are fatty acid primary oxidation products (Olafsdottir et al., 1997). The effect of dip treatment with *P. amarus* and *E. hirta* extracts on changes in PV of fish fillets are presented in Figure 2.

In the *P. amarus* experiment (Figure 2A), the PV of control fish fillets were 3.3 and 4.9 meq peroxide/kg fat at the beginning and the end of storage period, respectively, while in fish fillets dipped in water containing *P. amarus* extract, PV were 3.1 meq peroxide/kg fat for both day 0 and day 16, after treatment with 0.02% extract, and 2.4 and 2.3 meq peroxide/kg fat, respectively, after treatment with 0.04% extract. After 8 and 16 days of ice storage, PV were significantly lower ($p<0.05$) in fish fillets dipped in water containing both 0.02% or 0.04% *P. amarus* extract than in

control fish. Moreover, 0.04% *P. amarus* extract treatment seemed more effective to delay the fatty acid oxidation than 0.02%, as PV were significantly lower in fillets dipped in a solution containing 0.04% *P. amarus* extract. For some unexplained reason, the PV seem to increase after 12 days of storage in *P. amarus* extract treated fish fillets, while it remained stable in control fillets.

In the experiment of *E. hirta* dip treatment (Figure 2B), no significant difference was observed between PV of control and treated fish fillet after 0, 12 and 16 days of storage. The PV of fish fillets treated with both 0.06% and 0.2% *E. hirta* extract were significantly lower ($p<0.05$) than in control after 4 days of ice storage. Surprisingly, after 8 days of storage, only the PV of fish fillets treated with the lowest concentration of *E. hirta* extract (0.06%), was significantly lower ($p<0.05$) than the control.

Overall, the PV remained low, around 2.5 to 5 meq peroxide/kg fat, in all samples, including the controls. These PV values are well below the acceptable limit range of PV content for human consumption of 8-10 meq peroxide/kg fat in animal foods (Schormüller, 1968 cited by Linhartová et al., 2019). It was reported that the presence of phenolic compounds in the plant extract could inhibit the production of fatty acids free radicals and postpone the initiation of the autoxidative process in fat (Hraš et al., 2000). In previous studies, researchers evidenced that herbal extracts could help to extend the shelf life of fish products, regarding to their lipid oxidation status. Amoli et al. (2019) conducted successfully a dip experiment of rainbow trout using an alginate coating containing an ethanolic extract and/or essential oil of *Mentha aquatica*. They found that the PV of rainbow trout stored at 4^{o}C was below 7 meq peroxide/kg fish fat. Linhartová et al. (2019) demonstrated that rosemary extract could delay effectively the peroxidation in rainbow trout fillets, and maintain the PV in all samples below the maximal PV established for human consumption of 8-10 meq peroxide/kg fat (Schormüller, 1968 cited by Linhartová et al. 2019).

In this study, *P. amarus* extract was seen to be more active than *E. hirta* in protecting lipids of striped catfish flesh from oxidation.

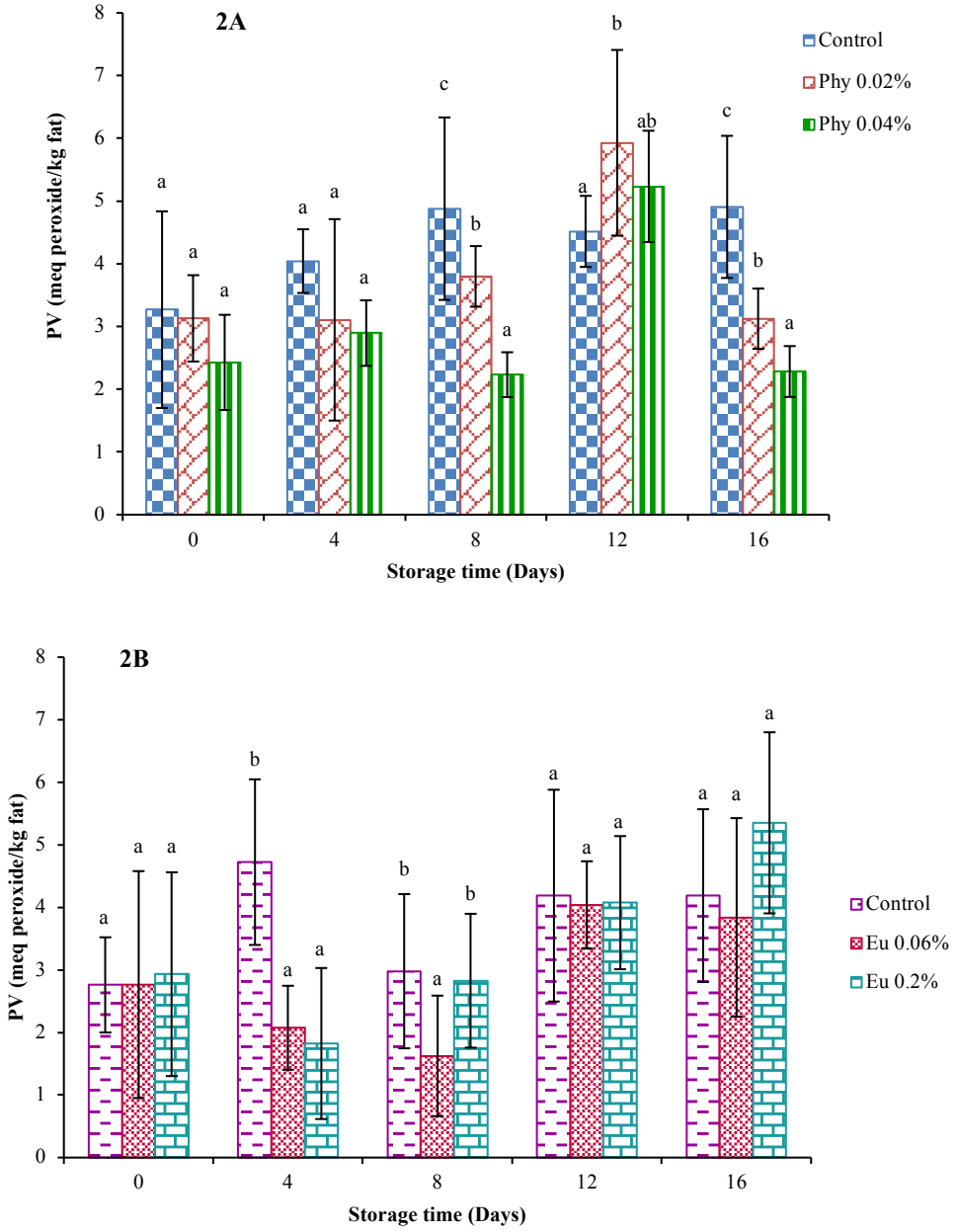


Figure 2. PV of striped catfish fillets after *P. amarus* (Phy) (A), *E. hirta* (Eu) (B) dip treatment during ice storage (mean ± SD, n=6). For the same day, same letters indicate insignificant differences between treatments (p > 0.05)

3.5. Sensory evaluation

3.5.1. Texture of fish fillets

In the present study, the texture of striped catfish fillets was determined by measuring the penetration strength of a probe. Changes in the penetration values of fish fillets during ice storage period are illustrated in Figure 3. In both experiments, no significant difference of texture was observed between control and treated fish (except one unexpected significant decrease of texture in fish treated with 0.02% *P. amarus*, after 4 days of storage, figure 3A), meaning that the plant extracts have no effect on the texture of the fish fillets in this study. Penetration values in both dip treatments tend to increase after 4 days of storage, apart from treatments of *P. amarus* 0.02% and *E. hirta* 0.2%. Afterward, a slight decreasing trend was seen over the storage period. Similar results were observed from the ice storage of sutchi catfish (Viji et al., 2015). The increase of firmness in fillets after 4 days of ice storage resulted from the stage of rigor mortis after the fish death. After more than 4 days, the firmness decreases due to the activity of autolytic enzymes (such as collagenase and ATPase) which degrade proteins from the connective tissue and the spoilage by bacteria (Lakshmanan et al., 2003).

3.5.2. Sensory properties

The changes in the total quality index (QI) of raw striped catfish fillets in different treated groups over the storage period are presented in Figure 4. On the scale of QI used here, zero represented absolutely fresh fish and 14 defined a completely deteriorated fish (see Appendix A). Overall, the scores of the sensory assessment exhibited a similar tendency of increasing unacceptability of the flesh samples for all groups (control and *P. amarus* and *E. hirta* extract dip treatments) (Figures 4A and 4B).

From day 0 to day 4, control and 0.02% *P. amarus* or 0.06% *E. hirta* treated fish presented the same quality index (of zero) whereas the group of 0.04% *P. amarus* and 0.2% *E. hirta* treated fish displayed a QI of 1, because of the higher concentration of extract that caused a negative effect on the fish fillets color (Figures 4A and 4B). During the remaining storage period, the groups treated with plant extracts exhibited a significant lower QI than the control samples ($p < 0.05$) (Figures 4A and 4B) and a similar pattern of increasing unacceptability. There was a noticeable change in the control group with the observable dull color, and loss of fresh odor in fillets, compared to the fish samples of plant extract treated groups in both experiments.

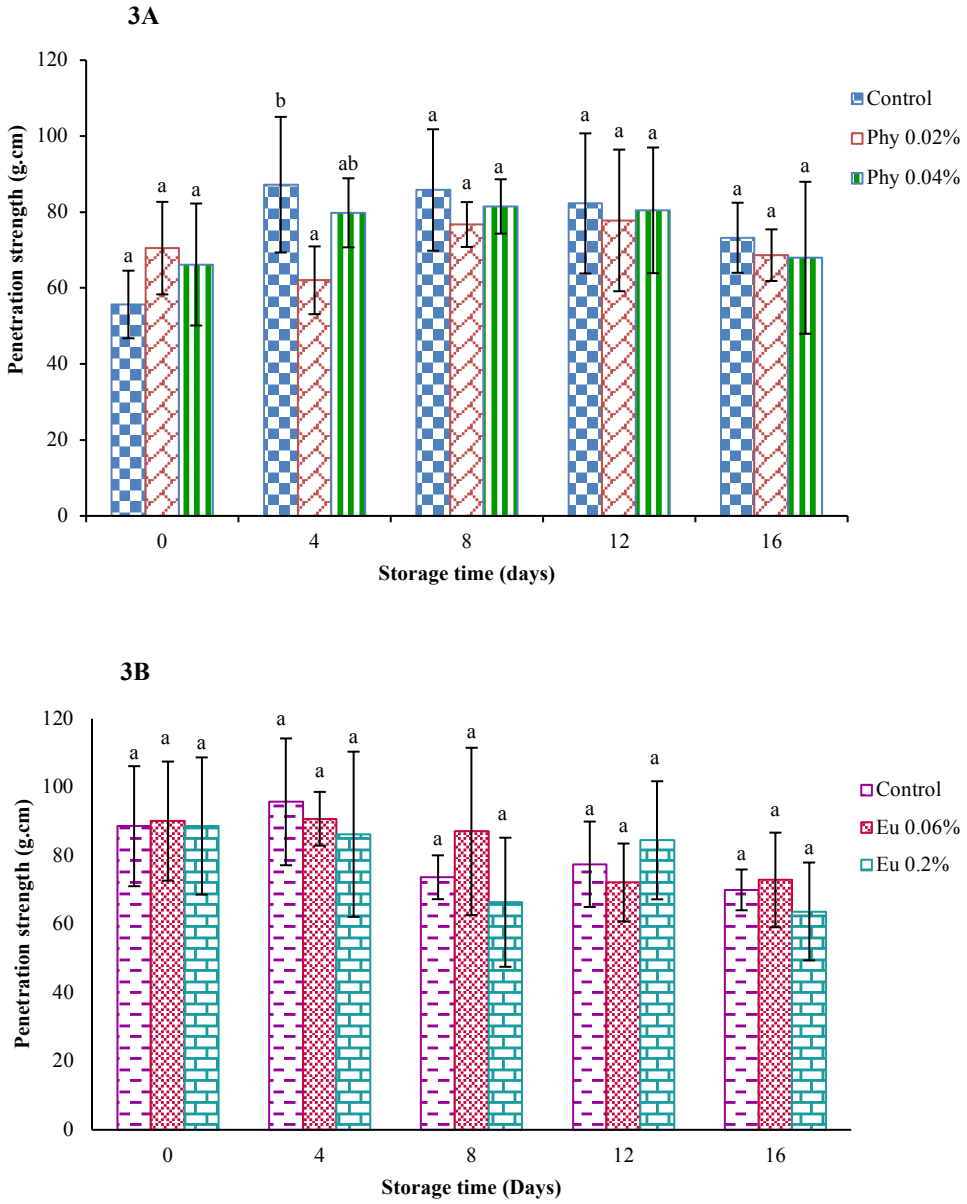


Figure 3. Texture property of striped catfish fillets after *P. amarus* (Phy) (A), *E. hirta* (Eu) (B) dip treatment during ice storage (mean ± SD, n=6). For the same day, same letters indicate insignificant differences between treatments (p > 0.05).

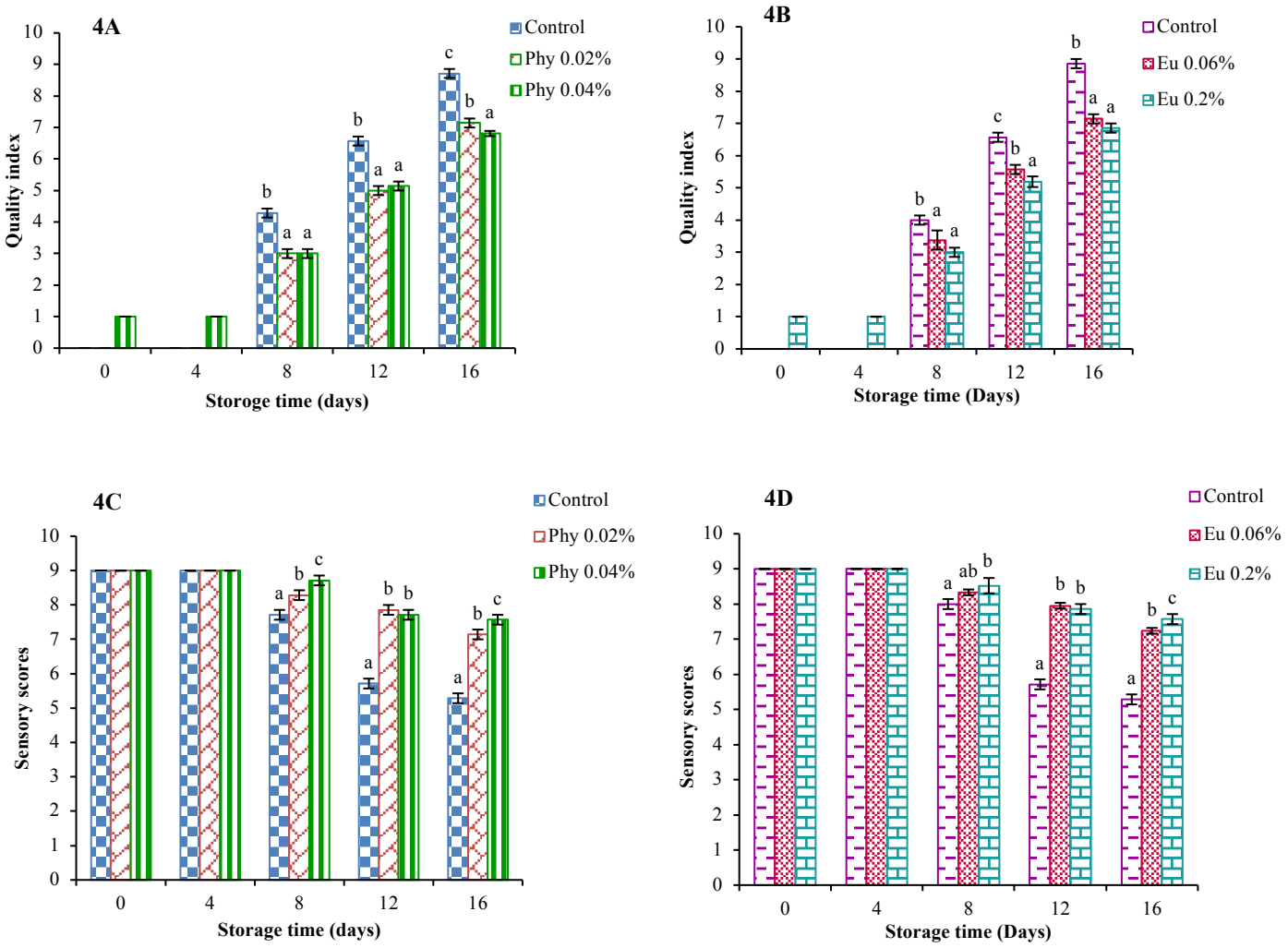


Figure 4. Sensory of striped catfish fillets during ice storage, *P. amarus* (Phy) treatment (A, C) and *E. hirta* (Eu) treatment (B, D) (mean ± SD, n=6). For the same day, same letters indicate insignificant differences between treatments (p > 0.05).

Acceptability score for taste of cooked striped catfish fillets was evaluated by using nine-point scale (see Appendix B). The striped fish samples were considered to be acceptable for human consumption until the sensory score reached 5 (Simeonidou et al., 1997). The scores of taste assessment of cooked fish are illustrated in Figure 4C and 4D. The results indicate that sensory scores showed a similar pattern of decreasing acceptability for the flesh samples of the control, *P. amarus* extract and *E. hirta* extract treated groups with increasing storage period. Both treated groups of fish had less fishy smells and the fish with plant extracts treatment were preferred by the panelists from day 8 onwards. In addition, there was no considerable differences between 0.06% and 0.2% *E. hirta* treated samples, but 0.06% could be the best option for the acceptable flavor and color of the fillets.

4. Conclusions

The treatment of 0.04% *P. amarus* extract could reduce the pH values during the initial storage period whereas *E. hirta* extract did not present this property. Neither plant extract dip treatments affected the texture of striped catfish fillets compared to the control group. *Pseudomonas* spp. and *Listeria monocytogenes* were not detected in the fish flesh, reflecting the safety of raw materials with regards to these specific spoilage and psychrotrophic pathogenic bacteria. Results showed that dipping fish fillets in a solution containing 0.04% *P. amarus* extract or 0.06% *E. hirta* extract allowed to prolong good sensory quality of fillets up to 8 days. From the data, it can be concluded that using *P. amarus* and *E. hirta* extracts dip treatments on striped catfish fillets could retain their good quality characteristics in terms of sensory assessment. These conclusions were supported by the results for chemical quality analyses (peroxide value).

Acknowledgements

This study was financed by the *ARES-CCD*, Académie de Recherche et d'Enseignement Supérieur, Commission de la Coopération au développement, through *AquaBioactive* project. The authors are grateful to Can Tho University (Vietnam) for providing necessary facilities for this study.

References

Abbas, K.A., A. Mohamed, B. Jamilah, and M. Ebrahimian, 2008. A review on correlations between fish freshness and pH during cold storage. American journal of biochemistry and biotechnology. 4, 416-421.

Ahmad, W., S. Singh, and S. Kumar, 2017. Phytochemical screening and antimicrobial study of *Euphorbia hirta* extracts. Journal of medicinal plants studies. 5, 183-186.

Al-Snafi, A.E., 2017. Pharmacology and therapeutic potential of *Euphorbia hirta* (Syn: *Euphorbia pilulifera*)-A review. IOSR Journal of Pharmacy. 7, 7-20.

Amoli, Z.A., T. Mehdizadeh, H. Tajik, and M. Azizkhani, 2019. Shelf life extension of refrigerated, vacuum-packed rainbow trout dipped in an alginate coating containing an ethanolic extract and/or the essential oil of *Mentha Aquatica.* Chemical Papers. 73, 2541-2550.

AOAC, 2016. Official methods of Analysis of AOAC International. 20th ed. Maryland, USA: Association of Official Agricultural Chemists.

Ashie, I., J. Smith, B. Simpson, and N.F. Haard, 1996. Spoilage and shelf-life extension of fresh fish and shellfish. Critical Reviews in Food Science & Nutrition. 36, 87-121.

Azam, K., S. Pervin, S. Naher, Y. Ali, M. Alam, K. Haque, and B. Ahmed, 2005. Quality Changes in Pangus (*Pangasius hypopthalamus*) in Relation to Size and season during storage in ice. Pakistan Journal of Biological Sciences. 8, 636-640.

Bach, L.T., L.T. Dung, N.T. Tuan, N.T. Phuong, P. Kestemont, J. Quetin-Leclercq, and B.T.B. Hue, 2018. Protective effect of pancreatic β-cells MIN6 by some medicinal plants in the Mekong Delta. Vietnam Journal of Chemistry. 56, 637-641.

Bagge-Ravn, D., Y. Ng, M. Hjelm, J.N. Christiansen, C. Johansen, and L. Gram, 2003. The microbial ecology of processing equipment in different fish industries—analysis of the microflora during processing and following cleaning and disinfection. International journal of food microbiology. 87, 239-250.

Bao, H., 2006. QIM Method Scores Quality, Shelf Life of Pangasius Fillets. Global Aquaculture Advocate. 28-30.

Chen, B.-Y., R. Pyla, T.-J. Kim, J.L. Silva, and Y.-S. Jung, 2010. Prevalence and contamination patterns of *Listeria monocytogenes* in catfish processing environment and fresh fillets. Food microbiology. 27, 645-652.

Devi, S., R. Rashid, and M. Kumar, 2017. Phytochemistry and pharmacological properties of *Phyllanthus amarus* Schum: A review. The Pharma Innovation Journal. 6, 169-172.

Direkbusarakom, S., 2004. Application of medicinal herbs to aquaculture in Asia. Walailak Journal of Science and Technology (WJST). 1, 7-14.

Donnelly, C., R. Brackett, S. Doores, W. Lee, and J. Lovett, 1992. *Listeria.* Compendium of methods for the microbiological examination of foods. 3, 637-663.

Duffes, F., 1999. Improving the control of *Listeria monocytogenes* in cold smoked salmon. Trends in Food Science & Technology. 10, 211-216.

Embuscado, M.E., 2015. Spices and herbs: Natural sources of antioxidants–a mini review. Journal of functional foods. 18, 811-819.

Erkan, N., Ş. Ulusoy, and Ş.Y. Tosun, 2011. Effect of combined application of plant extract and vacuum packaged treatment on the quality of hot smoked rainbow trout. Journal für Verbraucherschutz und Lebensmittelsicherheit. 6, 419-426.

Faleiro, M.L., 2011. The mode of antibacterial action of essential oils. Science against microbial pathogens: communicating current research and technological advances. 2, 1143-1156.

Falguera, V., J.P. Quintero, A. Jiménez, J.A. Muñoz, and A. Ibarz, 2011. Edible films and coatings: Structures, active functions and trends in their use. Trends in Food Science & Technology. 22, 292-303.

Fernandes, C.F., G.J. Flick, and T.B. Thomas, 1998. Growth of inoculated psychrotrophic pathogens on refrigerated fillets of aquacultured rainbow trout and channel catfish. Journal of food Protection. 61, 313-317.

Ghaly, A.E., D. Dave, S. Budge, and M. Brooks, 2010. Fish spoilage mechanisms and preservation techniques. American journal of applied sciences. 7, 859.

Gill, C., 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. Journal of Food Protection. 46, 444-452.

Gram, L., 1993. Inhibitory effect against pathogenic and spoilage bacteria of *Pseudomonas* strains isolated from spoiled and fresh fish. Applied and Environmental Microbiology. 59, 2197-2203.

Gram, L., J. Oundo, and J. Bon, 1989. Storage life of Nile perch (*Lates niloticus*) in relation to temperature and intial bacterial load. Tropical science.

Greeshma, S., K. Sarika, E. Priya, L. Kumar, T. Joseph, M. Pandey, and M. Prasad, 2019. Shelf life characteristics of *Pangasianodon hypophthalmus* fillets treated with *Moringa oleifera* (Lam) leaf extract.

Guha, G., V. Rajkumar, R.A. Kumar, and L. Mathew, 2010. Aqueous extract of *Phyllanthus amarus* inhibits chromium (VI)-induced toxicity in MDA-MB-435S cells. Food and chemical toxicology. 48, 396-401.

Hai, V.D., S.M.T. Hoang, N.T.Q. Hung, N.M. Ky, B. Gwi-Nam, P-h. Park, S.W. Chang, Q-V. Bach, T.N.T. Tran, and D.D. Nguyen, 2019. Characteristics of airborne bacteria and fungi in the atmosphere in Ho Chi Minh city, Vietnam-A case study over three years. International biodeterioration & biodegradation. 145, 104819.

Hraš, A.R., M. Hadolin, Ž. Knez, and D. Bauman, 2000. Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food chemistry. 71, 229-233.

Hultmann, L., T.M. Phu, T. Tobiassen, Ø. Aas-Hansen, and T. Rustad, 2012. Effects of pre-slaughter stress on proteolytic enzyme activities and muscle quality of farmed Atlantic cod (*Gadus morhua*). Food chemistry. 134, 1399-1408.

Huss, H.H., 1988. Fresh fish--quality and quality changes: a training manual prepared for the FAO/DANIDA Training Programme on Fish Technology and Quality Control. Food & Agriculture Org.

ICMSF, 1986. Sampling plans for fish and shellfish. International Commission on Microbiological Specifications for Foods, Microorganisms in Foods. Sampling for Microbiological Analysis: Principles and Scientific Applications. 2, 181-196.

ICMSF, 2005. Microbiological Ecology of Food Commodities, in Micro-organisms in Food 6, J.-L.C. T. A. Roberts, L. GramR. B. TompkinJ. I. Pitt, L. G. M. GorrisK. M. J. Swanson, Editor., Kluwer Academic/Plenum Publishers: New York. p. 174–249.

International IDF standards, 1991. Section 74A: 1991. International Dairy Federation, IDF-square Vergot 41.

Jeyakumari, A., R. Roy, G. Ninan, and V. Renuka, 2017. Effect of spice extracts on the biochemical, textural and sensory attributes of Pangasius (*Pangasius hypophthalmus*) chunks during ice storage.

Karl, H., I. Lehmann, H. Rehbein, and R. Schubring, 2010. Composition and quality attributes of conventionally and organically farmed Pangasius fillets (*Pangasius hypophthalmus*) on the German market. International journal of food science & technology. 45, 56-66.

Kumar, S., R. Malhotra, and D. Kumar, 2010. *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacognosy reviews. 4, 58.

Kumar Sarangi, M. and S. Padhi, 2017. Dengue and its phytotherapy: A review. International Journal of Pharmaceutical and Phytopharmacological Research. 4, 37-46.

Lakshmanan, R., J.R. Piggott, and A. Paterson, 2003. Potential applications of high pressure for improvement in salmon quality. Trends in Food Science & Technology. 14, 354-363.

Nguyen, L.A.D., T.M. Phu, C. Douny, J. Quetin-Leclercq, B.T.B. Hue, L.T. Bach, T. Quynh Nhu, B. Thi Bich Hang, D. Thi Thanh Huong, and N. Thanh Phuong, 2020. Screening and comparative study of *in vitro* antioxidant and antimicrobial activities of ethanolic extracts of selected Vietnamese plants. International Journal of Food Properties. 23, 481-496.

Lin, Y., R. Labbe, and K. Shetty, 2004. Inhibition of *Listeria monocytogenes* in fish and meat systems by use of oregano and cranberry phytochemical synergies. Applied and environmental microbiology. 70, 5672-5678.

Linhartová, Z., R. Lunda, P. Dvořák, J. Bárta, V. Bártová, J. Kadlec, E. Samková, J. Bedrníček, M. Pešek, and I. Laknerová, 2019. Influence of rosemary extract (*Rosmarinus officinalis*) Inolens to extend the shelf life of vacuum-packed rainbow trout (*Oncorhynchus mykiss*) fillets stored under refrigerated conditions. Aquaculture International. 27, 833-847.

Liu, X., Z. Wei, L. Fan, D.e. Ge, F. Wang, and J. Zhao, 2018. Effect of Chinese rice wine on proteolytic characterization originating from endogenous proteases in topmouth culter (*Culter alburnus*). PeerJ Preprints. 6, e26568v1.

Mazumder, A., A. Mahato, and R. Mazumder, 2006. Antimicrobial potentiality of *Phyllanthus amarus* against drug resistant pathogens. Natural product research. 20, 323-326.

Mitra, R. and S. Jain, 1985. Concept of *Phyllanthus niruri* (Euphorbiaceae) in Indian floras. Nelumbo-The Bulletin of the Botanical Survey of India. 27, 161-176.

Nguyen, V., J. Sakoff, and C. Scarlett, 2017. Physicochemical properties, antioxidant and cytotoxic activities of crude extracts and fractions from *Phyllanthus amarus.* Medicines. 4, 42.

Nguyen, V.T., Q. Van Vuong, M.C. Bowyer, I.A. Van Altena, and C.J. Scarlett, 2015. Effects of different drying methods on bioactive compound yield and antioxidant capacity of *Phyllanthus amarus.* Drying Technology. 33, 1006-1017.

Nhu, T.Q., B.T. Bich Hang, V. Cornet, M. Oger, L.T. Bach, N.L. Anh Dao, D.T. Thanh Huong, J. Quetin-Leclercq, M.-L. Scippo, and N.T. Phuong, 2020a. Single or combined dietary supply of *Psidium guajava* and *Phyllanthus amarus* extracts differentially modulate immune responses and liver proteome in striped catfish (*Pangasianodon hyphophthalmus*). Frontiers in Immunology. 11, 797.

Nhu, T.Q., N.P. Dam, B.T.B. Hang, B.T.B. Hue, M.-L. Scippo, N.T. Phuong, J. Quetin-Leclercq, and P. Kestemont, 2020b. Immunomodulatory potential of extracts, fractions and pure compounds from *Phyllanthus amarus* and *Psidium guajava* on striped catfish (*Pangasianodon hypophthalmus*) head kidney leukocytes. Fish & Shellfish Immunology.

Nhu, T.Q., B.T.B. Hang, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019a. Plant extract-based diets differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*). Fish & shellfish immunology. 92, 913-924.

Nhu, T.Q., B.T.B. Hang, A. Vinikas, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019b. Screening of immuno-modulatory potential of different herbal plant extracts using striped catfish (*Pangasianodon hypophthalmus*) leukocyte-based in vitro tests. Fish & shellfish immunology. 93, 296-307.

Nordic Committee on Food Analysis, 2006. Aerobic Plate Count in Foods. NMKL method 86, Nordic Committee on Food Analysis: Oslo, Norway.

Noseda, B., M.T. Islam, M. Eriksson, M. Heyndrickx, K. De Reu, H. Van Langenhove, and F. Devlieghere, 2012. Microbiological spoilage of vacuum and modified atmosphere packaged Vietnamese *Pangasius hypophthalmus* fillets. Food microbiology. 30, 408-419.

Novoslavskij, A., M. Terentjeva, I. Eizenberga, O. Valciņa, V. Bartkevičs, and A. Bērziņš, 2016. Major foodborne pathogens in fish and fish products: a review. Annals of microbiology. 66, 1-15.

Olafsdottir, G., E. Martinsdóttir, J. Oehlenschläger, P. Dalgaard, B. Jensen, I. Undeland, I. Mackie, G. Henehan, J. Nielsen, and H. Nilsen, 1997. Methods to evaluate fish freshness in research and industry. Trends in food science & technology. 8, 258-265.

Olatunde, O.O. and S. Benjakul, 2018. Natural preservatives for extending the shelf-life of seafood: A revisit. Comprehensive Reviews in Food Science and Food Safety. 17, 1595-1612.

Oluboyo, B., A. Oluboyo, and S. Kalu, 2016. Inhibitory effects of *Phyllanthus amarus* extracts on the growth of some pathogenic microorganisms. African Journal of Clinical and Experimental Microbiology. 17, 166-172.

Orban, E., T. Nevigato, G. Di Lena, M. Masci, I. Casini, L. Gambelli, and R. Caproni, 2008. New trends in the seafood market. Sutchi catfish (*Pangasius hypophthalmus*) fillets from Vietnam: Nutritional quality and safety aspects. Food Chemistry. 110, 383-389.

Patel, J.R., P. Tripathi, V. Sharma, N.S. Chauhan, and V.K. Dixit, 2011. *Phyllanthus amarus*: ethnomedicinal uses, phytochemistry and pharmacology: a review. Journal of Ethnopharmacology. 138, 286-313.

Perumal, S., R. Mahmud, S. Pillai, W.C. Lee, and S. Ramanathan, 2012. Antimicrobial activity and cytotoxicity evaluation of *Euphorbia hirta* (L.) extracts from Malaysia. APCBEE Procedia. 2, 80-85.

Phan, L.T., T.M. Bui, T.T. Nguyen, G.J. Gooley, B.A. Ingram, H.V. Nguyen, P.T. Nguyen, and S.S. De Silva, 2009. Current status of farming practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta, Vietnam. Aquaculture. 296, 227-236.

Rao, B.M., D. Jesmi, and P. Viji, 2017. Chilled storage of *Pangasianodon hypophthalmus* fillets coated with plant oil incorporated alginate gels: Effect of clove leaf, clove bud, rosemary and thyme oils. Journal of aquatic food product technology. 26, 744-755.

RASFF, 2011. The rapid alert system for food and feed - 2011 Annual Report. Available from: https://op.europa.eu/en/publication-detail/-/publication/79564291-a792-4c3b-aeb8-53455d22c9a8/language-en/format-PDF/source-200407129.

Roberts, T., J. Cordier, L. Gram, R. Tompkin, J. Pitt, L. Gorris, and K. Swanson, 2005. Micro-Organisms in Foods 6: Microbial Ecology of Food Commodities. Springer US.

Ruiz-Capillas, C. and A. Moral, 2001. Residual effect of CO_2 on hake (*Merluccius merluccius* L.) stored in modified and controlled atmospheres. European Food Research and Technology. 212, 413-420.

Schormüller, J., 1968. Handbuch der Lebensmittelchemie. Vol. Band III/2. New York: Springer Verlag, Berlin, Heidelberg.

Sen, A. and A. Batra, 2013. The study of *in vitro* and *in vivo* antioxidant activity and total phenolic content of *Phyllanthus amarus* Schum. & Thonn.: A medicinally important plant. International Journal of Pharmacy and Pharmaceutical Sciences. 5, 942-947.

Senjobi, C., A. Ettu, and C. Otujo, 2017. Antibacterial and antifungal activities of leaf extracts of *Phyllanthus amarus* Schum and Thonn. Journal of Pharmacognosy and Phytotherapy. 9, 6-10.

Simeonidou, S., A. Govaris, and K. Vareltzis, 1997. Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and Mediterranean hake (*Merluccius mediterraneus*). Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 204, 405-410.

Sveinsdottir, K., G. Hyldig, E. Martinsdottir, B. Jørgensen, and K. Kristbergsson, 2003. Quality Index Method (QIM) scheme developed for farmed Atlantic salmon (*Salmo salar*). Food Quality and Preference. 14, 237-245.

Tan, W.C., I.B. Jaganath, R. Manikam, and S.D. Sekaran, 2013. Evaluation of antiviral activities of four local Malaysian *Phyllanthus* species against herpes simplex viruses and possible antiviral target. International journal of medical sciences. 10, 1817.

Ushie, O., P. Neji, E. Etim, and G. Nsor, 2013. Phytochemical screening and antimicrobial activities of *Phyllanthus amarus* stem bark extracts. International Journal of Modern Biology and Medicines. 3, 101-112.

VASEP, 2019. Vietnam Association of Seafood Exporters and Producers. Fishery profile; Available from: http://vasep.com.vn/1192/OneContent/tong-quan-nganh.htm.

Vietnam Ministry of Public Health, 2012. National technical regulation of Microbiological contaminants in food (QCVN 8-3:2012/BYT). Available from: http://www.fsi.org.vn/pic/files/qcvn-8-3_2011-byt-ve-o-nhiem-vi-sinh-vattrong-tp_bia_merged.pdf.

Viji, P., S. Tanuja, G. Ninan, K. Lalitha, A. Zynudheen, P. Binsi, and T. Srinivasagopal, 2015. Biochemical, textural, microbiological and sensory attributes of gutted and ungutted sutchi catfish (*Pangasianodon hypophthalmus*) stored in ice. Journal of food science and technology. 52, 3312-3321.

Yang, X., W. Song, N. Liu, Z. Sun, R. Liu, Q.S. Liu, Q. Zhou, and G. Jiang, 2017. Synthetic phenolic antioxidants cause perturbation in steroidogenesis *in vitro* and *in vivo*. Environmental science & technology. 52, 850-858.

Yashin, A., Y. Yashin, X. Xia, and B. Nemzer, 2017. Antioxidant activity of spices and their impact on human health: A review. Antioxidants. 6, 70.

Zhuang, S., Y. Li, S. Jia, H. Hong, Y. Liu, and Y. Luo, 2019. Effects of pomegranate peel extract on quality and microbiota composition of bighead carp (*Aristichthys nobilis*) fillets during chilled storage. Food Microbiology. 82, 445-454.

Appendix A. Evaluation form for the sensory quality assessment of striped catfish fillets

Evaluation form for raw quality assessment of striped catfish fillets

Name: **Date:**

Code products:

Instruction: Please assess the fish fillets quality placing in front of you by using the list of descriptive terms provided below. Rate the fish on each of the quality parameters and circle the corresponding score on the scale (Table 4).

Table 4: Description and explanation of the properties in sensory analysis

Quality parameters	Description	Score
Texture	Firm and elastic	0
	Somewhat soft	1
	Soft	2
	Very soft	3
Surface	Very shiny	0
	Rather wrinkled and dried	1
	Wrinkled, dried	2
Odor	Fresh, seaweed	0
	Neutral, slightly fishy	1
	Fishy	2
	Sour, ammonia smell	3
Color	Cloudy white, bright	0
	Pinkish	1
	Yellowish	2
	Overall pink or yellow	3
Gaping	No Gaping	0
	Gaping, less than 25% of fillet	1
	Gaping, 25-75% of fillet	2
	Gaping, over 75% of fillet	3
Quality index (0-14)		

Appendix B. Evaluation form of cooked striped catfish fillets

Instruction: Please test the fish fillets placing in front of you and choose the best description to characterize the code by using the list of descriptive terms provided below. Write the corresponding score on the scale for the description (Table 5).

Table 5: Sensory evaluation of cooked striped catfish fillets

		Attribute score	Taste
Acceptable levels	No off- flavour	9	Typical fresh sweet taste for striped catfish fillets
		8	Some loss of sweetness
		7	Loss of the characteristic taste of striped catfish fillets
		6	Neutral taste, no off-flavours, slightly meaty
	Slight off-flavor	5	Trace of 'off-flavours', slightly putrid, slightly bitter
Reject levels	Severe off-flavor	4	Bitter, sour (citric)
		3	Sharp bitter taste, slight amine taste
		2	Sharp bitterness, strong sour
		1	Sharp 'off-flavour' of amines, rotten, defective fish fillets

5. SELECTED PLANT EXTRACTS AS NATURAL ANTIOXIDANTS FOR FISH FEED PRESERVATION AT AMBIENT TEMPERATURE

In general, fish feed contains a high amount of polyunsaturated fatty acids (PUFA) which are sensitive to lipid oxidation. Lipid oxidation in fish feed results in physicochemical changes and in a decrease of feed shelf life as well as in adverse effects on fish growth, health and nutritional quality. From the findings of the previous screening study of *in vitro* antioxidant and antimicrobial activities as well as immunostimulatory activities of twenty Vietnamese ethanolic plant extracts, five plant extracts were selected to be tested for their capacity to prevent lipid oxidation in fish feed throughout storage period at ambient temperature. Ethanolic extracts of *Phyllanthus amarus*, *Psidium guajava, Euphorbia hirta* and *Mimosa pudica* were selected due to their high *in vitro* antioxidant activity while *Azadiracha indica* extract, with no *in vitro* antioxidant activity. The concentrations of plant extracts supplemented to fish diet were established on the basis of a publication of Li et al. (2019) about fish feed storage, where *Angelica sinensis* plant extracts were used at concentrations ranging from 0.1 to 3.2 g/kg feed.

5. SELECTED PLANT EXTRACTS AS NATURAL ANTIOXIDANTS FOR FISH FEED PRESERVATION AT AMBIENT TEMPERATURE

Adapted from:

Nguyen Le Anh Dao, Tran Minh Phu, Caroline Douny, Joëlle Quetin-Leclercq, Bui Thi Buu Hue, Le Thi Bach, Do Thi Thanh Huong, Nguyen Thanh Phuong, Patrick Kestemont, Marie-Louise Scippo

Submitted to Animal Feed Science and Technology

Abstract

Five plants were selected to investigate the antioxidant activity of their ethanolic extracts in fish feed during its preservation at ambient temperature. Each extract was tested at two concentrations in the fish diet: 4 and 20 g/kg for ethanolic extracts of *Euphorbia hirta*, *Mimosa pudica* and *Azadiracha indica* and 2 and 10 g/kg for ethanolic extracts of *Phyllanthus amarus* and *Psidium guajava*. Negative control (fish diet without antioxidant or plant extract), positive control (fish diet without plant extract but with 0.2 g/kg of butylhydroxytoluene (BHT)) and fish diets containing plant extracts were stored at room temperature (30 – 35°C) in bags similar to those used by fish feed companies. The sampling was done after 0, 2, 4, 6 and 8 weeks of storage for analysis of moisture, total lipids and fatty acid profile, peroxide value (PV) and thiobarbituric acid reactive substances (TBARs). Eight secondary oxidation products (including malondialdehyde) were also measured using a liquid chromatography coupled to mass spectrometry method (LC-MS) validated for feed samples. The lipid content of the experimental diets was about 8%, and the fatty acid profile showed roughly an equal proportion of saturated, monounsaturated and polyunsaturated fatty acids. After 8 weeks of storage at room temperature, PV and TBARS values were significantly lower in fish diet containing plant extract than in the negative control diet. However, at this storage time, PV values were not significantly lower in the diets containing the plant extracts than in the positive control containing BHT, except for the diet containing 20 g/kg of ethanolic extract of *M. pudica*. Regarding the TBARS results after 8 weeks of storage, only two experimental diets showed significantly lower TBARS values than both the positive and the negative controls: the diet containing 10 g/kg of extract of *P. guajava* and the one containing 4 g/kg of *M. pudica*. Interestingly, the LC-MS measurement of malondialdehyde (MDA) did not match with the

TBARS results, showing that the TBARS values recorded here are due to other compounds than MDA. In conclusion, the ethanolic extract of *M. pudica* and *P. guajava* would be appropriate candidates as natural antioxidant to preserve fish feed during storage at room temperature.

Keywords: *Azadiracha indica, Euphorbia hirta*, *feed storage, Mimosa pudica, Phyllanthus amarus, Psidium guajava*

Abbreviations: BHT, butylhydroxytoluene; PV, peroxide value; TBARs, thiobarbituric acid reactive substances; LC-MS, liquid chromatography coupled to mass spectrometry method; MDA, malondialdehyde; PUFA, polyunsaturated fatty acids; MUFA, Monounsaturated fatty acids; BHA, butylhydroxyanisole; Psi, *Psidium guajava*; Phy, *Phyllanthus amarus*; Eup, *Euphorbia hirta*; Mim, *Mimosa pudica*; Aza, *Azadirachta indica*; NCtrl, Negative control; PCtrl, Positive control; 4-HHE, 4-hydroxy-2-hexenal; 4-HNE, 4-hydroxy-2-nonenal; CRT, crotonaldehyde; BNZ, Benzaldehyde; HXL, hexanal; 2,4-Nona, 2,4-Nonadienal; 2,4-Deca, 2,4-Decadienal; <LOQ, Lower than Limit of quantification.

1. Introduction

Fish feed generally contains high levels of polyunsaturated fatty acids (PUFA) which are particularly susceptible to lipid oxidation. It has been proved that lipid oxidation in fish feed is the cause of organoleptic changes and decrease of nutritional components content, of the formation of toxic metabolites and of the reduction of feed shelf life (Błaszczyk et al., 2013). This can negatively impact the fish growth and health, as well as its nutritional quality (Sutton et al., 2006; Grigorakis et al., 2010). Thus, controlling the oxidative stability of fish feed is needed to protect it against lipid oxidation. Antioxidants are considered as major inhibitors of free radical autoxidation effective to preserve polyunsaturated fatty acids against oxidative deterioration (Frankel, 2005). Synthetic antioxidants are usually used, such as ethoxyquin (EQ) in fish meal and butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) in fish oil (Hamre et al., 2010). However, it has been evidenced that they can cause adverse health effects to humans, who could be exposed via residues in fish flesh (Ito et al., 1985; Bohne et al., 2008; Lundebye et al., 2010). Moreover, there is growing interest in the substitution of synthetic antioxidants by natural ingredients (Lobo et al., 2010). In the previous decade, interesting studies were conducted to investigate the antioxidative capacity of plant extracts in fish feed. Hamre et al. (2010) found that adding a rosemary (*Rosmarinus officinalis*) extract at the concentration of 6 g/kg could show an optimal antioxidant effect on the diet during frozen storage for 18 weeks. Hernández et al. (2014) examined the protective capacity of natural antioxidants in extruded fish feed stored at different temperatures. These authors showed that 600 mg/kg of rosemary extract protected similarly extruded fish feed from oxidation than 200 mg/kg of butylhydroxytoluene, during storage for 24 weeks at ambient temperature (20–28^{o}C). In another study, the extract of *Angelica sinensis* appeared to be more effective than ethoxyquin to delay lipid oxidation in fish feed stored during 8 days at 45^{o}C (Li et al., 2019).

In a previous study, twenty plants, selected based on bibliography review data and on a survey in fish farms of Mekong Delta, were screened *in vitro* for their antioxidant (Nguyen et al., 2020) and immunostimulatory (Nhu et al., 2019) activities. In an *in vitro* screening of immuno-modulatory potential of these twenty plant extracts, five extracts including *Allium sativum*, *Azadirachta indica*, *Euphorbia hirta*, *Phyllanthus amarus* and *Zingiber officinale* induced significant changes in the expression of pro-inflammatory cytokine (il1β) antiviral cytokines (ifrγ

2a and b) and adaptive immune cytokine (mhc class II) in striped catfish cells. *P. amarus* always modulated the strongest expression of the four cytokines in peripheral blood mononuclear cells (PBMCs) and head kidney leukocytes (HKLs) over the whole experimental period (Nhu et al., 2019). In this study, the ethanolic extracts of five of these plants were tested for their capacity to limit lipid oxidation in fish feed during storage at ambient temperature. Four plants (*Phyllanthus amarus*, *Psidium guajava, Euphorbia hirta* and *Mimosa pudica*) were selected because their ethanolic extracts were shown to possess high *in vitro* antioxidant activity while a fifth one, *Azadiracha indica,* displayed no *in vitro* antioxidant activity.

2. Material and method

2.1. Plant extracts preparation

All plants in mature stage were collected from Mekong Delta (Vietnam) from October to November of 2016 and authenticated at the Department of Biology, College of Natural Science, Can Tho University. Fresh materials were obtained from whole plant of *E. hirta* and *M. pudica*, leaves of *P. guajava*, leaves and twigs of *P. amarus* and leaves, flowers and stem bark of *A. indica*. Ethanolic plant extracts were prepared as described by Bach et al. (2018). All extracted samples were lyophilized until dryness and stored at -20^{o}C before use.

2.2 Feed preparation

The formula was designed for striped catfish with a content of 30% crude protein, 6,7% crude lipid, 10.6% ash, 3.2% fiber and 4.4 Kcal/g of gross energy. Fish meal, soybean meal, cassava flour and rice bran were mixed and then sterilized at 110°C for 10 min. Vitamins, minerals and fish oil were well mixed with the sterilized mixture. BHT or each plant extract at two different concentrations was added (see below) and the final mixture was extruded using a mini-extrusion machine (College of Aquaculture and Fisheries, Can Tho University) at 70°C without steaming. All experimental diets (pellets of 2 mm in diameter) were dried at 60°C for 24 h.

2.3 Experimental design

The feed storage experiment was conducted to study the oxidative stability of fat in fish diet containing herbal extracts. Table 1 shows an overview of the experimental diets. Each extract was tested at two concentrations: extracts from *Euphorbia hirta*, *Mimosa pudica* and *Azadiracha*

indica were added to the fish diet at concentrations of 4 and 20 g/kg, while for *Phyllanthus amarus* and *Psidium guajava*, their extracts were tested at both concentrations of 2 and 10 mg/kg. A diet without plant extract was considered as negative control while feed containing 0.2 g/kg of BHT was the positive control (Hernández et al., 2014). All diets were stored at ambient temperature (30 – 35°C) in bags similar to those used by feed companies. The experimental design included 12 fish diets and each treatment was triplicated. Sampling was done after 0, 2, 4, 6 and 8 weeks of storage. At each sampling time, moisture (AOAC, 2016), PV (Hornero-Méndez et al., 2001) (fat content of each sample was determined at every sampling time) and TBARS (Raharjo et al., 1992) were determined. In addition, 8 aldehydes, as secondary oxidation products, were determined using a LC-MS/MS analytical method validated for feed samples (Douny et al., 2016). Lipid level in diet was determined at the beginning of the experiment (AOAC, 2016). The fatty acid profile was determined in the negative control diet at week 0 and in all samples after 8 weeks of storage, using a GC-MS method previously described (Douny et al., 2015).

2.4 Statistical analysis

The softwares Statistic SPSS (Version 16.0) and Microsoft Excel 2019 were used for the statistical analyses and calculation. All data were checked for normality and variance equality by using Kolmogorov-Smirnov and Leuven's tests, respectively. One-way analysis of variance (ANOVA) was used to determine the effects of antioxidant supplementation. Differences and effects were considered significant at $P<0.05$.

Table 1. Composition of experimental diets

Ingredients (1000 g of feed)	Negative Control Diet	Positive Control Diet	Experimental diets									
			Psi2	Psi10	Phy2	Phy10	Eup4	Eup20	Mim4	Mim20	Aza4	Aza20
[1]Soybean meal (g)	326	326.2	326	326	326	326	326	326	326	326	326	326
[2]Rice bran (g)	295	295	295	295	295	295	295	295	295	295	295	295
[3]Cassava (g)	184	183.6	182	174	182	174	180	164	180	164	180	164
[4]Fish meal (g)	150	150	150	150	150	150	150	150	150	150	150	150
[5]Fish oil (g)	10	10	10	10	10	10	10	10	10	10	10	10
[6]Premix* (g)	30	30	30	30	30	30	30	30	30	30	30	30
[7]Gelatin (g)	5	5	5	5	5	5	5	5	5	5	5	5
[8]Butylated hydroxytoluene (BHT)		0.2										
Plant extract (g)			2	10	2	10	4	20	4	20	4	20

Psi: *Psidium guajava*, Phy: *Phyllanthus amarus*, Eup: *Euphorbia hirta*, Mim: *Mimosa pudica*, Aza: *Azadirachta indica*.

[1] Wilpromil R Soy Protein Concentrate, Yihai (Fangchenggang) Soybeans Industries, (Wilmar Group), Fangchenggang, China.

[2] Cai Lan Oils & Fats Industries Company, Can Tho Branch, Can Tho City, Vietnam.

[3] Hong Ha Company, Can Tho City, Vietnam.

[4] Minh Tam, Can Tho, Vietnam.

[5] Vegetable oil (Simply, Vietnam) and squid oil (Vemedim, Vietnam) at a ratio of 1:1.

[6] The vitamin/mineral premix (Unit/kg) from Vemedim, Can Tho, VietNam: vitamin A, 6000 IU; vitamin D3, 5600 IU; vitamin E, 160 IU; vitamin B1, 10 mg; vitamin B6, 20 mg; vitamin B12, 0.03 mg; vitamin K, 0.3 mg; riboflavin, 60 mg; vitamin C, 300 mg; pantothenic acid, 60 mg; folic acid, 8 mg; nicotinic acid, 184 mg; biotin, 0.3 mg; iron, 50 mg; copper, 10 mg; iodine, 9 mg; zinc, 34 mg; selenium, 0.4 mg; manganese, 30 mg.

[7] Xilong Chemical Industry Incorporated (China).

[8] Honshu Chemical Industry Company, Japan.

3. Results and Discussion

3.1 Moisture, lipid contents and fatty acid profiles of fish feed

The initial moisture content of each diet ranged from 6.1 to 9.6% (Table 2). These results were in agreement with the publication of Royes and Chapman (2003), regarding the final moisture content in formulated dried fish feeds from 6 to 10%. During storage, the moisture of each diet increased, and was ranging from 10.5% to 13.6% for all experimental diets (Table 2). It is likely that fish diets were absorbing some water during their storage. The upper limit recommended for moisture content of extruded pet feeds is 12% (Rokey et al., 1985). In this study, extruded and pelleted feeds were produced at noticeably lower moisture contents, allowing a safety margin in case of reabsorption of water during storage. Only one experimental diet, containing 2 g/kg of *P. amarus*, reached a moisture content slightly above this limit after weeks of storage.

Table 2. Lipid content (%, fresh weight) of experimental diets before storage and moisture content (%) during storage at ambient temperature

	Moisture content					Lipid content
Diet	**Week 0**	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 0**
Psi2	7.5±0.2	7.8±0.2	8.8±0.2	9.5±0.3	10.6±0.2	8.65 ±0.5
Psi10	7.6±0.2	9.2±0.3	9.6±0.4	10.4±0.2	11.1±0.5	7.94 ±0.6
Phy2	9.6±0.2	10.3±0.4	11.4±0.4	12.2±0.1	13.6±0.2	7.90 ±0.4
Phy10	6.1±0.3	7.3±0.2	8.2±0.2	10.4±0.2	11.0±0.2	8.38 ±0.5
Eup4	7.9±0.2	9.3±0.3	10.2±0.2	10.2±0.3	11.2±0.4	8.34 ±1.3
Eup20	6.0±0.2	7.9±0.2	8.4±0.3	8.9±0.2	10.5±0.4	7.31 ±1.0
Mim4	9.5±0.2	10.1±0.7	10.2±0.8	10.9±0.2	11.3±0.6	8.09 ±0.3
Mim20	9.0±0.5	7.5±0.4	8.4±0.1	9.4±0.3	10.5±0.4	8.03 ±1.0
Aza4	6.6±0.8	8.6±0.3	8.6±0.2	9.7±0.3	10.6±0.2	8.30 ±0.5
Aza20	8.6±0.3	8.2±0.2	8.7±0.4	9.9±0.2	10.9±0.5	8.19 ±0.8
NCtrl	7.7±0.3	9.6±0.3	9.6±0.3	11.6±0.3	12.3±0.2	8.12 ±0.7
PCtrl	8.5±0.3	9.6±0.2	9.6±0.3	11.4±0.3	12.3±0.3	8.58 ±0.4

Values are mean ± standard deviation (n= 3)

The lipid levels of fish feed samples were measured at the beginning of the storage time (week 0). Results showed that lipid contents fluctuated from 7.3% to 8.7% among all experimental diets (Table 2). In general, the lipid content of fish feed should be approximately 7 to 15%, to

provide essential fatty acids as well as allowing to solubilize fat soluble vitamins (Craig et al., 2017).

From the results of fatty acid profile (Supplementary material 1), saturated fatty acids were mainly represented by palmitic acid (C16:0) (about 20% of total fatty acids) and stearic acid (C18:0) (about 4% of total fatty acids), whereas oleic acid (C18:1,9c, ω9) (about 38% of total fatty acids) was the major monounsaturated fatty acid. Linoleic acid (C18:2,9c,12c, ω6) was the principal polyunsaturated fatty acid (PUFA) (about 28% of total fatty acids), whereas alpha linolenic acid (C18:3, ω3) (about 3% of total fatty acids) was the principal ω3 PUFA in the diets. Long-chain PUFA (C20 to C22) were present in the diets in low amounts (around 1% of total fatty acids). When comparing the fatty acid profile of the negative control diet before storage (week 0) and after 8 weeks of storage and of the experimental diets, no dramatic change was observed (Supplementary material 1).

3.2 Lipid oxidation

Fish feeds contain high amount of PUFA that could make them susceptible to lipid oxidation (Hernández et al., 2014). Lipid oxidation in feed during storage was evaluated through the determination of peroxide values (PV), which are related to primary oxidation products (Fig. 1). As expected, peroxide values were increasing during the 8 weeks storage period, but they remained below the level of 10 meq peroxide/kg which is considered as acceptable level for vegetable oil (Babalola and Apata, 2011). When comparing to the negative control diet (without any antioxidant), it is observed that all experimental diets containing BHT or plant extracts display significantly lower PV ($P<0.05$), after 8 weeks of storage, except the experimental diet containing 4 g/kg of extract of *Azadiracha indica* (which is the one, from the five selected plants, from which the ethanolic extract displayed no antioxidant activity *in vitro* according to Nguyen et al. (2020)). In comparison with the positive control diet (containing BHT as antioxidant), the PV was significantly lower in only one experimental diet, the one containing 20 g/kg of *Mimosa pudica* after 8 weeks of storage at room temperature. This finding may indicate that the higher the extract concentration, the more effective the inhibition of peroxidation during storage. Moreover, dietary incorporated with 20 g/kg *Mimosa pudica* seems to better prevent lipids from oxidation than 0.2

g/kg BHT. Similarly, Li et al. (2019) showed that on one hand, peroxide values in experimental fish diets stored at 45°C were lower, the higher the content of extract of *Angelica sinensis*, and on another hand, peroxide values in experimental diet containing *Angelica sinensis* extract were lower than in fish feed containing ethoxyquin as antioxidant.

Also, in a study about poultry feed, Luna et al. (2017) showed that lipid peroxidation was slowered during 60 days of storage at room temperature by adding thymol or BHT at the concentration of 0.4 g/kg.

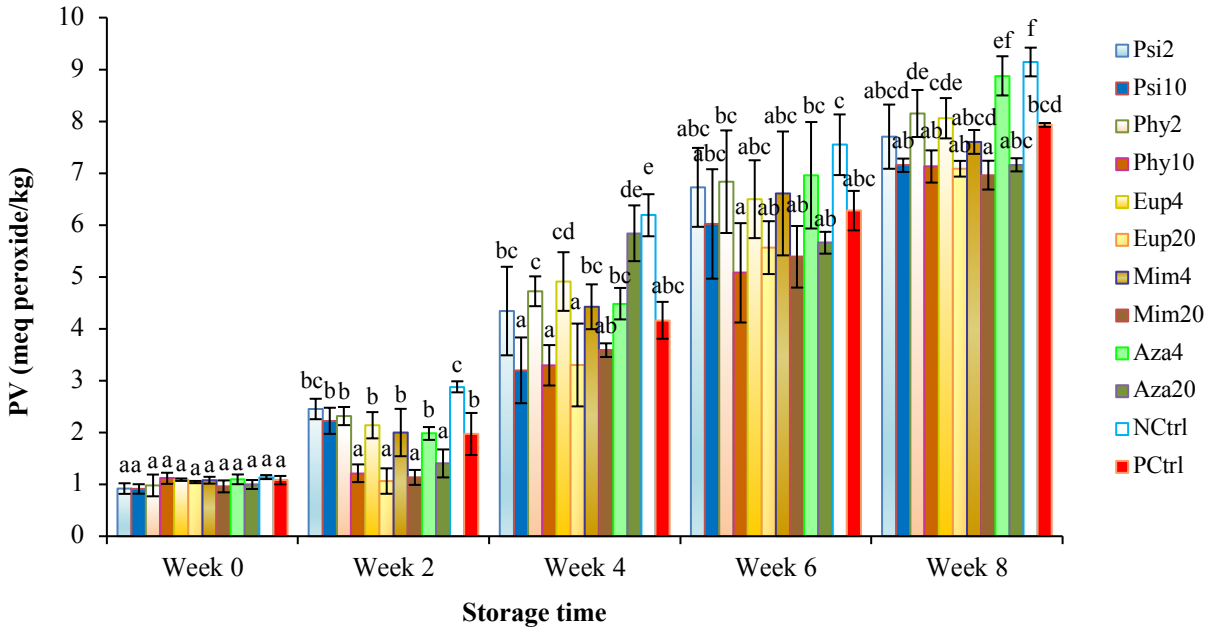


Figure 1. Peroxide value (meq peroxide/kg) during storage of feed (mean +/- SD, n=3)

Psi: *Psidium guajava*, Phy: *Phyllanthus amarus*, Eup: *Euphorbia hirta*, Mim: *Mimosa pudica*, Aza: *Azadirachta indica*, NCtrl: Negative control, PCtrl: Positive control, 2: 2 g/kg, 10: 10 g/kg, 4: 4 g/kg, 20: 20 g/kg. Values in the same day followed by different letter indicate significant differences between treatments on that day ($p < 0.05$).

The secondary oxidation products were assessed through thiobarbituric acid reactive species (TBARS) as well as LC-MS analysis of 8 specific aldehydes. As observed for PV, the TBARS values tend to increase in all experimental diets during storage at room temperature (Fig. 2). After 8 weeks of storage, the negative control (diet without antioxidant) showed the highest TBARS value of 8.59 mg MDA/kg feed, while the lowest values of TBARS, significantly lower

(P<0.05) compared to the BHT positive control, were observed in diets containing 10 g/kg of *Psidium guava* or 4 g/kg of *Mimosa pudica*. Hernández et al. (2014) reported that TBARS was 11.6 mg and 10.7 mg MDA/kg feed after 8 weeks of storage at ambient temperature in fish feed supplemented with BHT and rosemary extract (*Rosmarinus officinalis*), respectively. Furthermore, other authors already found that plant extracts such as rosemary extracts display similar or higher antioxidant activity than butylhydroxytoluene or butylhydroxyanisole (Richheimer et al., 1996; Formanek et al., 2001; Sebranek et al., 2005; Estevez et al., 2007). Also, Li et al. (2016) observed that ethyl acetate extract of *Ginkgo biloba* leaves was able to inhibit lipid oxidation in fish feed.

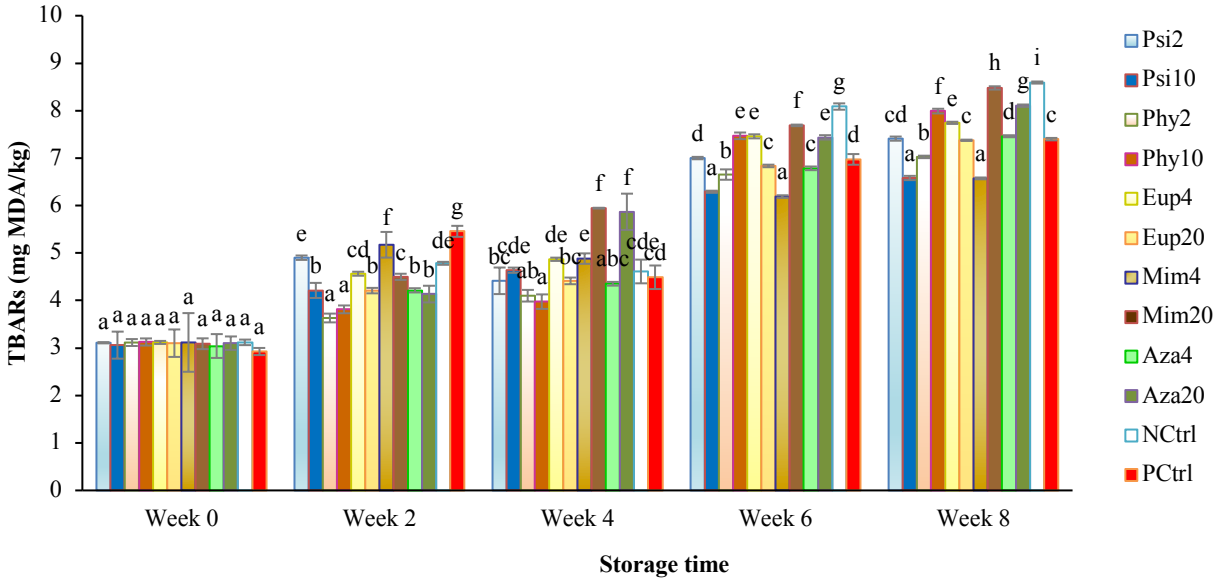


Figure 2. The TBARs value (mg MDA/kg) during storage of feed (mean +/- SD, n=3)

Psi: *Psidium guajava*, Phy: *Phyllanthus amarus*, Eup: *Euphorbia hirta*, Mim: *Mimosa pudica*, Aza: *Azadirachta indica*, NCtrl: Negative control, PCtrl: Positive control, 2: 2 g/kg, 10: 10 g/kg, 4: 4 g/kg, 20: 20 g/kg. Values in the same day followed by different letter indicate significant differences between treatments on that day ($p < 0.05$).

Among the 8 individual aldehydes (as secondary oxidation products) determined in the experimental fish diets using a LC-MS technique, malondialdehyde, hexanal, 2,4-nonadienal and 2,4-decadienal showed levels above their respective limit of quantification (LOQ) at various storage times (Supplementary material 2). As expected, no aldehyde was detected at week 0 of storage (Supplementary material 2). Table 3 shows the LC-MS results expressed as the sum of the

8 aldehydes determined. Table 3 shows that this sum is increasing during storage in the negative control diet (without antioxidant), while it remained low (below 1 mg/kg) in all other experimental diets, containing BHT or plant extracts, except in experimental diets containing *Azadiracha indica* extracts (those displaying no antioxidant activity *in vitro*) after 8 weeks of storage. Particularly, in the diets containing *Mimosa pudica* extracts, total levels of aldehydes remained below the LOQ of the LC-MS method, until the end of the experimental storage time at ambient temperature. This result could be related to the low level of TBARS in the experimental diet containing 4 g/kg of *Mimosa pudica* extract, but it cannot in the case of the diet containing 20 g/kg of this extract, which displayed higher TBARS levels. Besides, aldehydes levels remained also below the LOQ, until 6 weeks of storage, in experimental diets containing *Psidium guajava* extracts and BHT (positive control). For experimental diets containing *Phyllanthus amarus* and *Euphorbia hirta* extracts, the aldehydes levels remained below the LOQ until 4 weeks of storage only, showing a lower antioxidant effect of these extracts (Table 3).

Table 3. Concentrations of the sum of 8 aldehydes* (mg/kg) measured by LC-MS in fish feed during 8 weeks of storage at ambient temperature

	Week 0	Week 2	Week 4	Week 6	Week 8
Psi2	<LOQ	<LOQ	<LOQ	<LOQ	0.77
Psi10	<LOQ	<LOQ	<LOQ	<LOQ	0.42
Phy2	<LOQ	<LOQ	<LOQ	0.42	0.65
Phy10	<LOQ	<LOQ	<LOQ	0.40	0.64
Eup4	<LOQ	<LOQ	<LOQ	0.67	0.83
Eup20	<LOQ	<LOQ	<LOQ	0.62	0.79
Mim4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Mim20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Aza4	<LOQ	0.43	0.55	0.67	1.41
Aza20	<LOQ	<LOQ	0.63	0.86	1.08
NCtrl	<LOQ	1.02	4.00	4.15	4.92
PCtrl	<LOQ	<LOQ	<LOQ	<LOQ	0.70

* Names of the 8 aldehydes: MDA = Malondialdehyde, 4-HHE = 4-hydroxy-2-hexenal; 4-HNE = 4-hydroxy-2-nonenal; CRT = crotonaldehyde; BNZ = Benzaldehyde; HXL= hexanal; 2,4-Nona= 2,4-Nonadienal; 2,4-Deca = 2,4-Decadienal. <LOQ: Lower than Limit of Quantification

When comparing TBARS and LC-MS data, it can be concluded that the same trends are globally observed: the negative control diets display the higher results with both techniques, while after 8 weeks of storage at ambient temperature, the experimental diet containing 4 g/kg of extract of *Mimosa pudica* showed the lowest level with both techniques. However, the low levels of aldehydes determined by LC-MS analysis, including malondialdehyde (MDA), compared to TBARS levels, seems to indicate that MDA was probably not the main contributor to the TBARS results, as it is generally accepted. Indeed, Tsikas (2017) showed that HPLC analysis of TBA-treated extracts of oxidized methyl esters of linoleic acid and arachidonic acid revealed formation not only of the TBA-MDA derivative, but also several not identified TBA derivatives. Furthermore, according to Yoden and Iio (1989), lipophilic TBARS produced in oxidized lipids *in vitro* are major TBARS and the production of free MDA is small. Since the fatty acid profile of the feed revealed a content of nearly 30% of linoleic acid (see supplementary material 1), the TBARS results are probably the result of the formation of other thiobarbituric reactive species, maybe not all detected in the LC-MS method. In particular, in the case of feed containing 20 g/kg of *M. pudica* ethanolic extract, the increase of TBARS values was not explained by the MDA LC-MS analysis (where MDA was not detected). This TBARS result might be associated to some pro-oxidant activity of *M. pudica* extract at high concentration under the storage condition at ambient temperature, as observed in the study of Hernández et al. (2014) about fish feed preservation or the one of Ozkan and Erdogan (2012), when using an *in vitro* cell based model.

From the above results, it can be concluded that ethanolic extracts from *P. guajava*, *P. amarus* and *M. pudica* were able to inhibit lipid oxidation in feed materials, which seems to be a new finding, as no available reference reported yet their oxidative prevention in feeds. The antioxidant effects of these plant extracts may be closely correlated with their active components. Our earlier study pointed out a positive association between the antioxidant properties and the total phenolic content of these plant extracts (Nguyen et al., 2020).

4. Conclusions

Adding plant extracts into feed could reduce lipid oxidation during the storage at ambient temperature. Among the 5 plant extracts tested, *Mimosa pudica* ethanolic extract (at the

concentration of 4 or 20 g/kg) would be the most appropriate natural preservative for use as a feed preservative and would be able to lower lipid oxidation compared to 0.2 g/kg of BHT after 8 weeks of storage at ambient temperature.

Acknowledgements

This study was financed by the ARES-CCD, Académie de Recherche et d'Enseignement Supérieur, Commission de la Coopération au développement, through AquaBioactive project.

References

AOAC, 2016. Official methods of Analysis of AOAC International. 20th ed. Maryland, USA: Association of Official Agricultural Chemists.

Babalola, T. and D. Apata, 2011. Chemical and quality evaluation of some alternative lipid sources for aqua feed production. Agriculture and Biology Journal of North America. 2, 935-943.

Bach, L.T., L.T. Dung, N.T. Tuan, N.T. Phuong, P. Kestemont, J. Quetin-Leclercq, and B.T.B. Hue, 2018. Protective effect of pancreatic β-cells MIN6 by some medicinal plants in the Mekong Delta. Vietnam Journal of Chemistry. 56, 637-641.

Błaszczyk, A., A. Augustyniak, and J. Skolimowski, 2013. Ethoxyquin: an antioxidant used in animal feed. International journal of food science. 2013,

Bohne, V.J.B., A.-K. Lundebye, and K. Hamre, 2008. Accumulation and depuration of the synthetic antioxidant ethoxyquin in the muscle of Atlantic salmon (*Salmo salar* L.). Food and Chemical Toxicology. 46, 1834-1843.

Craig, S., L.A. Helfrich, D. Kuhn, and M.H. Schwarz, 2017. Understanding fish nutrition, feeds, and feeding.

Douny, C., P. Bayram, F. Brose, G. Degand, and M.L. Scippo, 2016. Development of an LC-MS/MS analytical method for the simultaneous measurement of aldehydes from polyunsaturated fatty acids degradation in animal feed. Drug testing and analysis. 8, 458-464.

Douny, C., R. El Khoury, J. Delmelle, F. Brose, G. Degand, N. Moula, F. Farnir, A. Clinquart, G. Maghuin-Rogister, and M.L. Scippo, 2015. Effect of storage and cooking on the fatty acid

profile of omega-3 enriched eggs and pork meat marketed in Belgium. Food Science & Nutrition. 3, 140-152.

Estevez, M., R. Ramírez, S. Ventanas, and R. Cava, 2007. Sage and rosemary essential oils versus BHT for the inhibition of lipid oxidative reactions in liver pâté. LWT-Food Science and Technology. 40, 58-65.

Formanek, Z., J. Kerry, F. Higgins, D. Buckley, P. Morrissey, and J. Farkas, 2001. Addition of synthetic and natural antioxidants to α-tocopheryl acetate supplemented beef patties: effects of antioxidants and packaging on lipid oxidation. Meat science. 58, 337-341.

Frankel, E., 2005. Lipid oxidation. Bridgewater, United Kingdom): The Oily Press.

Grigorakis, K., I. Giogios, A. Vasilaki, and I. Nengas, 2010. Effect of the fish oil, oxidation status and of heat treatment temperature on the volatile compounds of the produced fish feeds. Animal feed science and technology. 158, 73-84.

Hamre, K., K. Kolås, and K. Sandnes, 2010. Protection of fish feed, made directly from marine raw materials, with natural antioxidants. Food Chemistry. 119, 270-278.

Hernández, A., B.G. García, M. Jordán, and M. Hernández, 2014. Natural antioxidants in extruded fish feed: Protection at different storage temperatures. Animal Feed Science and Technology. 195, 112-119.

Hornero-Méndez, D., A. Pérez-Gálvez, and M.I. Mínguez-Mosquera, 2001. A rapid spectrophotometric method for the determination of peroxide value in food lipids with high carotenoid content. Journal of the American Oil Chemists' Society. 78, 1151-1155.

Ito, N., S. Fukushima, and H. Tsuda, 1985. Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. CRC Critical reviews in Toxicology. 15, 109-150.

Nguyen, L.A.D., T.M. Phu, C. Douny, J. Quetin-Leclercq, B.T.B. Hue, L.T. Bach, T. Quynh Nhu, B. Thi Bich Hang, D. Thi Thanh Huong, and N. Thanh Phuong, 2020. Screening and comparative study of *in vitro* antioxidant and antimicrobial activities of ethanolic extracts of selected Vietnamese plants. International Journal of Food Properties. 23, 481-496.

Li, H., D. Yang, Z. Li, M. He, F. Li, J. Jiang, S. Tang, P. Peng, W. Du, and Y. Ma, 2019. Effects of Angelica sinensis extracts on lipid oxidation in fish feeds and growth performance of juvenile Jian carp (*Cyprinus carpio* var. Jian). Animal Nutrition. 5, 109-114.

Li, H., X. Zhou, P. Gao, Q. Li, H. Li, R. Huang, and M. Wu, 2016. Inhibition of lipid oxidation in foods and feeds and hydroxyl radical-treated fish erythrocytes: a comparative study of *Ginkgo biloba* leaves extracts and synthetic antioxidants. Animal Nutrition. 2, 234-241.

Lobo, V., A. Patil, A. Phatak, and N. Chandra, 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews. 4, 118.

Luna, A., R. Lema-Alba, J.S. Dambolena, J.A. Zygadlo, M.C. Lábaque, and R.H. Marin, 2017. Thymol as natural antioxidant additive for poultry feed: oxidative stability improvement. Poultry Science. 96, 3214-3220.

Lundebye, A.-K., H. Hove, A. Måge, V. Bohne, and K. Hamre, 2010. Levels of synthetic antioxidants (ethoxyquin, butylated hydroxytoluene and butylated hydroxyanisole) in fish feed and commercially farmed fish. Food Additives & Contaminants: Part A. 27, 1652-1657.

Nhu, T.Q., B.T.B. Hang, A. Vinikas, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019. Screening of immuno-modulatory potential of different herbal plant extracts using striped catfish (*Pangasianodon hypophthalmus*) leukocyte-based in vitro tests. Fish & shellfish immunology. 93, 296-307.

Ozkan, A. and A. Erdogan, 2012. A comparative study of the antioxidant/prooxidant effects of carvacrol and thymol at various concentrations on membrane and DNA of parental and drug resistant H1299 cells. Natural product communications. 7, 1934578X1200701201.

Raharjo, S., J.N. Sofos, and G.R. Schmidt, 1992. Improved speed, specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C18 method for measuring lipid peroxidation in beef. Journal of Agricultural and Food Chemistry. 40, 2182-2185.

Richheimer, S.L., M.W. Bernart, G.A. King, M.C. Kent, and D.T. Beiley, 1996. Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary. Journal of the American Oil Chemists' Society. 73, 507-514.

Rokey, G.J., J. Krehbiel, K. Matson, and G. Huber, 1985. Production of pet food, in Feed manufacturing technology III, R.R. McEllhiney, Editor., American Feed Industry Association: Arlington, VA. p. 222-237.

Royes, J.-A.B. and F.A. Chapman, 2003. Preparing your own fish feeds. EDIS. 2003,

Sebranek, J., V. Sewalt, K. Robbins, and T. Houser, 2005. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat science. 69, 289-296.

Sutton, J., S. Balfry, D. Higgs, C.-H. Huang, and B. Skura, 2006. Impact of iron-catalyzed dietary lipid peroxidation on growth performance, general health and flesh proximate and fatty acid composition of Atlantic salmon (*Salmo salar* L.) reared in seawater. Aquaculture. 257, 534-557.

Tsikas, D., 2017. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Analytical biochemistry. 524, 13-30.

Yoden, K. and T. Iio, 1989. Determination of thiobarbituric acid-reactive substances in oxidized lipids by high-performance liquid chromatography with a postcolumn reaction system. Analytical biochemistry. 182, 116-120.

Supplementary material 1. Fatty acid profile of fish feed collected from week 0 and week 8 of ambient storage (% of total fatty acids)

	Week 0					Week 8							
	NCtrl	**Psi2**	**Psi10**	**Phy2**	**Phy10**	**Eup4**	**Eup20**	**Mim4**	**Mim20**	**Aza4**	**Aza20**	**NCtrl**	**PCtrl**
Saturated Fatty Acid													
Capric acid, C10:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lauric acid, C12:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tridecyclic acid, C13:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Myristic acid, C14:0	1.2	1.0	1.1	1.1	1.1	1.0	1.1	1.1	1.1	1.1	1.0	1.1	0.9
Palmitic acid, C16:0	21.6	18.8	20.8	20.1	20.8	20.4	23.3	20.5	21.1	20.1	19.8	22.0	18.9
Heptadecanoic acid, C17:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Stearic acid, C18:0	4.8	3.7	4.4	3.8	4.1	3.9	5.7	4.1	4.0	4.0	3.7	4.2	3.7
Arachidic acid, C20:0	<LOQ	0.7	<LOQ	0.8	0.7	0.6	0.7	0.7	0.7	0.6	0.6	0.7	0.7
Docosanoic acid, C22:0	<LOQ	<LOQ	<LOQ	<LOQ	0.4	0.3	0.4	0.4	0.4	<LOQ	0.3	0.4	0.3
Lignoceric acid, C24:0	0.7	0.7	0.9	1.0	0.8	0.6	0.7	0.9	0.8	0.6	0.6	0.8	0.7
MUFA													
Palmitoleic acid, C16:1, ω7	1.0	1.0	0.9	1.2	1.0	1.1	1.2	1.1	1.0	1.2	1.0	1.0	1.0
Heptadecenoic acid, C17:1, ω7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Elaidic acid C18:1,9t, ω9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Oleic acid C18:1,9c, ω9	37.5	37.8	36.8	37.9	35.5	36.1	34.6	38.2	38.4	36.1	36.4	36.7	37.4
ω6 PUFA													
Linoleic acid, C18:2,9c,12c, ω6	27.2	29.7	23.7	27.4	27.3	31.3	26.0	27.7	27.1	30.7	30.5	28.7	29.6
Linolelaidic acid, C18:2,9t,12t, ω6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Rumenicaid acid, C18:2,9c,11t, ω6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Isolinoleic acid, C18:2,9t,11t, ω6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Gamma Linolenic acid, C18:3, ω6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Eicosadienoic acid, C20:2, ω6	<LOQ	<LOQ	3.4	<LOQ	1.6	<LOQ	1.3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Arachidonic acid, C20:4, ω6	0.7	0.7	0.5	0.6	0.5	0.4	0.3	0.6	0.5	0.5	0.4	0.5	0.7
ω3 PUFA													

alpha Linolenic acid, C18:3, ω3	2.7	2.5	5.0	2.7	4.2	2.4	3.5	2.3	2.3	2.3	3.5	2.1	2.6
Octadecatetraenoic acid C18:4, ω3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
di-homo-γ-Linolenic acid, C20:3, ω3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Eicosapentaenoic acid (EPA), C20:5, ω3	0.8	0.9	0.7	0.9	0.7	0.5	0.4	0.7	0.7	0.8	0.6	0.6	1.0
Docosapentaenoic acid (DPA), C22:5, ω3	0.8	0.8	0.6	0.7	0.5	0.5	0.3	0.7	0.6	0.7	0.7	0.5	0.7
Docosahexaenoic acid (DHA), C22:6, ω3	1.0	1.7	1.0	1.8	0.9	0.7	0.5	1.2	1.3	1.5	0.9	0.8	1.8
Saturated FA (% total FA)	28.3	24.8	27.3	26.8	27.9	27.0	31.8	27.7	28.1	26.4	26.0	29.2	25.2
MUFA (% total FA)	38.5	38.8	37.7	39.1	36.5	37.2	35.8	39.3	39.4	37.2	37.5	37.7	38.4
PUFA (% total FA)	33.2	36.3	35.0	34.1	35.6	35.8	32.3	33.1	32.5	36.4	36.5	33.2	36.4
PUFA/saturated	1.17	1.46	1.28	1.27	1.27	1.33	1.02	1.20	1.16	1.38	1.40	1.14	1.44
ω6 (% total FA)	27.9	30.4	27.7	28.0	29.3	31.7	27.6	28.2	27.6	31.2	30.9	29.2	30.2
ω3 (% total FA)	5.3	6.0	7.3	6.1	6.2	4.1	4.7	4.8	5.0	5.2	5.6	4.0	6.1
ω6/ω3	5.3	5.1	3.8	4.6	4.7	7.7	5.9	5.9	5.6	6.0	5.5	7.3	4.9

<LOQ: Lower than limit of quantification; LOD and LOQ were 0.05 and 0.1% of total fatty acids, respectively
Psi: *Psidium guajava*, Phy: *Phyllanthus amarus*, Eup: *Euphorbia hirta*, Mim: *Mimosa pudica*, Aza: *Azadirachta indica*, NCtrl: Negative control, PCtrl: Positive control, 2: 2 g/kg, 10: 10 g/kg, 4: 4 g/kg, 20: 20 g/kg
MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

Supplementary material 2. Contents of aldehydes (mg/kg) in fish feed during storage at ambient temperature

		Concentration (mg/kg)								
		MDA	**4HHE**	**CRT**	**BNZ**	**4HNE**	**HXL**	**2,4NONA**	**2,4DECA**	**Summary**
	LOD (mg/kg)	**0.20**	**0.08**	**0.20**	**0.20**	**0.08**	**0.25**	**0.20**	**0.20**	
	LOQ (mg/kg)	**0.40**	**0.16**	**0.40**	**0.40**	**0.16**	**0.50**	**0.40**	**0.40**	
Week 0	Psi2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Psi10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Phy2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Phy10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Eup4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Eup20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Mim4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Mim20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Aza4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Aza20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	NCtrl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	PCtrl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
Week 2	Psi2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Psi10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Phy2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Phy10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Eup4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Eup20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Mim4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Mim20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Aza4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.43	<LOQ	**0.43**
	Aza20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	NCtr	1.02	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**1.02**
	PCtrl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**

Week 4	Psi2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Psi10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Phy2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Phy10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Eup4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Eup20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Mim4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Mim20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Aza4	<LOQ	<LOQ	0.55	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.55**
	Aza20	0.63	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.63**
	NCtrl	<LOQ	<LOQ	0.42	<LOQ	<LOQ	<LOQ	2.81	0.76	**4.00**
	PCtrl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
Week 6	Psi2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Psi10	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Phy2	<LOQ	<LOQ	0.42	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.42**
	Phy10	0.40	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.40**
	Eup4	0.67	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.67**
	Eup20	0.62	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.62**
	Mim4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Mim20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Aza4	0.67	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.67**
	Aza20	0.86	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.86**
	NCtrl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.15	<LOQ	<LOQ	**4.15**
	PCtrl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
Week 8	Psi2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.77	<LOQ	**0.77**
	Psi10	0.42	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.42**
	Phy2	0.65	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.65**
	Phy10	0.64	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.64**
	Eup4	0.83	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**0.83**

	Eup20	0.41	<LOQ	<LOQ	<LOQ	0.38	<LOQ	<LOQ	<LOQ	**0.79**
	Mim4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.15	<LOQ	<LOQ	**4.15**
	Mim20	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	**<LOQ**
	Aza4	0.95	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.46	<LOQ	**1.41**
	Aza20	0.64	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.44	<LOQ	**1.08**
	NCtrl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4.92	<LOQ	**4.92**
	PCtrl	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.70	<LOQ	**0.70**

<LOQ : Lower than limit of quantification

Psi: *Psidium guajava*, Phy: *Phyllanthus amarus*, Eup: *Euphorbia hirta*, Mim: *Mimosa pudica*, Aza: *Azadirachta indica*, NCtrl: Negative control, PCtrl: Positive control, 2: 2 g/kg, 10: 10 g/kg, 4: 4 g/kg, 20: 20 g/kg

MDA = Malondialdehyde, 4-HHE = 4-hydroxy-2-hexenal; 4-HNE = 4-hydroxy-2-nonenal; CRT = crotonaldehyde; BNZ = Benzaldehyde; HXL= hexanal; 2,4-Nona= 2,4-Nonadienal; 2,4-Deca = 2,4-Decadienal

6. PLANT EXTRACTS SUPPLEMENTATION OF STRIPED CATFISH (*PANGASIANODON HYPOPHTHALMUS*) DIET IMPROVES MICROBIOLOGICAL, CHEMICAL AND ORGANOLEPTIC QUALITY OF FISH FILLETS AFTER ICE STORAGE

Our previous studies showed that among five plant extracts selected from the screening of *in vitro* antioxidant, antimicrobial activity as well as immunostimulatory activities, ethanolic extract of *Mimosa pudica* was the most appropriate natural preservative for feed storage compared to butylhydroxytoluene (BHT) after 8 weeks of storage at ambient temperature. In addition, earlier investigations in Can Tho (Vietnam) proved the effectiveness of plant extract-based diets in stimulating immune responses and resistance to bacterial infection in striped catfish. In this fifth study, these five ethanolic plant extracts were supplemented to striped catfish in their diets to study their impact on the quality changes of fish fillets during ice storage. The choice of plant extract concentrations used in this study was made according to the combination of information from publications regarding *in vivo* feeding trials for validating immune responses (Nhu et al., 2020a; 2020b) and improving the quality of fish muscle during post-harvest storage at low temperature (Bao et al., 2009).

6. PLANT EXTRACTS SUPPLEMENTATION OF STRIPED CATFISH (*PANGASIANODON HYPOPHTHALMUS*) DIET IMPROVES MICROBIOLOGICAL, CHEMICAL AND ORGANOLEPTIC QUALITY OF FISH FILLETS AFTER ICE STORAGE

Adapted from:

Nguyen Le Anh Dao, Tran Minh Phu, Caroline Douny, Joëlle Quetin-Leclercq, Aurore Richel, Bui Thi Buu Hue, Le Thi Bach, Truong Quynh Nhu, Bui Thi Bich Hang, Do Thi Thanh Huong, Nguyen Thanh Phuong, Patrick Kestemont & Marie-Louise Scippo

Submitted to Aquaculture International

Abstract

To assess the impact of striped catfish herbal extracts supplementation on fish filets quality during ice storage, 2-month *in vivo* feeding experiments were conducted. In a first experiment, extracts from five different plants (*Euphorbia hirta*, *Mimosa pudica*, *Azadiracha indica*, *Phyllanthus amarus* and *Psidium guajava)* were tested individually at two concentrations in the diet (2 or 4 and 10 or 20 g extract/kg feed). In a second experiment, *P. guajava* and *P. amarus* extracts were tested at 3 concentrations, separately or together (0.8, 2 and 5 g/kg). Results of the analysis of fish filet during a period of 16 days of ice storage showed that *E. hirta, P. guajava* and *P. amarus* extract supplementation allowed to decrease the bacterial load and the fatty acid oxidation products content of fish filets and to improve their organoleptic quality. This positive effect was not observed when *P. guajava* and *P. amarus* extracts were given together to fish. In conclusion, *P. guajava* extract used at 5 g/kg feed could be the best choice, as feed additive in aquaculture, to improve the quality of striped catfish fillet during post-harvest ice storage.

Keywords: *Euphorbia hirta*, feeding trial, ice storage, *Phyllanthus amarus, Psidium guajava*

1. Introduction

The striped catfish (*Pangasianodon hypophthalmus*) is a migrant riverain species which is extensively cultured as a key commercial fish in several Asian countries. The annual production of striped catfish aquaculture in Vietnam was increasing rapidly the late years, from more than 300 thousand tons in 2004 to approximately 1.42 million tons in 2019 (Phuong and Oanh 2010; VASEP 2021).

Nowadays, the intensive catfish production leads to an increasing pressure on aquaculture to reduce or eradicate the use of feed antibiotics used as growth enhancers, resulting in new research to figure out safe and efficient natural alternatives. Besides, synthetic antioxidant additives, such as butylated hydroxytoluene (BHT), which are usually used in fish feed, become more and more suspect about their possible negative impact on the consumer health (Liu and Mabury 2020).

Herbal additives used in fish aquaculture are acknowledged to contain constituents which display immunostimulant, antibacterial and antioxidant properties (Reverter et al. 2014). It has been proven that various medicinal plant products possess a variety of active constituents, primarily alkaloids, steroids, phenols, tannins, terpenoids, saponins, glycosides and flavonoids (Ortega-Ramirez et al., 2014; Pinto et al., 2021; Škrovánková et al., 2012). Various researches have presented that these valuable ingredients could stimulate appetite (Reverter et al. 2014), impulse metabolism, stimulate immunity (Harikrishnan et al., 2011; Kirubakaran et al., 2010), improve disease resistance (Zhu 2020) and enhance fish flesh quality (Pu et al. 2017).

Recent studies indicated that the common trend of supplementing fish with plant extracts could effectively improve aquatic immunomodulation and disease resistance (Abdel-Tawwab et al., 2018; Gobi et al., 2016; Na-Phatthalung et al., 2018). Several studies were also conducted about the impact of fish supplementation with plant extracts on fish flesh quality during post-harvest storage at low temperature. For example, Bao et al. (2009) studied the dietary supplementation with an extract prepared from mushroom (*Flammulina velutipes*) culture medium for preventing discoloration and lipid oxidation in dark muscle of yellowtail. The impact of organic plant ingredients in the diet on rainbow trout muscle composition and oxidative stability was investigated in the research of Baron et al. (2013).

Earlier studies in Can Tho (Vietnam) demonstrated that plant extract-based diets were effective to stimulate immune responses and resistance to bacterial infection in striped catfish (*P. hypophthalmus*) (Nhu et al. 2019a). In that previous study, twenty plants, selected on literature review data and on a survey in fish farms in the Mekong Delta, were screened *in vitro* for their antioxidant (Nguyen et al. 2020) and immunostimulatory (Nhu et al. 2019b) activities.

In this study, the ethanolic extracts of five of these plants were used for diet supplementation of striped catfish to study their impact on the microbiological, chemical and organoleptic quality of fish fillets during ice storage. Four plants (*Phyllanthus amarus*, *Psidium guajava*, *Euphorbia hirta* and *Mimosa pudica*) were selected because their ethanolic extracts were shown to possess *in vitro* antioxidant and antimicrobial activities while a fifth one, *Azadiracha indica*, displayed no *in vitro* antioxidant nor antimicrobial activity.

2. Material and method

2.1. Plant extracts preparation

All plants were obtained from the Mekong Delta (Vietnam) and authenticated at the Department of Biology, College of Natural Science of the Can Tho University. Fresh materials were obtained from the whole plant of *E. hirta* and *M. pudica,* leaves of *P. guajava,* leaves and twigs of *P. amarus*, leaves, flower and stem bark of *A. indica*. Ethanolic extracts were prepared as described by Bach et al. (2018). All extracts were lyophilized to remove residual water and stored at -20 ^{o}C before use.

2.2 Fish diet preparation

The feed formula was designed for striped catfish with a content of 30.00 % crude protein, 6.66 % crude lipid, 10.58 % ash, 3.21 % fiber and 4.41 Kcal/g of energy. Fish meal, soybean meal, cassava flour and rice bran were mixed and then sterilized at 110 °C for 10 min. Vitamins, minerals and fish oil were then mixed with the sterilized mixture. BHT (0.2 g/kg) and each plant extract at various concentrations (see below) were added and the final mixture was extruded using a mini-extrusion machine (College of Aquaculture and Fisheries, Can Tho University) at 70 °C without steaming. All experimental diets (pellets of 2 mm in diameter) were dried at 60 °C for 24 h.

2.3 Experimental design

Juvenile striped catfish (15–20 g) were collected from a local fish farm in Vinh Long province (Vietnam). The fish were transported to the laboratory in plastic bags filled with oxygenated water. The laboratory conditions were set for fish acclimatization during 15 days.

For the first feeding trial using five plant extracts, each extract was tested at two concentrations: extracts from *Euphorbia hirta*, *Mimosa pudica* and *Azadiracha indica* were added to the fish diet at concentrations of 4 and 20 g/kg feed, while *Phyllanthus amarus* and *Psidium guajava* extracts were tested at both concentrations of 2 and 10 g/kg feed (Table 1A). The decision of plant extract concentrations was made from the combination of publications in *in vivo* feeding trial for validating immune response and improving the quality of fish muscle during post-harvest storage at low temperature.

In the second feeding trial, two plant extracts (*P. guajava* and *P. amarus*) were used individually or combined, at concentrations of 0.8, 2 and 5 g/kg feed (Table 1B). From the work of immunology in our project, *P. guajava* was the best promising plant in activating fish health as well as disease resistance of striped catfish. In addition, *P. amarus* extract induced the highest antioxidant activity in striped catfish after 8-weeks of extract-based diets.

Fish were randomly assigned into 11 treatments (first feeding experiment) or 10 treatments (second feeding experiment), with stocking density of 33 fish/tank (250 L). Each treatment was performed in triplicate (3 tanks/treatment). The photoperiod was designed with 12 h of light and 12 h of dark. Fish were fed three times daily (8 am, 12 am, and 5 pm) with an amount of feed corresponding to 2% of the fish body weight per day for 10 weeks.

During the experimental period, water temperature, dissolved oxygen and pH were controlled daily and maintained at 30 ± 2°C, 5.7 ± 0.01 mg/L and 7.5 ± 0.02, respectively. After 10 weeks, fillets (with skin) of 3 fish were taken from each tank for analysis of proximate composition (moisture, protein, ash and lipid content) (AOAC, 2016). Other fish (30) in each tank were harvested, weighed and fish flesh (including skin) was collected. Fillets (60 pieces) of fish from each tank were divided into 5 polyethylene (PE) bags and stored under ice for 16 days. Samples were taken at 0, 4, 8, 12 and 16 days of storage. At each sampling time, one bag containing 12 fillets was collected. In each bag, three fillets were used for sensory analysis, while the other

fish fillets were used for the triplicate determination of total viable count (TVC), peroxide value (PV) (International IDF standards, 1991) (fat content of each sample was determined at every sampling time) and thiobarbituric acid reactive species (TBARS) (Raharjo et al., 1992). Amino acid profile and biogenic amines were analyzed in selected samples following the methods of Paul et al. (2016) and Douny et al. (2019), respectively. The fatty acid profile was determined in the control diet at day 0 and in all samples after 16 days of storage, using a GC-MS method previously described (Douny et al., 2015).

Sensory analyses were performed at each sampling time thanks to a panel of seven trained members who evaluated the freshness of striped catfish fillets using the quality index method (QIM) (Sveinsdottir et al., 2003) and the taste of cooked striped catfish fillets according to Simeonidou et al. (1997). The QIM is based on significant, well-defined changes of appearance attributes that occur in raw fish after storage, such as odor, texture, color, gaping and surface. A score from 0 to 3 demerit points was given for these 5 parameters, the final QI being the sum of these 5 scores, which can vary from 0 (very fresh) to a maximum of 14 (very bad) (Bao, 2006). For sensory evaluation of cooked fish fillets (cooked in a steam oven at 100ºC for 10 minutes), a nine points scale was used (nine corresponding to the best taste), the threshold of acceptability being the score of 5.

2.4 Statistical analysis

The software Statistic (Version 20.0) and Microsoft Excel 2019 were used for the statistical analyses and calculation. One-way analysis of variance (ANOVA) was used to determine effects of plant extract supplementation. Differences and effects were considered significant at $P< 0.05$.

Table 1. Composition of experimental diets of striped catfish supplementation with plant extracts: first (A) and second (B) experiment

A

Ingredients (per kg feed)	Control Diet	Experimental diets									
		Psi2	Psi10	Phy2	Phy10	Eup4	Eup20	Mim4	Mim20	Aza4	Aza20
[1]Soybean meal (g)	326.2	326.2	326.2	326.2	326.2	326.2	326.2	326.2	326.2	326.2	326.2
[2]Rice bran (g)	295.0	295.0	295.0	295.0	295.0	295.0	295.0	295.0	295.0	295.0	295.0
[3]Cassava (g)	183.6	181.6	173.6	181.6	173.6	179.6	163.6	179.6	163.6	179.6	163.6
[4]Fish meal (g)	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0	150.0
[5]Fish oil (g)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
[6]Premix (g)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
[7]Gelatin (g)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
[8]Butylated hydroxytoluene (BHT) (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Plant extract (g)											
Psi		2	10	-	-	-	-	-	-	-	-
Phy		-	-	2	10	-	-	-	-	-	-
Eup		-	-	-	-	4	20	-	-	-	-
Mim		-	-	-	-	-	-	4	20	4	20
Aza		-	-	-	-	-	-	-	-	-	-

Psi: *Psidium guajava*, Phy: *Phyllanthus amarus*, Eup: *Euphorbia hirta*, Mim: *Mimosa pudica*, Aza: *Azadirachta indica*.
[1] Wilpromil R Soy Protein Concentrate, Yihai (Fangchenggang) Soybeans Industries, (Wilmar Group), Fangchenggang, China; [2] Cai Lan Oils & Fats Industries Company, Can Tho Branch, Can Tho City, Vietnam; [3] Hong Ha Company, Can Tho City, Vietnam; [4] Minh Tam, Can Tho, Vietnam; [5] Vegetable oil (Simply, Vietnam) and squid oil (Vemedim, Vietnam) at a ratio of 1:1; [6] The vitamin/mineral premix (Unit/kg) from Vemedim, Can Tho, VietNam: vitamin A, 6000 IU; vitamin D3, 5600 IU; vitamin E, 160 IU; vitamin B1, 10 mg; vitamin B6, 20 mg; vitamin B12, 0.03 mg; vitamin K, 0.3 mg; riboflavin, 60 mg; vitamin C, 300 mg; pantothenic acid, 60 mg; folic acid, 8 mg; nicotinic acid, 184 mg; biotin, 0.3 mg; iron, 50 mg; copper, 10 mg; iodine, 9 mg; zinc, 34 mg; selenium, 0.4 mg; manganese, 30 mg; [7] Xilong Chemical Industry Incorporated (China).; [8] Honshu Chemical Industry Company, Japan.

B

Ingredients (per kg feed)	Control diet	Experimental diets								
		Psi0.8	Psi2	Psi5	Phy0.8	Phy2	Phy5	Mix0.8	Mix2	Mix5
[1]Soybean meal (g)	240.0	240.0	240.0	240.0	240.0	240.0	240.0	240.0	240.0	240.0
[2]Rice bran (g)	295.0	295.0	295.0	295.0	295.0	295.0	295.0	295.0	295.0	295.0
[3]Cassava (g)	179.6	178.8	177.6	174.6	178.8	177.6	174.6	178.0	175.6	169.6
[4]Fish meal (g)	240.0	240.0	240.0	240.0	240.0	240.0	240.0	240.0	240.0	240.0
[5]Fish oil (g)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
[6]Premix (g)	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
[7]Gelatin (g)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
[8]Butylated hydroxytoluene (BHT) (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Plant extract (g)										
Psi	-	0.8	2	5	-	-		0.8	2	5
Phy	-	-	-	-	0.8	2	5	0.8	2	5

Psi: *Psidium guajava*, Phy: *Phyllanthus amarus*

[1] Wilpromil R Soy Protein Concentrate, Yihai (Fangchenggang) Soybeans Industries, (Wilmar Group), Fangchenggang, China; [2] Cai Lan Oils & Fats Industries Company, Can Tho Branch, Can Tho City, Vietnam; [3] Hong Ha Company, Can Tho City, Vietnam; [4] Minh Tam, Can Tho, Vietnam; [5] Vegetable oil (Simply, Vietnam) and squid oil (Vemedim, Vietnam) at a ratio of 1:1; [6] The vitamin/mineral premix (Unit/kg) from Vemedim, Can Tho, VietNam: vitamin A, 6000 IU; vitamin D3, 5600 IU; vitamin E, 160 IU; vitamin B1, 10 mg; vitamin B6, 20 mg; vitamin B12, 0.03 mg; vitamin K, 0.3 mg; riboflavin, 60 mg; vitamin C, 300 mg; pantothenic acid, 60 mg; folic acid, 8 mg; nicotinic acid, 184 mg; biotin, 0.3 mg; iron, 50 mg; copper, 10 mg; iodine, 9 mg; zinc, 34 mg; selenium, 0.4 mg; manganese, 30 mg; [7] Xilong Chemical Industry Incorporated (China).; [8] Honshu Chemical Industry Company, Japan.

3. Results and discussion

3.1 Proximate composition of striped catfish fillets before ice storage

Proximate analysis was determined in the fish fillets before ice storage. Moisture was ranging between 80.7 and 83.1 %, protein content between 14.0 and 15.8 %, and lipid content between 0.2 and 1.5 % (Supplementary material, table S1A and B). The lipid content of the fish fillets was generally above 1 %, but in the first trial, fish fillets coming from treatments Eup20 and Aza20 (i.e. fish fed with a diet containing 20 g/kg of *E. hirta* or *A. indica* extract, respectively), contained less lipid (0.7% and 0.2%, respectively) than the samples coming from the 9 other treatments (Supplementary material, Table S1A and B). Besides, no significant difference was observed between proximal composition of fish fillets from the different treatments. In studies performed on rainbow trout by Farahi and co-workers, it was observed that fish feed supplementation with *Melissa officinalis* or *Aloe vera* had no remarkable impact on fish body composition (Farahi et al., 2012), while adding garlic to the fish diet (30 g/kg) seemed to result in an increase of protein content and a decrease of lipid levels (Farahi et al., 2010).

3.2. Changes on total viable counts during striped catfish fish fillets ice storage

In the fish fillets coming from both experiments, the initial total viable counts (TVC) (day 0) were similar (about 3.5 log cfu/g), and the TVC showed an increasing trend during the 16-day ice storage period in all treatments (Figures 1A and 1B). At the first sampling day (day 0), there were no significant difference of TVC among the 11 treatments of the first experiment (Figure 1A). In the second experiment, day 0 sampled fish fillets coming from fish fed with feed containing 5 g/kg of extract from *P. guajava* (3.29 $\log_{10}$ CFU/g) or any concentration of *P. amarus* extract (3.32 $\log_{10}$ CFU/g, 3.33 $\log_{10}$ CFU/g, 3.27 $\log_{10}$ CFU/g) or the highest tested concentration of the mixture of *P. guajava* and *P. amarus* extracts (5g/kg each) (3.34 $\log_{10}$ CFU/g) already showed a significantly lower TVC than the control (3.72 $\log_{10}$ CFU/g), even if the difference in TVC was small (< 0.5 $\log_{10}$ CFU/g). This lower TVC remained significantly lower than the control until the end of the storage period, in case of treatment with 5 g/kg of extract from *P. guajava* and the mixture of *P. guajava* and *P. amarus* extracts (5g/kg each) (Figure 1B). The mean TVC in fish fillets coming from fish supplemented with *A. indica* extract were significantly higher than TVC

in all other treatments after 4 days of ice storage and not different from the control after 8, 21 and 16 days of ice storage (Figure 1A). This was expected as *A. indica* displayed no antimicrobial property in a previous *in vitro* screening (Nguyen et al. 2020). For the other treatments, significantly lower TVC were observed in fish fillets coming from fish supplemented with 10 g/kg feed of *P. amarus* extract (after 8 days of ice storage, Figure 1A) or any concentration of *E. hirta*, *P. amarus* or *P. guajava* extract (after 12 and 16 days of storage, Figure 1A). In the first experiment, treatment with 10 g/kg feed of *P. amarus* or *P. guajava* extract resulted in the lowest TVC values of 4.21 log cfu/g on day 8 and 5.17 log cfu/g on day 12, respectively (Figure 1A).

These results show the potential valorization of *P. amarus* and *P. guajava* extract in fish feed for microorganism inhibition during cold storage and confirm the antibacterial activity of these plant extracts observed in previous *in vitro* assays (Nguyen et al. 2020).

These results are also confirmed by those obtained in the second *in vivo* experiment, where only two plant extracts (*P. amarus* and *P. guayava*) were used for feed supplementation, separately or in combination. Among the various concentrations tested, it seems that only the highest tested concentration is able to significantly lower the TVC (and thus inhibit microbial growth) compared to the control, at all sampling times, though lower concentrations may have significant effects at some sampling times. Indeed, the TVC were significantly lower in fish fillets coming from fish supplemented with 5 g/kg feed of *P. guajava or P. amarus* extract compared to the control, at all sampling days (Figure 1B). The lowest TVC (3.25 and 3.87 log cfu/g) were observed in fillets from fish supplemented with 5 g/kg feed of *P. guajava* extract, sampled at day 4 and at day 8 of ice storage, respectively (Figure 1B).

In both experiments, from the twelfth day of storage (for the control) or the sixteenth day of storage, for all treatments, the average TVC values exceeded 6 log cfu/g (Figures 1A and 1B), which is the microbiological acceptability limit value for raw fish (Vietnam Ministry of Public Health 2012) and was proposed as a criterion for human consumption by Chang et al. (1998). This indicates a microbiological shelf-life of about 8 days for the striped catfish fillets during ice storage, when no plant extract supplementation is applied. However, after 12 days of storage, plant extract supplementation allowed to keep the TVC below 6 log cfu/g, which indicates that plant extract supplementation seems to permit to prolong by 4 days the shelf-life of fish fillets.

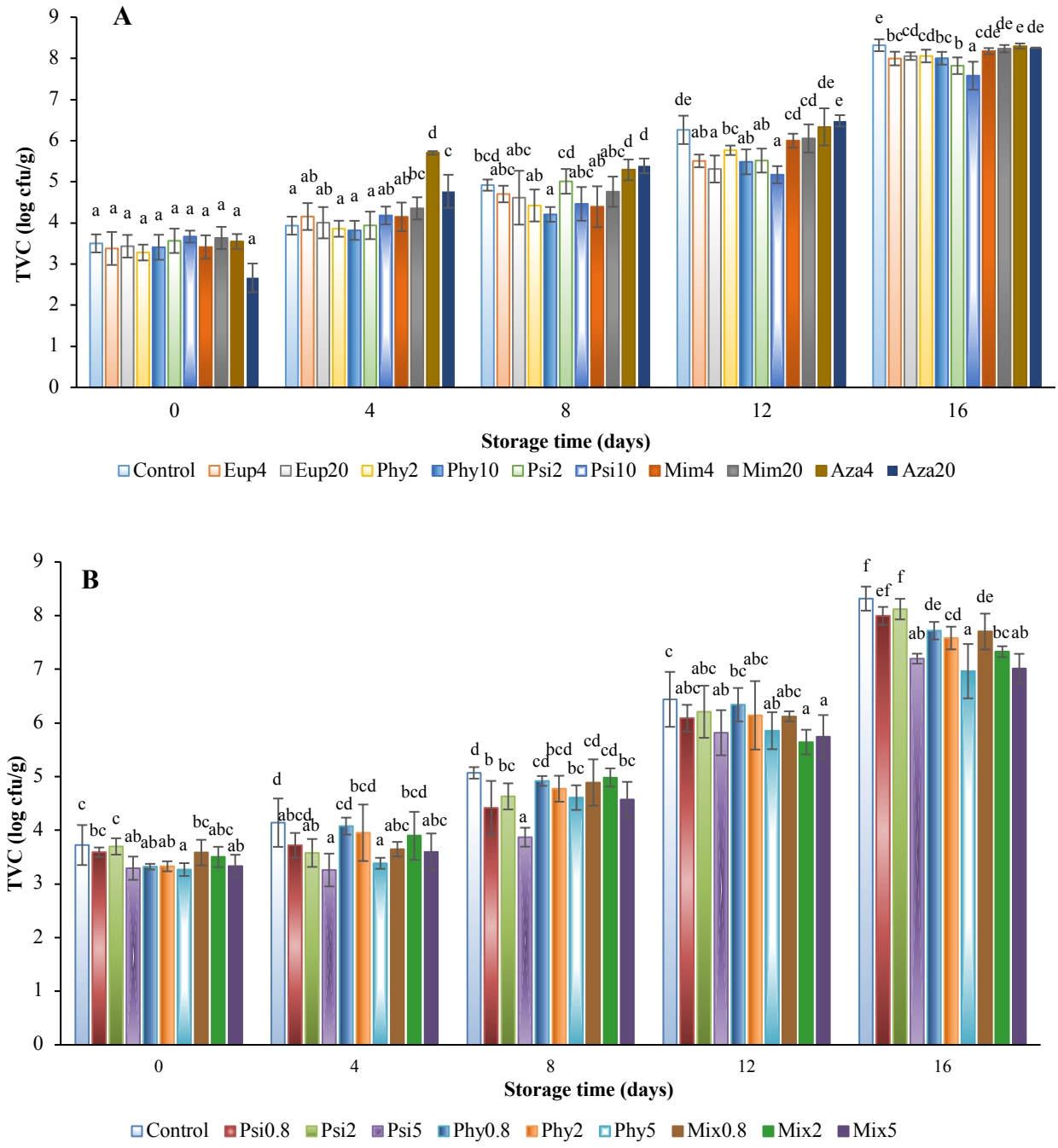


Figure 1: Changes in total viable count (TVC) of striped catfish filet during ice storage after *in vivo* fish supplementation with ethanolic extracts from 5 (figure 1A) or 2 (figure 1B) different plants (mean +/- SD, n=3)

See tables 1A and 1B for details about the various treatment. Values in the same day followed by different letter present significant differences between treatments each day ($p < 0.05$).

These antimicrobial properties can be due to plant secondary metabolites, which can generally be classified, according to their chemical structure into alkaloids, terpenoids (triterpenes and steroid saponins), phenolic compounds (including flavonoids, and tannins), glycosides and polysaccharides (Ortega-Ramirez et al., 2014). Phytochemical constituents of the extracts from *Phyllanthus* species, for instance, lignans (such as phyllanthin and hypophylanthin), flavonoids, triterpenoids, glycosides and tannins have already shown to possess antimicrobial activity (Rajeshkumar et al. 2002).

Regarding the application of plants in aquaculture, Hernández et al. (2014) studied the influence of rosemary extract added to feed of gilthead seabream (*Sparus aurata*) on the improvement of its preservation during ice storage. Fish fed with the highest tested dose of 2400 mg of rosemary extract per kg of feed showed the lowest total aerobic counts (3.57 log cfu/g) after 14 days of ice storage. In the study of Álvarez et al. (2012), the supplementation of gilthead seabream (*Sparus aurata*) with thyme essential oil at a level of 500 mg/kg feed resulted in lower TVC compared to control. It was found that most of the active compounds responsible for antimicrobial activity in rosemary extract and essential oils from the Thymus genus were related to phenolic compounds (Hernández et al., 2014; Rota et al., 2008). The plants used in this study (i.e., *P. amarus* and *P. guajava*) are known to contain, as active constituents, lignans (in *P. amarus)* and phenolic and triterpenic compounds (in both species) (Dhiman et al., 2011; Patel et al., 2011). Specifically, the antibacterial efficiency of ethanolic extracts of *P. amarus* and *P. guajava* has been documented both by *in vitro* screening (Nguyen et al. 2020) and *in vivo* in striped catfish (Nhu et al. 2020a).

3.3. Changes in fatty acid profiles during fish fillets storage

From the results of fatty acid profile in control fish fillets at day 0 of storage of the second experiment (Supplementary material, Table S2), it appears that saturated fatty acids (SFA) were dominant in this profile (about 40%), while monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were equally represented (about 30% each). The main saturated fatty acids were palmitic acid (C16:0) (about 26% of total fatty acids) and stearic acid (C18:0) (about 9% of total fatty acids), whereas oleic acid (C18:1,9c, ω9) (about 30% of total fatty acids) was the

principal monounsaturated fatty acid. Linoleic acid (C18:2,9c,12c, ω6), an ω6 fatty acid, was the major polyunsaturated fatty acid (PUFA) (about 17% of total fatty acids), whereas the crucial ω3 PUFA docosahexaenoic acid (C22:6, ω3) represented about 4% of total fatty acids. Comparing this fatty acid profile with literature is not very useful as it is known that the fish flesh fatty acid profile is depending of mainly of the fish feed composition (Khalili Tilami and Sampels, 2018).

About the comparison of the fatty acid profile of the striped catfish flesh from the control treatment (day 0) and after 16 days of storage with those from the experimental diets, it is not possible to perform a statistical analysis of the results as, for budgetary reasons, only one sample per treatment group was analyzed. However, we can see a downward trend of the PUFA content after 16 days of storage in both control and experimental groups, which is accompanied by an increasing trend of the SFA. This decrease of PUFA might be due to their susceptibility to oxidation over the storage period (Thiansilakul et al., 2010). Chaijan et al. (2006) reported depletion of PUFA and MUFA during storage due to the hydrolysis of triglycerides and phospholipids into free fatty acids, among which PUFA and MUFA are further oxidated to a higher extent than SFA. The PUFA decrease in fish fillets after 16 days of storage due to oxidation is confirmed by the increase of the peroxide value (PV) after 16 days of storage compared to day 0 (Figure 2B). However, it is not possible to draw conclusions about the effect of the plant extract on the PUFA oxidation by only looking at the fatty acid profile, due to the lack of repeated analysis (see above).

3.4. Changes in lipid oxidation during fish fillets storage

The peroxide value (PV) is an indicator for measuring the primary lipid oxidation while thiobarbituric reactive species (TBARS) are due to the second stage of oxidation during which peroxides are oxidized to aldehydes and ketones. Both parameters were employed for evaluating the oxidative stability of fish flesh during ice storage.

In both *in vivo* experiments in this study, the control diet contained 0.2 g/kg of BHT, as an antioxidant, while experimental diets contained both BHT (0.2 g/kg feed) and plant extracts (at various doses). Here below, significant changes compared to control mean thus a specific effect of the plant extract given to the fish as a feed supplement.

In all experimental groups, the PV was below the recommended acceptable range of 10 - 20 meq peroxide/kg fish fat (Huss, 1995) (Figures 2A and 2B).

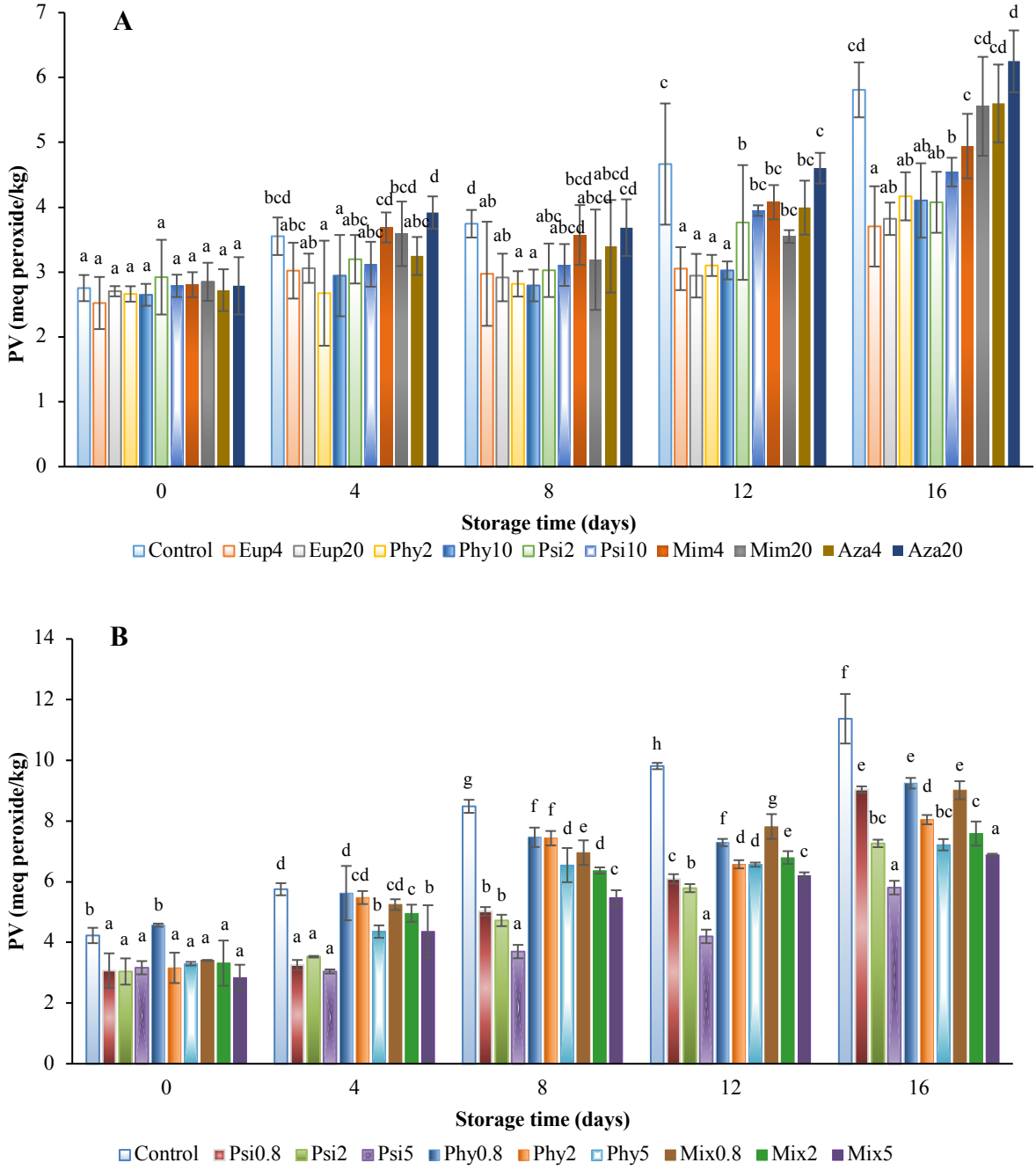


Figure 2: Changes in peroxide value (PV) of striped catfish filet during ice storage after *in vivo* fish supplementation with ethanolic extracts from 5 (figure 2A) or 2 (figure 2B) different plants (mean +/- SD, n=3). See tables 1A and 1B for details about the various treatment. Values in the same day followed by different letter present significant differences between treatments each day ($p < 0.05$).

In both experiments and as expected, the PV of all samples progressively increased during the 16 days of ice storage (Figures 2A and 2B), in agreement with the decrease of PUFA content observed after 16 days of storage, probably because of their oxidation (see above). It can be observed that the PV of fillets coming from fish supplemented with any dose of individual extracts of *E. hirta*, *P. amarus, P. guajava* after 8, 12 and 16 days (except the dose 10 g/kg feed of *P. guajava* at day 8 and 12) (Figures 2A and 2B) or those of a combination of extracts of *P. guajava* and *P. amarus* (Figure 2B) at each sampling day were significantly lower than in control fish fillets. It was also observed after 8, 12 and 16 days that the PV was significantly lower in the experimental groups supplemented with the highest amount (5 g/kg feed) of plant extract of *P. guajava* and *P. amarus* applied either separately or in combination, compared to treatment with lowest doses of plant extract (Figure 2B).

These results show the capacity of these plant extracts to inhibit fat oxidation and peroxides production, which is in agreement with previous findings showing the *in vitro* antioxidant capacity in a DPPH assay of *P. amarus, P. guajava* and *E. hirta*. ethanolic plant extracts (Nguyen et al., 2020), where the tested extracts were shown to contain 18.8, 14.5 and 10.3 % of total phenolic contents, respectively. Extracts from *M. pudica* and *A. indica* did not induce any significant difference in PV, compared to control, which is also in agreement with previous *in vitro* results showing lower antioxidant activity and total phenol contents for these two plant extracts (Nguyen et al., 2020).

The TBARS levels in all fish fillet samples were very low, below 1 mg/kg (Figures 3A and 3B). Because of these low values (and a subsequent low repeatability of the results), it is difficult to draw conclusions about the impact of plant extracts on TBARS reduction during fish fillet ice storage, in particular from the first *in vivo* experiment (Figure 3A). From the second *in vivo* experiment however, it is clear that TBARS are significantly lower in fish fillets coming from fish supplemented with any dose of *P. guajava* or *P. amarus* extract or their mixture and ice stored for 12 and 16 days, compared to the control (Figure 3B). In comparison with the first *in vivo* trial, it was shown that high doses did not yield the inhibition efficiency observed at lower doses and were sometimes found to be less effective (Bulfon et al., 2015). Similar results were reported by Mohebbi et al. (2012) who showed that the lowest TBARS values were recorded in rainbow trout fed with a diet added with 30 g/kg garlic compared to higher doses (40 and 50 g/kg diet). In short,

these results show again that *P. guajava* and *P. amarus* extract had a positive effect on preventing lipid oxidation in fish flesh during ice storage. Contrary to what was observed for the PV of fish fillets coming from the second *in vivo* experiment, no clear significant differences in TBARS levels were observed between the experimental groups treated with different plant extracts doses (Figure 3B).

In the research of Farahi et al. (2012), after 7 days of chilling storage (4 ^{o}C) of rainbow trout, the lowest TBARS content was recorded in samples from fish supplemented with *Aloe vera* at a dose of 10 g per 1 kg of diet. In another study, Hernández et al. (2014) investigated the influence of gilthead seabream (*Sparus aurata*) supplementation with different doses of rosemary extract on the quality of fish flesh during ice storage. A dose of 600 mg/kg rosemary extract was shown to allow a preservation of 21 days, with TBARS levels around 0.5 mg/kg.

According to Arsyad et al. (2018), the prevention of oxidation in cultured fish flesh could be improved by applying natural antioxidants derived from edible plants as feed supplements. Antioxidant compounds could help cells to fight against the multiple impacts of reactive oxygen species, i.e superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (Crespy and Williamson, 2004). Ilangkovan et al. (2015) and Ashraf et al. (2016) demonstrated that *P. guajava* and *P. amarus* possessed antioxidant and immunomodulatory properties. It was reported that the antioxidant capacity was mainly due to the presence of phenolic compounds in plant extracts (Saeed et al., 2012; Simamora et al., 2018). Furthermore, Nhu et al. (2020b) showed that hypophyllanthin, a chemical component highly responsible for antioxidant activity, was contained in the crude extracts of *P. amarus* before and after removing tannins. Moreover, these authors observed the presence of two major groups of metabolites in the crude extract of *P. guajava*, such as phenolic and triterpenic compounds. In combination with these findings, it can be suggested that the ethanol crude extracts of *P. amarus* and *P. guajava* can act as antioxidants and immunomodulators in striped catfish as well as optimistic candidates in fishery sciences.

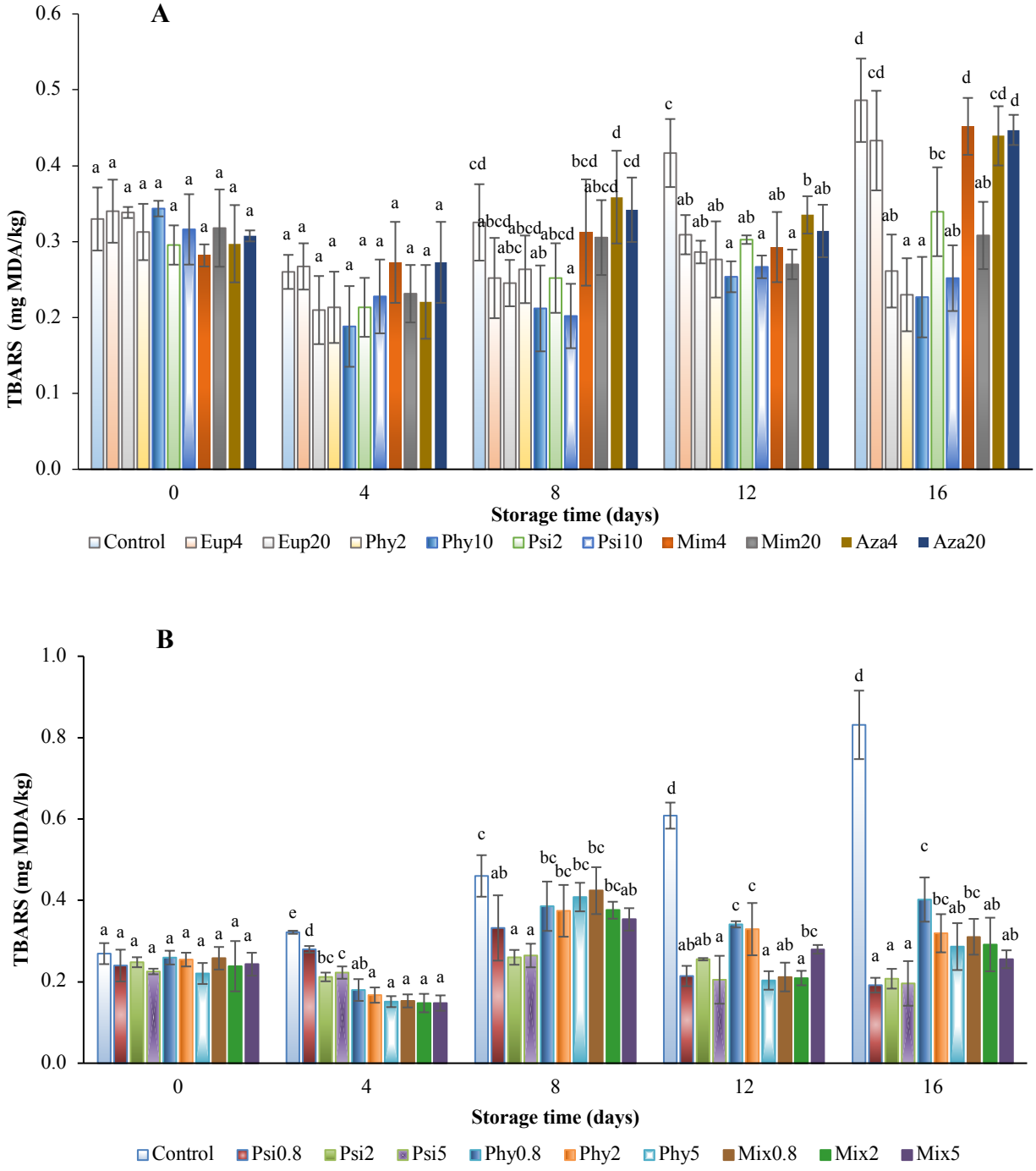


Figure 3: Changes in thiobarbituric reactive species (TBARS) levels in striped catfish filet during ice storage after *in vivo* fish supplementation with ethanolic extracts from 5 (figure 3A) or 2 (figure 3B) different plants (mean +/- SD, n=3)

See tables 1A and 1B for details about the various treatment. Values in the same day followed by different letter present significant differences between treatments each day ($p < 0.05$).

3.5. Changes of sensory properties during fish fillets storage

Sensory properties of raw striped catfish fillets (color, odor, gapping and texture) and cooked fish fillets were evaluated by a panel of seven trained members. The changes in the quality index (QI) of the freshness of striped catfish fillets from different treatments over the storage period are presented in Figures 4A and 4B. The value of QI is inversely proportional to the freshness of the fish fillet, as evaluated by the panel of assessors.

The freshness of the fish flesh generally displayed a decreasing trend for all treatments. At the first sampling day (day 0), the fish fillets from all experimental groups display a QI of 0, meaning that they were evaluated as very fresh by the panel. However, after 4 days of ice storage, a perceptible change was witnessed in control samples but not in fish fillets coming from fish supplemented with plant extracts (Figures 4A and 4B). After 12 and 16 days of ice storage, fish fillets coming from fish supplemented with plant extracts (all tested conditions, including supplementation with *A. indica* interestingly) displayed a significantly lower (i.e. better) QI than control samples. Among all conditions of plant extract supplementation, from the first *in vivo* experiment, the group receiving *E. hirta* extract at the concentration of 20 g/kg feed showed the significantly lower QI after 12 and 16 days of fish fillets ice storage (Figure 4A), while from the second *in vivo* experiment, *P. guajava* extract (5 g/kg feed) seemed to be the more efficient to significantly decrease the QI of fish fillets after 12 and 16 days of ice storage (Figure 4B).

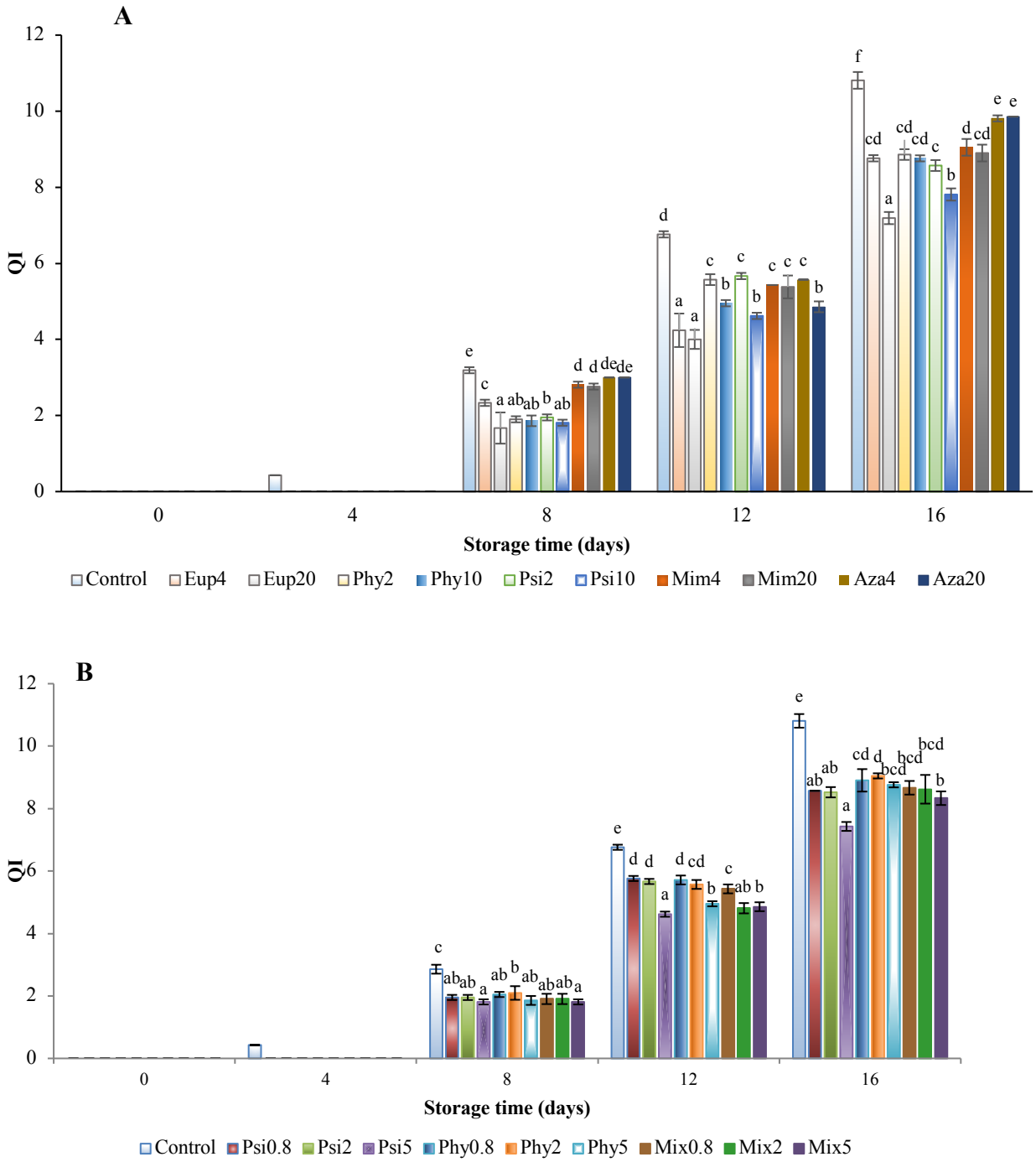


Figure 4: Changes in quality index (QI) of striped catfish filet during ice storage after *in vivo* fish supplementation with ethanolic extracts from 5 (figure 4A) or 2 (figure 4B) different plants (mean +/- SD, n=3).

See tables 1A and 1B for details about the various treatment. Values in the same day followed by different letter present significant differences between treatments each day ($p < 0.05$).

The taste of cooked fish fillets was assessed using a nine-point scale, nine being the best taste (Simeonidou et al., 1997). As observed for the QI of freshness of the fish fillets, the sensory scores of cooked fish fillets showed a decrease over the ice storage period, for all experimental groups (Figures 5A and 5B). The striped fish fillets were considered to be acceptable for human consumption when their sensory score was above or equal to 5 (Simeonidou et al., 1997), which was not the case anymore for the control group after 16 days of ice storage. After 4 days of ice storage, the sensory score was significantly better in fish filets after supplementation with both tested concentrations of extracts of *E. hirta*, *P. amarus* and *P. guajava* and one concentration of *A. indica* extract (4 g/kg feed) (Figure 5A). After 8, 12 and 16 days of fish fillets ice storage, the sensory scores of cooked fish fillets were significantly better than in control in almost all tested plant extract supplementation conditions (Figures 5A and 5B).

These results are in accordance with those of similar studies using herbal extracts for fish supplementation. For example, Hernández et al. (2014) observed an improvement of the sensory quality of gilthead seabream supplemented with rosemary extract at a dose of 600 mg/kg. Arsyad et al. (2018) documented that adding olive leaf powder to aquaculture fish feed could improve the texture characteristics of the muscle (it becomes harder) due to the quantity and the quality of collagen fibers in the endomysium. The addition of tochu leaf powder in fish diet was effective in developing the muscle of eels (*Anguilla japonica*) which were 1.8 times harder than without tochu (Tanimoto et al., 1993). Gatlin (2007) showed that the texture of young yellowtail was improved when fish were supplemented with green tea polyphenols.

Several unexpected results were seen in case of *A. indica* extract treatments, for example, which showed higher sensory scores of cooked fish fillets at lower concentration (Figure 5A). It is likely that the fish did not take enough experimental feed in these treatments due to the unfamiliar flavor of feed affected by higher concentration of this plant extract and thereby impact on the nutritional and organoleptic quality of fish flesh. Similarly, a few observations of sensory scores occurred in single or mixture administrations of some plants (Figure 5B) that could not be explained since it is possibly correlated to biological metabolism of individual fed fish and might lead to the dissimilarity of proximate composition among fish administrated plant-based diets.

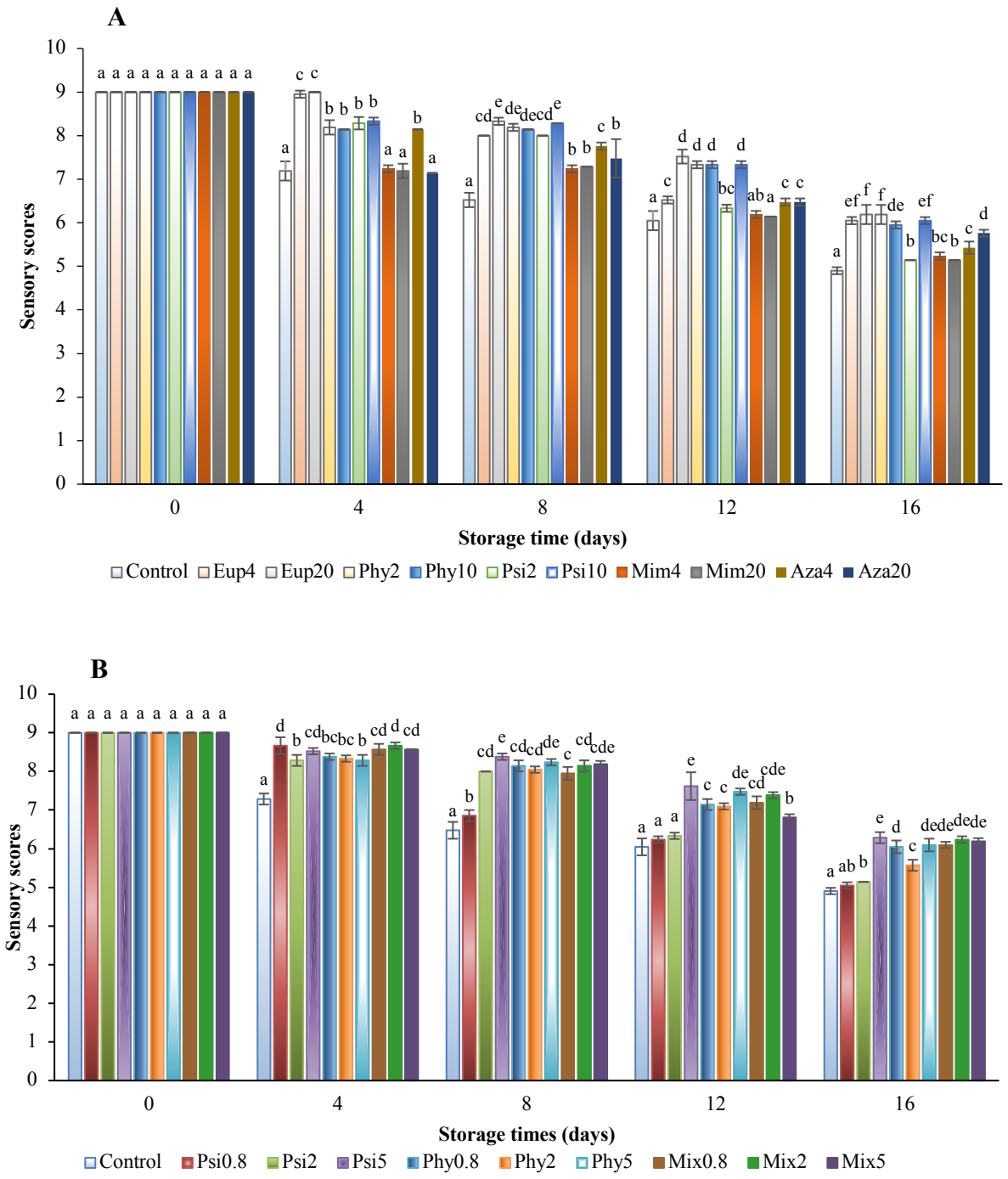


Figure 5: Changes in sensory scores of striped catfish filet during ice storage after *in vivo* fish supplementation with ethanolic extracts from 5 (figure 5A) or 2 (figure 5B) different plants (mean +/- SD, n=3).

See tables 1A and 1B for details about the various treatment. Values in the same day followed by different letter present significant differences between treatments each day ($p < 0.05$).

Regarding causes associated to sensory quality changes, the presence of lipid oxidation products (TBARS) and spoilage bacteria result in the formation of off-odors and off-flavors, slime production and discoloration which could decrease the sensory quality of fish fillets (Giménez et al., 2004; Lone and Hans, 1996). In this study, samples coming from fish supplemented with 5 g/kg feed of *P. guajava* displayed the lowest PV and TBARS values and showed the best sensory properties.

3.6. Amino acids profile and biogenic amines content of striped catfish fillets during ice storage

The total amino acid composition of striped catfish fillet was determined on a limited number of samples to check for biogenic amines precursors abundance and make the link with biogenic amines analysis results. It would have been interesting to know the amount of free amino acids formed after ice storage but unfortunately, due to insufficient quantity of sample, it was not possible to determine the free amino acids. Table 2 shows the total amino acid composition of muscle from non supplemented fish (control) at day 0 and after 16 days of ice storage, and from fish supplemented with a mixture *P. guajava* and *P. amarus* extracts (5 g/kg feed, each), after 16 days of ice storage. The major amino acids in fish flesh were lysine and leucine and total amino acid levels ranged between 15.34 and 15.52 g/100 g fresh material. The amino acid composition is similar in the three groups. Similarly, in the study of Lee et al. (2012), no differences of juvenile sterlet sturgeon (*Acipenser ruthenus*) whole body amino acid composition was observed between the experimental groups supplemented with garlic extracts and the controls. No data was found in the literature about the amino acid profile of striped catfish fillet, but for the 9 essential amino acids, the amounts are close to what was found by Danuwat et al. (2016) in juveniles of *P. bocourti*.

Table 2. Amino acids composition (grams of amino acids per 100 grams of fish fillet wet weight) of ice stored (0 and 16 days) striped catfish *(P. hypophthalmus)* fillets after *in vivo* supplementation with *P. guajava* and *P. amarus* ethanolic extracts.

Amino acid	Control (Day 0)	Control (Day 16)	Mix5 (Day 16)
Cysteine	0.22 ± 0.02	0.24 ± 0.02	0.22 ± 0.00
Aspartic	1.49 ± 0.02	1.54 ± 0.24	1.49 ± 0.14
Methionine*	0.56 ± 0.06	0.53 ± 0.11	0.49 ± 0.10
Threonine*	0.76 ± 0.01	0.78 ± 0.07	0.73 ± 0.01
Serine	0.71 ± 0.05	0.72 ± 0.07	0.69 ± 0.03
Glutamic acid	2.30 ± 0.04	2.34 ± 0.80	2.30 ± 0.16
Proline	0.69 ± 0.00	0.63 ± 0.07	0.67 ± 0.14
Glycine	0.81 ± 0.03	0.71 ± 0.10	0.87 ± 0.14
Alanin	0.87 ± 0.02	0.87 ± 0.04	0.86 ± 0.15
Valine*	0.80 ± 0.01	0.81 ± 0.05	0.81 ± 0.09
Isoleucine*	0.72 ± 0.00	0.73 ± 0.09	0.73 ± 0.06
Leucine*	1.21 ± 0.02	1.24 ± 0.15	1.21 ± 0.16
Tyrosine	0.58 ± 0.02	0.62 ± 0.06	0.58 ± 0.05
Phenylalanine*	0.71 ± 0.01	0.75 ± 0.01	0.71 ± 0.05
Histidine*	0.49 ± 0.01	0.49 ± 0.01	0.47 ± 0.01
Lysine*	1.37 ± 0.06	1.40 ± 0.14	1.36 ± 0.15
Tryptophan*	0.15 ± 0.00	0.13 ± 0.0	0.16 ± 0.01
Arginine	1.00 ± 0.09	0.99 ± 0.09	0.99 ± 0.13
Total	15.47	15.52	15.34

*essential amino acid

See table 1B for details about the plant extract supplementation.

Ten biogenic amines, as indicators of fish flesh spoilage, were determined in fish fillets from experimental groups of the second *in vivo* experiment at day 0 (data not shown) and after 16 days of ice storage (Table 3). At the start of the storage period (day 0), there was no biogenic amines quantified above the limit of quantification in fish flesh collected from all experimental groups, except spermidine and spermine, which were detected, at concentrations of 10.3 and 21 mg/kg, respectively (data not shown). This can be explained by the fact that spermidine and spermine are native components of living cells (Bardócz, 1995). Spermidine in fish flesh samples was observed at lower concentrations than those of spermine, which is normal for food from animal origin (Kalac and Krausová, 2005). After 16 days of the storage, putrescine was present in fish fillets from all treatment groups at levels ranging from 4.3 to 8.3 mg/kg. Cadaverine and serotonine

were also observed in fish fillets of some treatments at low levels. Tyramine was found in the control sample and surprisingly in only one treatment group at levels of 31 and 38.9 mg/kg, respectively (Table 3).

Table 3. Biogenic amines contents (mg/kg fresh weight) of 16 days ice stored striped catfish *(P. hypophthalmus)* fillets after *in vivo* supplementation with *P. guajava* and *P. amarus* ethanolic extracts.

	Putrescine	Cadaverine	Histamine	Serotonine	Tyramine	Spermidine	Spermine
Control	8.3	<LOQ	<LOQ	<LOQ	31.0	7.0	20.9
Psi0.8	5.6	<LOQ	<LOQ	2.9	38.9	5.8	15.5
Psi2	6.0	1.4	<LOQ	<LOQ	<LOQ	6.5	20.6
Psi5	4.4	<LOQ	<LOQ	2.6	<LOQ	6.8	19.6
Phy0.8	6.3	1.6	<LOQ	<LOQ	<LOQ	8.1	17.3
Phy2	6.7	1.6	<LOQ	<LOQ	<LOQ	7.5	20.1
Phy5	4.5	<LOQ	<LOQ	<LOQ	<LOQ	6.6	17.8
Mix0.8	4.3	<LOQ	<LOQ	<LOQ	<LOQ	4.3	19.4
Mix2	5.9	<LOQ	<LOQ	<LOQ	<LOQ	7.2	16.5
Mix5	6.3	1.9	<LOQ	10.6	<LOQ	6.6	12.2
LOD	1.1	0.7	5.2	1.2	1.8	0.65	1.25
LOQ	2.2	1.4	10.4	2.4	3.6	1.3	2.5

<LOQ: Lower than limit of quantification
See table 1B for details about the plant extract supplementation.

Among the ten biogenic amines measured in this study, histamine is the most concerning one, because of its acute adverse health effects such as low blood pressure and headache, diarrhea typical allergic reactions such as skin reddening and edema (EFSA Panel on Biological Hazards, 2011, Marissiaux et al., 2018; Visciano et al., 2020). Histamine levels determined from fish flesh of all treatment groups were below the limit of quantification (<10 mg/kg fresh weight), in agreement with the results of a survey showing low levels of histamine in this fish species (Tao et al., 2011), as histamine is known to be mostly produced in scombroid fish species, which are rich in histidine, the histamine precursor (Houicher et al., 2021). Our results are in agreement with those of Guimarães et al. (2016) presenting that histamine was not detected in striped catfish flesh or much lower than the histamine limit established by the European legislation (100 mg/kg) (EC, 2005) and Food and Drug Administration (50 mg/kg) (FDA, 1995). According to Baixas-Nogueras et al. (2001) and Rezaei et al. (2007), no reference was found about the association between

histamine intoxication and consumption of histidine-poor fish. The use of histamine as an indicator for evaluating the quality of histidine-poor fish seems thus inappropriate (Prester, 2011), while putrescine, cadaverine, spermidine and spermine seem more relevant, in particular for freshwater fish (Rodrigues et al., 2013). However, there are no maximal limits for putrescine, cadaverine or other biogenic amines, but several recommendations have been proposed, such as for instance, the limit proposed by the Food and Drug Administration for tyramine was 100 – 800 mg/ kg (FDA, 2001). Du et al. (2002) also proposed a biogenic amine index (sum of concentrations of tyramine, histamine, putrescine and cadaverine) which, when above 90 mg/kg, indicates an advanced decomposition of fish. The biogenic amine index was well below this threshold in all samples of Table 3.

4. Conclusion

In conclusion, our results pointed out the relevance of plant extracts supplementation of juvenile striped catfish to prolong shelf life of fish fillet under ice storage. Both *P. guajava* and *P. amarus* extracts used individually or in mixture, as well as *E. hirta,* were effective to decrease TVC and fatty acid oxidation parameters and increase sensory quality, in fish fillets during ice storage, compared to the control. No improvement was noted when using a mixture of both plant extracts compared to individual treatments. Among all tested treatment, fish supplementation with 5 g/kg feed of *P. guajava* appeared to be the best choice.

Acknowledgments

The authors are grateful to the Académie de Recherche et d'Enseignement supérieur - Commission de la Coopération au Développement (ARES-CCD) for financial support through AquaBioActive (Natural bio-active plant products for environmental friendly aquaculture production in the Mekong Delta) project.

References

Abdel-Tawwab, M., I. Adeshina, A. Jenyo-Oni, E.K. Ajani, and B.O. Emikpe, 2018. Growth, physiological, antioxidants, and immune response of African catfish, *Clarias gariepinus* (B.), to dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to *Listeria monocytogenes* infection. Fish & shellfish immunology. 78, 346-354.

Álvarez, A., B.G. García, M. Jordán, C. Martínez-Conesa, and M. Hernández, 2012. The effect of diets supplemented with thyme essential oils and rosemary extract on the deterioration of farmed gilthead seabream (*Sparus aurata*) during storage on ice. Food Chemistry. 132, 1395-1405.

AOAC, 2016. Official methods of Analysis of AOAC International. 20th ed. Maryland, USA: Association of Official Agricultural Chemists.

Arsyad, M.A., T. Akazawa, C. Nozaki, M. Yoshida, K. Oyama, T. Mukai, and M. Ogawa, 2018. Effects of olive leaf powder supplemented to fish feed on muscle protein of red sea bream. Fish physiology and biochemistry. 44, 1299-1308.

Ashraf, A., R.A. Sarfraz, M.A. Rashid, A. Mahmood, M. Shahid, and N. Noor, 2016. Chemical composition, antioxidant, antitumor, anticancer and cytotoxic effects of *Psidium guajava* leaf extracts. Pharmaceutical biology. 54, 1971-1981.

Bach, L.T., L.T. Dung, N.T. Tuan, N.T. Phuong, P. Kestemont, J. Quetin-Leclercq, and B.T.B. Hue, 2018. Protective effect of pancreatic β-cells MIN6 by some medicinal plants in the Mekong Delta. Vietnam Journal of Chemistry. 56, 637-641.

Baixas-Nogueras, S., S. Bover-Cid, M. Vidal-Carou, and M. Veciana-Nogues, 2001. Volatile and nonvolatile amines in Mediterranean hake as function of their storage temperature. Journal of food science. 66, 83-88.

Bao, H., 2006. QIM Method Scores Quality, Shelf Life of Pangasius Fillets. Global Aquaculture Advocate. 28-30.

Bao, H.N., Y. Shinomiya, H. Ikeda, and T. Ohshima, 2009. Preventing discoloration and lipid oxidation in dark muscle of yellowtail by feeding an extract prepared from mushroom (*Flammulina velutipes*) cultured medium. Aquaculture. 295, 243-249.

Bardócz, S., 1995. Polyamines in food and their consequences for food quality and human health. Trends in Food Science & Technology. 6, 341-346.

Baron, C.P., G.H. Svendsen, I. Lund, A. Jokumsen, H.H. Nielsen, and C. Jacobsen, 2013. Organic plant ingredients in the diet of Rainbow trout (*Oncorhynchus mykiss*): Impact on fish muscle composition and oxidative stability. European Journal of Lipid Science and Technology. 115, 1367-1377.

Bulfon, C., D. Volpatti, and M. Galeotti, 2015. Current research on the use of plant-derived products in farmed fish. Aquaculture Research. 46, 513-551.

Chaijan, M., S. Benjakul, W. Visessanguan, and C. Faustman, 2006. Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. Food chemistry. 99, 83-91.

Chang, K.L.B., J. Chang, C.-Y. Shiau, and B.S. Pan, 1998. Biochemical, microbiological, and sensory changes of sea bass (*Lateolabrax japonicus*) under partial freezing and refrigerated storage. Journal of Agricultural and Food Chemistry. 46, 682-686.

Crespy, V. and G. Williamson, 2004. A review of the health effects of green tea catechins in in vivo animal models. The Journal of nutrition. 134, 3431S-3440S.

Danuwat, P., P. Rimruthai, C. Phattanawan, and D. Peerarat, 2016. Determination of essential amino acids in *Pangasius bocourti.* Journal of Food Processing and Technology. 7,

Dhiman, A., A. Nanda, S. Ahmad, and B. Narasimhan, 2011. *In vitro* antimicrobial activity of methanolic leaf extract of *Psidium guajava* L. Journal of Pharmacy and Bioallied Sciences. 3, 226.

Douny, C., S. Benmedjadi, F. Brose, O.H.I. Afé, A. Igout, D.J. Hounhouigan, V.B. Anihouvi, and M.-L. Scippo, 2019. Development of an analytical method for the simultaneous measurement of 10 biogenic amines in meat: application to Beninese grilled pork samples. Food Analytical Methods. 12, 2392-2400.

Douny, C., R. El Khoury, J. Delmelle, F. Brose, G. Degand, N. Moula, F. Farnir, A. Clinquart, G. Maghuin-Rogister, and M.L. Scippo, 2015. Effect of storage and cooking on the fatty acid profile of omega-3 enriched eggs and pork meat marketed in Belgium. Food science & nutrition. 3, 140-152.

Du, W.X., C.M. Lin, A.T. Phu, J. Cornell, M. Marshall, and C.I. Wei, 2002. Development of biogenic amines in yellowfin tuna (*Thunnus albacares*): effect of storage and correlation with decarboxylase-positive bacterial flora. Journal of Food Science. 67, 292-301.

EC, 2005. European Commission, Commission Regulation 2073/2005/EC of 15th November 2005 on microbiological criteria for foodstuffs. Official Journal. L 338, 1-26.

EFSA Panel on Biological Hazards, 2011. Scientific opinion on risk based control of biogenic amine formation in fermented foods. Efsa Journal. 9, 2393.

Farahi, A., M. Kasiri, M. Sudagar, M.S. Iraei, and M.D. Shahkolaei, 2010. Effect of garlic (*Allium sativum*) on growth factors, some hematological parameters and body compositions in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, Aquarium, Conservation & Legislation. 3, 317-323.

Farahi, A., M. Kasiri, M. Sudagar, M. Soleimani Iraei, and S. Zorriehzahra, 2012. Effect of dietary supplementation of *Melissa officinalis* and *Aloe vera* on hematological traits, lipid oxidation of carcass and performance in rainbow trout (*Oncorhynchus mykiss*). Online journal of animal and feed research. 1, 1-5.

FDA, 1995. US Food and Drug Administration, Decomposition and histamine—raw, frozen tuna and mahi-mahi, canned tuna, and related species; revised compliance guide; availability. Federal Register. 149, 39754-39756.

FDA, 2001. US Food and Drug Administration, Scombrotoxin (histamine) formation, in Fish and fishery products hazards and controls guide. Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood: Washington, DC. p. 73-93.

Gatlin, D.M., 2007. Dietary supplements for the health and quality of cultured fish, ed. H. Nakagawa, M. Sato, and D.M. Gatlin. Oxon: CABI Publishing. 244.

Giménez, B., P. Roncalés, and J.A. Beltrán, 2004. The effects of natural antioxidants and lighting conditions on the quality characteristics of gilt-head sea bream fillets (*Sparus aurata*) packaged in a modified atmosphere. Journal of the Science of Food and Agriculture. 84, 1053-1060.

Gobi, N., C. Ramya, B. Vaseeharan, B. Malaikozhundan, S. Vijayakumar, K. Murugan, and G. Benelli, 2016. *Oreochromis mossambicus* diet supplementation with *Psidium guajava* leaf extracts enhance growth, immune, antioxidant response and resistance to *Aeromonas hydrophila.* Fish & Shellfish Immunology. 58, 572-583.

Guimarães, C.F.M., E.T. Máralém, M.L.G. Monteiro, M. Lemos, S.B. Mano, and C.A. Conte Junior, 2016. The chemical quality of frozen Vietnamese *Pangasius hypophthalmus* fillets. Food science & nutrition. 4, 398-408.

Harikrishnan, R., C. Balasundaram, and M.-S. Heo, 2011. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture. 317, 1-15.

Hernández, A., B.G. García, M. Jordán, and M. Hernández, 2014. Natural antioxidants in extruded fish feed: Protection at different storage temperatures. Animal Feed Science and Technology. 195, 112-119.

Houicher, A., A. Bensid, J.M. Regenstein, and F. Özogul, 2021. Control of biogenic amine production and bacterial growth in fish and seafood products using phytochemicals as biopreservatives: A review. Food Bioscience. 39, 100807.

Huss, H.H., 1995. Quality and quality changes in fresh fish. Vol. 348. FAO Rome.

Ilangkovan, M., I. Jantan, M.A. Mesaik, and S.N.A. Bukhari, 2015. Immunosuppressive effects of the standardized extract of *Phyllanthus amarus* on cellular immune responses in Wistar-Kyoto rats. Drug design, development and therapy. 9, 4917.

International IDF standards, 1991. Section 74A: 1991. International Dairy Federation, IDF-square Vergot 41.

Kalac, P. and P. Krausová, 2005. A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods. Food Chemistry. 90, 219-230.

Khalili Tilami, S. and S. Sampels, 2018. Nutritional value of fish: lipids, proteins, vitamins, and minerals. Reviews in Fisheries Science & Aquaculture. 26, 243-253.

Kirubakaran, C.J.W., C.P. Alexander, and R.D. Michael, 2010. Enhancement of non-specific immune responses and disease resistance on oral administration of *Nyctanthes arbortristis* seed extract in *Oreochromis mossambicus* (Peters). Aquaculture Research. 41, 1630-1639.

Lee, D.-H., S.-R. Lim, C.-S. Ra, and J.-D. Kim, 2012. Effects of dietary garlic extracts on whole body amino acid and fatty acid composition, muscle free amino acid profiles and blood plasma changes in juvenile sterlet sturgeon, *Acipenser ruthenus*. Asian-Australasian journal of animal sciences. 25, 1419.

Liu, R. and S.A. Mabury, 2020. Synthetic phenolic antioxidants: A review of environmental occurrence, fate, human exposure, and toxicity. Environmental science & technology. 54, 11706-11719.

Lone, G. and H. Hans, 1996. Microbiological spoilage of fish and fish products. Int. J. Fd. Microbiol. 33, 121-137.

Marissiaux, L., M.-L. Scippo, G. Daube, N. Botteldoorn, S. Gobert, A. Ghuysen, S. Denayer, and B. Scholtes, 2018. An awkward fishing expedition. Acta Anaesthesiologica Belgica. 69,

Mohebbi, A., A. Nematollahi, E.E. Dorcheh, and F.G. Asad, 2012. Influence of dietary garlic (*Allium sativ*um) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research. 43, 1184-1193.

Na-Phatthalung, P., M. Teles, S.P. Voravuthikunchai, L. Tort, and C. Fierro-Castro, 2018. Immunomodulatory effects of *Rhodomyrtus tomentosa* leaf extract and its derivative compound, rhodomyrtone, on head kidney macrophages of rainbow trout (*Oncorhynchus mykiss*). Fish physiology and biochemistry. 44, 543-555.

Nguyen, L.A.D., M.P. Tran, C. Douny, J. Quetin-Leclercq, T.B.H. Bui, T.B. Le, Q.N. Truong, T.B.H. Bui, T.T.H. Do, T.P. Nguyen, P. Kestemont, and M.-L. Scippo, 2020. Screening and comparative study of *in vitro* antioxidant and antimicrobial activities of ethanolic extracts of selected Vietnamese plants. International Journal of Food Properties. 23, 481-496.

Nhu, T.Q., B.T. Bich Hang, V. Cornet, M. Oger, L.T. Bach, N.L. Anh Dao, D.T. Thanh Huong, J. Quetin-Leclercq, M.-L. Scippo, and N.T. Phuong, 2020a. Single or combined dietary supply of *Psidium guajava* and *Phyllanthus amarus* extracts differentially modulate immune responses and liver proteome in striped catfish (*Pangasianodon hyphophthalmus*). Frontiers in Immunology. 11, 797.

Nhu, T.Q., N.P. Dam, B.T.B. Hang, B.T.B. Hue, M.-L. Scippo, N.T. Phuong, J. Quetin-Leclercq, and P. Kestemont, 2020b. Immunomodulatory potential of extracts, fractions and pure compounds from *Phyllanthus amarus* and *Psidium guajava* on striped catfish (*Pangasianodon hypophthalmus*) head kidney leukocytes. Fish & Shellfish Immunology.

Nhu, T.Q., B.T.B. Hang, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019a. Plant extract-based diets differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*). Fish & shellfish immunology. 92, 913-924.

Nhu, T.Q., B.T.B. Hang, A. Vinikas, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019b. Screening of immuno-modulatory potential of different herbal plant extracts using striped catfish (*Pangasianodon hypophthalmus*) leukocyte-based *in vitro* tests. Fish & shellfish immunology. 93, 296-307.

Ortega-Ramirez, L.A., I. Rodriguez-Garcia, J.M. Leyva, M.R. Cruz-Valenzuela, B.A. Silva-Espinoza, G.A. Gonzalez-Aguilar, M.W. Siddiqui, and J.F. Ayala-Zavala, 2014. Potential of medicinal plants as antimicrobial and antioxidant agents in food industry: a hypothesis. Journal of food science. 79, R129-R137.

Patel, J.R., P. Tripathi, V. Sharma, N.S. Chauhan, and V.K. Dixit, 2011. *Phyllanthus amarus*: ethnomedicinal uses, phytochemistry and pharmacology: a review. Journal of Ethnopharmacology. 138, 286-313.

Paul, A., M. Frederich, R. Uyttenbroeck, P. Malik, S. Filocco, A. Richel, S. Heuskin, T. Alabi, R.C. Megido, and T. Franck, 2016. Nutritional composition and rearing potential of the meadow grasshopper (*Chorthippus parallelus* Zetterstedt). Journal of Asia-Pacific Entomology. 19, 1111-1116.

Phuong, N.T. and D.T.H. Oanh, 2010. Striped catfish aquaculture in Vietnam: a decade of unprecedented development, in Success stories in Asian aquaculture. Springer. p. 131-147.

Pinto, T., A. Aires, F. Cosme, E. Bacelar, M.C. Morais, I. Oliveira, J. Ferreira-Cardoso, R. Anjos, A. Vilela, and B. Gonçalves, 2021. Bioactive (Poly) phenols, Volatile Compounds from Vegetables, Medicinal and Aromatic Plants. Foods. 10, 106.

Prester, L., 2011. Biogenic amines in fish, fish products and shellfish: a review. Food Additives & Contaminants: Part A. 28, 1547-1560.

Pu, H., X. Li, Q. Du, H. Cui, and Y. Xu, 2017. Research progress in the application of Chinese herbal medicines in aquaculture: a review. Engineering. 3, 731-737.

Raharjo, S., J.N. Sofos, and G.R. Schmidt, 1992. Improved speed, specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C18 method for measuring lipid peroxidation in beef. Journal of Agricultural and Food Chemistry. 40, 2182-2185.

Rajeshkumar, N., K. Joy, G. Kuttan, R. Ramsewak, M.G. Nair, and R. Kuttan, 2002. Antitumour and anticarcinogenic activity of *Phyllanthus amarus* extract. Journal of Ethnopharmacology. 81, 17-22.

Reverter, M., N. Bontemps, D. Lecchini, B. Banaigs, and P. Sasal, 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture. 433, 50-61.

Rezaei, M., N. Montazeri, H.E. Langrudi, B. Mokhayer, M. Parviz, and A. Nazarinia, 2007. The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. Food Chemistry. 103, 150-154.

Rodrigues, B.L., T.S. Alvares, M.d. Costa, G.S.L. Sampaio, C. Lázaro De La Torre, E.T. Mársico, and C.A. Conte Júnior, 2013. Concentration of biogenic amines in rainbow trout (*Oncorhynchus mykiss*) preserved in ice and its relationship with physicochemical parameters of quality. Journal of Aquaculture Research & Development. 4, 174-177.

Rota, M.C., A. Herrera, R.M. Martínez, J.A. Sotomayor, and M.J. Jordán, 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food control. 19, 681-687.

Saeed, N., M.R. Khan, and M. Shabbir, 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary and alternative medicine. 12, 1-12.

Simamora, A., L. Paramita, N. Azreen, A.W. Santoso, and K.H. Timotius, 2018. *In vitro* Antidiabetic and Antioxidant Activities of Aqueous Extract from the Leaf and Fruit of *Psidium guajava* L. The Indonesian Biomedical Journal. 10, 156-64.

Simeonidou, S., A. Govaris, and K. Vareltzis, 1997. Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and Mediterranean hake (*Merluccius mediterraneus*). Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 204, 405-410.

Škrovánková, S., L. Mišurcová, and L. Machů, 2012. Antioxidant activity and protecting health effects of common medicinal plants. Advances in food and nutrition research. 67, 75-139.

Sveinsdottir, K., G. Hyldig, E. Martinsdottir, B. Jørgensen, and K. Kristbergsson, 2003. Quality Index Method (QIM) scheme developed for farmed Atlantic salmon (*Salmo salar*). Food Quality and Preference. 14, 237-245.

Tanimoto, S.-y., K. Koike, and S. Takahashi, 1993. Improvement in broiled meat texture of cultured eel by feeding of tochu leaf powder. Bioscience, biotechnology, and biochemistry. 57, 325-327.

Tao, Z., M. Sato, H. Zhang, T. Yamaguchi, and T. Nakano, 2011. A survey of histamine content in seafood sold in markets of nine countries. Food Control. 22, 430-432.

Thiansilakul, Y., S. Benjakul, and M.P. Richards, 2010. Changes in heme proteins and lipids associated with off-odour of seabass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus*× *O. niloticus*) during iced storage. Food Chemistry. 121, 1109-1119.

VASEP, 2021. Production of raw pangasius in H1. Vietnam Association of Seafood Exporters and Producers Available from: http://seafood.vasep.com.vn/key-seafood-sectors/pangasius/news/production-of-raw-pangasius-in-h1-16369.html.

Vietnam Ministry of Public Health, 2012. National technical regulation of Microbiological contaminants in food (QCVN 8-3:2012/BYT). Available from: http://www.fsi.org.vn/pic/files/qcvn-8-3_2011-byt-ve-o-nhiem-vi-sinh-vattrong-tp_bia_merged.pdf.

Visciano, P., M. Schirone, and A. Paparella, 2020. An overview of histamine and other biogenic amines in fish and fish products. Foods. 9, 1795.

Zhu, F., 2020. A review on the application of herbal medicines in the disease control of aquatic animals. Aquaculture. 526, 735422.

Table S1. Proximate composition of striped catfish fillets from the first (A) and second (B) *in vivo* experiment, before ice storage

A

Treatments	Proximate composition (%)			
	Moisture	Ash	Lipid	Protein
Control	82.4 ± 0.6	1.3 ± 0.3	1.5 ± 0.4	14.5 ± 0.2
Eup4	81.3 ± 1.2	1.1 ± 0.1	1.3 ± 0.4	14.9 ± 0.8
Eup20	82.9 ± 1.1	1.1 ± 0.0	0.7 ± 0.1	14.7 ± 1.2
Phy2	82.4 ± 1.1	1.0 ± 0.1	1.1 ± 0.1	14.3 ± 1.2
Phy10	81.0 ± 0.3	1.6 ± 0.5	1.4 ± 0.5	15.3 ± 0.7
Psi2	80.7 ± 0.2	1.3 ± 0.3	1.1 ± 0.3	15.4 ± 0.6
Psi10	81.0 ± 0.3	1.1± 0.0	1.1 ± 0.2	15.4 ± 0.4
Mim4	81.7 ± 0.2	1.3 ± 0.3	1.2 ± 0.2	15.2 ± 0.5
Mim20	81.4 ± 0.4	1.1 ± 0.1	1.4 ± 0.2	14.9 ± 0.8
Aza4	81.0 ± 0.7	1.4 ± 0.9	1.3 ± 0.5	15.8 ± 0.9
Aza20	83.0 ± 0.1	0.8 ± 0.4	0.2 ± 0.1	14.8 ± 0.7

B

Treatments	Proximate composition (%)			
	Moisture	Ash	Lipid	Protein
Control	81.7 ± 0.5	1.1 ± 0.0	1.2 ± 0.1	15.2 ± 0.8
Psi0.8	82.0 ± 0.3	1.1 ± 0.0	0.8 ± 0.4	15.3 ± 0.5
Psi2	82.0 ± 0.4	1.1 ± 0.0	0.8 ± 0.4	15.5 ± 0.3
Psi5	82.5 ± 0.5	1.1 ± 0.0	0.8 ± 0.2	14.9 ± 0.9
Phy0.8	81.7 ± 0.2	1.1 ± 0.0	1.4 ± 0.1	15.2 ± 0.4
Phy2	82.5 ± 0.7	1.1 ± 0.0	1.2 ± 0.2	14.0 ± 0.3
Phy5	82.5 ± 0.7	1.1 ± 0.0	1.4 ± 0.5	14.9 ± 0.2
Mix0.8	82.6 ± 0.4	1.1 ± 0.0	1.1 ± 0.5	14.6 ± 0.1
Mix2	82.8 ± 0.2	1.1 ± 0.0	1.0 ± 0.1	14.3 ± 0.1
Mix5	83.1 ± 0.7	1.0 ± 0.1	1.4 ± 0.4	14.2 ± 0.7

Values are mean ± standard deviation (n= 3).

See tables 1A and 1B for details about the various plant extracts supplementation conditions.

Table S2. Fatty acid profile of striped catfish flesh collected from the second *in vivo* experiment at day 0 and day 16 of ice storage (% of total fatty acids)

	Day 0	Day 16									
	Control	**Control**	**Psi0.8**	**Psi2**	**Psi5**	**Phy0.8**	**Phy2**	**Phy5**	**Mix0.8**	**Mix2**	**Mix5**
Saturated Fatty Acid											
Capric acid, C10:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lauric acid, C12:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Tridecyclic acid, C13:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Myristic acid, C14:0	2.8	2.31	3.1	3.2	3.2	3.9	3.50	3.8	3.80	3.8	3.4
Palmitic acid, C16:0	25.9	29.48	27.8	27.1	26.4	29.3	28.38	27.3	29.57	28.2	27.4
Heptadecanoic acid, C17:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Stearic acid, C18:0	8.8	12.99	8.2	9.0	8.8	8.3	9.47	9.1	7.99	9.0	9.2
Arachidic acid, C20:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Docosanoic acid, C22:0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Lignoceric acid, C24:0	<LOQ	0.77	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
MUFA											
Palmitoleic acid, C16:1, ω7	1.0	0.90	1.0	1.1	1.1	1.1	1.02	1.1	1.00	1.0	0.9
Heptadecenoic acid, C17:1, ω7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Elaidic acid C18:1,9t, ω9	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Oleic acid C18:1,9c, ω9	30.4	29.16	36.0	31.9	32.3	33.2	31.27	33.7	33.39	34.1	32.1
ω6 PUFA											
Linoleic acid, C18:2,9c,12c, ω6	16.6	13.29	15.7	15.2	15.3	14.6	13.91	13.0	15.46	15.2	15.4
Linolelaidic acid, C18:2,9t,12t, ω6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Rumenic acid, C18:2,9c,11t, ω6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Isolinoleic acid, C18:2,9t,11t, ω6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Gamma Linolenic acid, C18:3, ω6	0.8	<LOQ	0.6	0.6	0.7	0.7	0.64	0.8	0.60	0.6	0.6
Eicosadienoic acid, C20:2, ω6	1.0	0.88	0.7	0.9	0.9	0.7	0.88	0.8	0.75	0.8	0.8
Arachidonic acid, C20:4, ω6	4.3	3.65	1.9	2.9	3.4	2.2	3.34	2.8	2.30	2.2	3.4
ω3 PUFA											

alpha Linolenic acid, C18:3, ω3	1.2	0.98	1.1	1.3	1.2	1.1	1.02	1.3	0.95	1.1	1.0
Octadecatetraenoic acid C18:4, ω3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
di-homo-γ-Linolenic acid, C20:3, ω3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Eicosapentaenoic acid (EPA), C20:5, ω3	0.9	0.98	0.6	0.9	1.0	0.7	0.91	1.0	0.58	0.6	0.9
Docosapentaenoic acid (DPA), C22:5, ω3	2.4	1.69	1.4	2.3	2.1	1.5	2.14	2.0	1.44	1.4	1.8
Docosahexaenoic acid (DHA), C22:6, ω3	4.1	2.93	1.9	3.4	3.5	2.6	3.51	3.3	2.17	1.9	3.1
Saturated FA (% total FA)	37.6	45.6	39.1	39.3	38.4	41.6	41.4	40.2	41.4	41.0	40.0
MUFA (% total FA)	31.3	30.1	37.0	33.1	33.4	34.3	32.3	34.8	34.4	35.1	33.0
PUFA (% total FA)	31.1	24.4	24.0	27.6	28.2	24.1	26.4	25.0	24.3	23.8	27.0
PUFA/saturated	0.83	0.54	0.61	0.70	0.73	0.58	0.64	0.62	0.59	0.58	0.68
ω6 (% total FA)	22.6	17.8	18.9	19.7	20.3	18.3	18.8	17.4	19.1	18.8	20.2
ω3 (% total FA)	8.5	6.6	5.0	7.9	7.9	5.8	7.6	7.6	5.1	5.0	6.8
ω6/ω3	2.7	2.7	3.8	2.5	2.6	3.1	2.5	2.3	3.7	3.7	3.0

<LOQ : Lower than limit of quantification; LOD and LOQ are 0.05 and 0.1% of total fatty acids, respectively

MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

See tables 1A and 1B for details about the various plant extracts supplementation conditions.

7. DISCUSSION, CONCLUSIONS AND PERSPECTIVES

7.1. DISCUSSION

7.1.1. Use of drugs, chemicals, plants and plant extract products in grow-out farms of striped catfish in the Mekong Delta, Vietnam

The common occurrence of bacterial diseases (1 to 12 episodes per crop) was reported in the investigation of 60 striped catfish grow-out farms in An Giang and Dong Thap provinces (the Mekong Delta, Vietnam) (Chapter 2). Despite applying feeding immunostimulant products and feed additives as health enhancing methods, farmers have to use antimicrobials for disease treatment. Pale gill and liver syndrome, parasite infection as well as yellow fillet syndrome have been arisen habitually. Therefore, fish health management practices in striped catfish farming need to be addressed by different stakeholders which contributes to the sustainable development of this value chain.

Twelve types of antibiotics (used individually or as mixtures of two active substances) were applied in striped catfish farming. Specifically, enrofloxacin and ciprofloxacin, which are banned antibiotics according to Vietnamese authority regulations, were found on the list of antibiotics use in the survey. According to an analysis of notifications reported in the European Rapid Alert System for Feed and Food (RASFF) over the years 2001-2021, several antibiotic residues were found in striped catfish products originating from Vietnam, including chloramphenicol, ciprofloxacin, enrofloxacin, doxycycline, enrofloxacin, ofloxacin and quinolones (Guardone et al. 2022). In some cases, the antibiotic application is so close to the harvest period that the withdrawal time is not respected leading to the presence of antibiotic residues in fish tissues. Thinh and Phu (2021) reported that among the antibiotic used in the striped catfish farming in the Mekong Delta, 12 types of antibiotics were allowed based on the regulations of the Vietnamese Ministry of Agriculture and Rural Development and 2 were banned (enrofloxacin and ciprofloxacin). The most used antibiotics were amoxicillin, followed by doxycycline and sulfamethoxazole + trimethoprim in striped catfish farming in the Mekong Delta region (Thinh and Phu, 2021). Many types of chemicals were repeatedly used to disinfect water (Iodine and Benzalkonium chloride), control ecto-parasites (copper sulfate and chlorine) and improve water quality, without considering updated practices. Furthermore, most of farmers used

probiotics and other nutritious supplementation products (such as vitamins and minerals) even though their efficiency was unreliable.

Regarding the use of plants and plant extract products, farmers used various commercial products (*Cleome chelidonii*, *Combretum quadrangulare* and *Allium sativum*) for different types of targets, although the quality and the effectiveness of these products were doubtful. In addition, the conventional remedies based on medicinal plants for human were empirically used whether farmers know about the applied doses or not. Additionally, no validation and certification of the quality of the plant extract products were available, in terms of qualitative and quantitative composition. Thus, it would be recommended to an in-depth study of the efficacy of the use of plants as on-farm therapy.

As mentioned in Section 3, this study reported the high *in vitro* antioxidant activity of extracts from *P. amarus, P. betle*, *P. guajava*, *E. hirta* and *M. pudica*, whereas *P. amarus* extract showed the highest antibacterial capacity against two different strains of *Aeromonas hydrophila*. Nhu et al. (2019a) reported that plant extract supplemented diets modulate differently immune responses and resistance to microbial infection in striped catfish. Earlier studies showed the ability of supplementation with ginger (*Zingiber officinale*) (Sukumaran et al., 2016), garlic (*Allium sativum*) (Ghehdarijani et al., 2016) and date palm fruit (*Phoenix dactylifera*) (Hoseinifar et al., 2015) to promote skin mucosal immune responses in rohu (*Labeo rohita*), Caspian roach (*Rutilus rutilus*), and common carp (*Cyprinus carpio*), respectively.

To date, the trend of using plant extracts is more spread out as it reduces treatment costs and is an environmentally friendly solution. Also, plant extracts, characterized by the high diversity of their molecule metabolite, tend to be more decomposable than synthetic molecules and show a lower risk of drug resistance in parasites (Reverter et al., 2014).

7.1.2. Antioxidant and antimicrobial activities of plant extracts in Vietnam

In the context of human concerns towards synthetic compounds, a comprehensive view of natural alternative from plant extracts will bring acceptability based on consumers' prism and regulations. Ethanolic extracts from 20 regionally available plants and 3 commercial products used by fish farmers in the Mekong Delta (Vietnam) were assessed for their *in vitro* antioxidant and antimicrobial activities (against *A. hydrophila* and *E. ictaluri*). In the assay of DPPH free radical scavenging, *P. amarus* extract possessed the highest antioxidant activity, followed by, in

descending order, *P. betle*, *P. guajava*, *E. hirta*, and *M. pudica*. A positive association between total phenolic content and antioxidant activity of the 23 extracts was shown. However, among 5 plant extracts with high antioxidant activity, *P. amarus* extract also showed high antibacterial activity against two different strains of *A. hydrophila*; whereas, *P. betle* displayed moderate activity against *E. ictaluri*. Antioxidant activity is associated with the presence of phenolic compounds, which may be present in larger quantities in leaves to defend their tissues and protect the plant against the stress from solar exposure (Veiga et al., 2020). As possessing aromatic or phenolic rings, phenolic compounds are capable of donating hydrogen atoms to free radicals formed during oxidation. These radical intermediates are regulated by the resonance delocalization of the electron on the aromatic ring and generate quinone structures (Nawar, 1996). Meanwhile, the mode of action for antimicrobial activity of plant extracts is different according to the group of microorganisms (Brillet et al., 2004). The bacteria can be mainly killed by physical disruption of their membrane, by dissipation of their proton motive force, or by the inhibition of membrane-associated enzymes (Gyawali et al., 2015).

In our study, plant extracts displaying high antioxidant activity were in accordance with previous publications (Almalki, 2016; Asha et al., 2016; Ashraf et al., 2016; Jayalakshmi et al., 2015; Nguyen et al., 2016). However, several plant extracts such as garlic (*Allium sativum*), neem (*Azadirachta indica*), Asiatic pennywort (*Centella asiatica*), ginger (*Zingiber officinale*) did not show antioxidant properties, although earlier studies mentioned potential antioxidant activity of these plants when tested in DPPH radical scavenging assay (Chekki et al., 2014; Mošovská et al., 2015; Narendhirakannan et al., 2012; Rahman et al., 2012). Indeed, the bioactive compounds content of the plant extract responsible for the antioxidant capacity is influenced by several factors, such as, for instances, plant physiological stage, genetic background, pedoclimatic conditions and geographical origin, without forgetting the extraction methods (Blasi et al., 2016; Lombardi et al., 2017; Rocchetti et al., 2020).

Interestingly, no significant antibacterial activity was found for the five plant extracts (*P. amarus, P. betle*, *P. guajava*, *E. hirta*, and *M. pudica*) displaying the highest antioxidant activity. Most of the bioactive compounds displaying an antimicrobial activity act by modifying the permeability and the integrity of the bacterial cell. It is extensively ascertained in the literature that the presence of the periplasmic membrane in gram-negative bacteria made them more tolerant to

plant bioactive compounds than gram-positive ones (Fernández-Pérez, 2018). Moreover, the low antibacterial activity of plant extracts reported in this work could be explained by the envelope structure (Rocchetti et al., 2020) of the examined gram-negative bacteria *A. hydrophila* and *E. ictaluri*.

Another noticeable point in our work is the decrease of antioxidant and antimicrobial activities after removing tannins from *P. amarus* and *E. hirta* extracts. Tannins were shown to mostly contribute to the antioxidant and antimicrobial activities of *P. amarus* and *E. hirta* extracts. The basic mode of action in antioxidant activity of tannins is free radical scavenging activity, chelation of transition metals and prevention of prooxidative enzymes (Koleckar et al., 2008). Chung et al. (1998) and Akiyama et al. (2001) reported that the antibacterial mechanism of tannins was the incorporation of several factors including (i) the binding and precipitation with enzymes and proteins, (ii) the influence on microorganic membranes and (iii) the formation of metal ions complexes, for example, the complexation of tannins and vital iron could render iron unavailable for the microorganisms. Besides our findings that tannins are responsible for the inhibition of *A. hydrophila*, other studies reported the action of tannins against numerous microbial strains, i.e. *Salmonella*, *Staphylococcus aureus*, *Helicobacter*, *Escherichia coli*, *Clostridium*, *Campylobacter*, *Bacillus*, *Proteous bulgaris, Candida parapsilosis*, *Staphylococcus aureus* and *Streptococcus mutans* (Daglia, 2012; Doss et al., 2009).

7.1.3. Effect of plant extracts on the shelf-life of striped catfish fillets under ice storage

One possible application of plant extracts to increase the shelf life of fish products during storage is using an immersion technique. In our study, *P. amarus* and *E. hirta* extracts were assessed for their potential application in improving ice storage of striped catfish fillets. From the organoleptic viewpoint, treatments of striped catfish fillets by immersion in water containing *P. amarus* or *E. hirta* extracts did not negatively impact their texture, compared to the control group. According to the sensory parameters of fish fillets, a dip treatment in a solution containing 0.04% (w/v) *P. amarus* extract or 0.06% (w/v) *E. hirta* extract could allow maintaining the shelf life of striped catfish fillets up to 8 days. Furthermore, these treatments remarkably decreased the primary lipid oxidation in fish samples.

Besides protection against lipid oxidation and bacterial contamination, consumers pay attention to the impact of plant extracts dip treatments on the organoleptic characteristic of fish fillets. When dipping solutions contain a large amount of plant extracts, a strong flavor or an unpleasant color can appear, which are not well appreciated by the consumers, and lead to restrictions on plant extracts applications in foods (Kim et al., 2013), although those concentrations showed the capacity to inhibit lipid oxidation and microbial growth. Thus, it is important to decide on an adequate concentration of plant extract that can ensure a mild flavor as well as an acceptable color for the consumers. In the study of Tran et al. (2021), the dip treatment in water containing *P. guajava* leaf ethanolic extract at the concentration of 0.03% (w/v) did not affect the color of cobia (*Rachycentron canadum*) fillets during ice storage whereas, the presence of *P. guajava* flavor could improve the sensory properties of fish fillets.

Despite various benefits, plants may contain toxic compounds that be harmful to humans. Therefore, it is essential to control possible toxic effects of plant components, and, if needed, to establish safe levels without adverse effects on consumer health (Zhao et al., 2015). The acute oral toxicity of *P. amarus* and *E. hirta* ethanolic extracts were evaluated in rats. The median lethal dose (LD_{50}) of these extracts was estimated to be more than 5,000 mg/kg as there were no symptoms of toxicity in the animals（Adolor et al., 2019; Akomas et al., 2015; Yuet Ping et al., 2013). Based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS), a chemical is not categorized as toxic, mortal or injurious for test animals if its LD_{50} is greater than 5,000 mg/kg (GHS, 2017). However, information regarding a "no-observed-adverse effect-level" (NoAEL) as well as a tolerable daily intake (TDI) for these plant extracts are lacking. *P. amarus* and *E. hirta* are being used as popular medicinal plants in traditional medicine for human in several countries over the world. Thus, the crude ethanolic extracts of *E. hirta* and *P. amarus* were found to be safe and non-toxic and could be used for fish product preservation.

Besides the immersion technique, plant extracts may be applied directly on the surface of the fish and spread on both sides, or applied via the use of impregnated filter paper, or films or coating, via marination (Baptista et al., 2020). However, one of the purposes of our experimental design with dip treatment of plant extracts is a simulation of the tumbling step in the processing of striped catfish company (Tong Thi et al., 2014). In this step, striped catfish fillets were dipped in a spinning basin containing polyphosphate, an additive controlled by good manufacturing practices

that enhances the water binding of fish flesh. However, their excessive use leads to adverse chemical alterations in products (Guimarães et al., 2016). Consequently, positive results shown in this study when using *P. amarus* and *E. hirta* extracts in a dip experiment of striped catfish fillets suggest a promising alternative to the use of commercial additives.

7.1.4. Potential use of plant extracts as feed additives to improve fish feed quality and fish flesh during storage

7.1.4.1 Antioxidant capacity of plant extracts for feed preservation at ambient temperature

Our previous screening study of *in vitro* antioxidant (Nguyen et al., 2020) and immunostimulatory (Nhu et al., 2019b) activities from twenty plants collected in the Mekong Delta, ethanolic extracts of four plants possessing *in vitro* antioxidant and antimicrobial activities (*P. amarus*, *P. guajava*, *E. hirta* and *M. pudica*) were selected to test their capacity to inhibit lipid oxidation in fish feed during storage at ambient temperature. A fifth plant displaying no *in vitro* antioxidant nor antimicrobial activity (*A. indica*) was also included in the experiment as a negative control.

After 8 weeks of storage at room temperature, the peroxide value (PV) and thiobarbituric acid reactive species (TBARS) were significantly lower in feed containing plant extract than in the negative control feed. However, at this storage time, PV was not significantly lower in the feeds containing the plant extracts than in the positive control containing the synthetic antioxidant BHT, except for the feed containing 20 g/kg of ethanolic extract of *M. pudica*. Likewise, peroxide values in the experimental fish feeds stored at 45^{o}C were found to be lower in case they contained a high amount of 1.55 g/kg *A. sinensis* extract (Li et al., 2019). Moreover, these authors showed that *A. sinensis* extract could be lower peroxide values in the experimental feed than ethoxyquin used as antioxidant feed. Additionally, in the work of Luna et al. (2017) on poultry feed, supplementing with thymol or BHT at the concentration of 0.4 g/kg could retard lipid peroxidation in feed stored at room temperature for 60 days.

Regarding the TBARS content after 8 weeks of storage, among 12 experimental feeds, only the feed containing extracts from *P. guajava* (10 g/kg) and *M. pudica* (4 g/kg) presented considerably lower TBARS values than both the positive (with BHT) and the negative controls. Hernández et al. (2014b) showed that adding rosemary extract (*Rosmarinus officinalis*) in fish feed

significantly decreased the TBARS value (10.7 mg MDA/kg feed) if compared to fish feed supplemented with BHT (11.6 mg MDA/kg) after 8 weeks of storage at ambient temperature. In addition, rosemary extracts exhibited analogous or higher antioxidant activity than BHT or butylhydroxyanisole (Estevez et al., 2007; Formanek et al., 2001; Richheimer et al., 1996; Sebranek et al., 2005). Similarly, Li et al. (2016) showed that *Ginkgo biloba* leaf ethyl acetate extract was capable of impeding lipid oxidation in fish feed.

Another attractive finding of this study was that no malondialdehyde (MDA) was found in feed by LC-MS measurement, indicating that what was measured in the TBARS assay was probably not MDA but other secondary fatty acid oxidation products (such as 4-hydroxy-2-hexenal, 4-hydroxy-2-nonenal, crotonaldehyde, benzaldehyde, hexanal, 2,4-Nonadienal, 2,4-Decadienal) able to react with thiobarbituric acid. Since the fatty acid profile of the feed revealed a content of nearly 30% of linoleic acid; the TBARS found are probably the result of the formation of other thiobarbituric reactive species, maybe not all detected in the LC-MS method. Indeed, Tsikas (2017) showed that HPLC analysis of TBA-treated extracts of oxidized methyl esters of linoleic acid and arachidonic acid revealed formation not only of the TBA-MDA derivative, but also of several not identified TBA derivatives. Furthermore, according to Yoden and Iio (1989), lipophilic TBARS produced in oxidized lipids *in vitro* are major TBARS and the production of free MDA is small.

Among five ethanolic plant extracts, *P. guajava*, *P. amarus* and *M. pudica* were able to prevent lipid oxidation in feed materials. However, no report has been published on the effects of these plant extracts on lipid oxidation in fish feeds. Their antioxidant impact may be intimately associated with their active components. Our previous publication emphasized a positive relation between the antioxidant capacity and the total phenolic content of these plant extracts (Nguyen et al., 2020). This was consistent with results from the literature showing a correlation between antioxidant activity and phenolic content of *A. sinensis* together with its capacity to inhibit lipid oxidation in fish feeds (Li et al., 2019).

7.1.4.2 Effect of plant extracts *in vivo* supplementation on the quality of striped catfish fillets

In this study, our results demonstrated the relevance of plant extracts supplementation in juvenile striped catfish to increase the shelf life of fish fillets during ice storage. The supplementation with *E. hirta*, *P. guajava* and *P. amarus* extracts resulted in a considerable

decrease in TVC and fatty acid oxidation products (PV and TBARS) as well as an increase in the organoleptic quality of fish fillets under ice storage, compared to the control. The promising stabilization impact of *P. amarus* and *P. guajava* extracts added to fish feed was confirmed by the lower fish fillet microorganism load during cold storage, and agreed with the antimicrobial activity of these plant extracts shown earlier in *in vitro* assays (Nguyen et al, 2020) and in *in vivo* experiments on striped catfish (Nhu et al., 2020). In both *in vivo* feeding experiments, plant extract addition allowed to keep the TVC below the acceptable limit of 6 log cfu/g (Vietnamese Ministry of Public Health, 2012), indicating a shelf life of 12 days for fish fillets under ice storage. Hernández et al. (2014b) also pointed out that the highest tested dose of rosemary extract (2,400 mg/kg of feed) in gilthead seabream (*Sparus aurata*) feed presented the lowest total aerobic counts (3.57 log cfu/g) after 14 days of ice storage. Considering inhibition of fat oxidation, our results are in accordance with previous *in vitro* antioxidant capacity findings in a DPPH screening of *P. amarus, P. guajava* and *E. hirta* ethanolic plant extracts (Nguyen et al., 2020), where the tested extracts possessed 18.8, 14.5 and 10.3% of total phenolic contents, respectively.

In the application of edible plants as feed additives, antioxidant compounds could inhibit the oxidation in farmed fish *in vivo* by inhibiting reactive oxygen species (Crespy and Williamson, 2004; Descalzo and Sancho, 2008). Furthermore, the addition of antioxidant compounds in feed may lead to their accumulation in fish flesh (Álvarez et al., 2012). Another study of Ha et al. (2021), striped catfish was fed with feed containing 10% concentrated *Flammulina velutipes* extract for 90 days, the results showed that the highest ergothioneine accumulation from *F. velutipes* in striped catfish muscle could limit lipid oxidation as well as improve color stability during cryopreservation. For the amelioration of sensory quality, several studies proved the improvement of sensory quality gilthead seabream supplemented with rosemary extract at a dose of 600 mg/kg (Hernández et al., 2014b). Also, Arsyad et al. (2018), on one hand, documented that olive leaf powder addition to fish feed could ameliorate the texture characteristics of the muscle; on the other hand, Tanimoto et al. (1993) and Gatlin et al. (2007) acknowledged the development of muscle of eels and young yellowtail when fed with tochu leaf powder and green tea polyphenols, respectively.

The amino acid composition is similar in the three groups, indicating that the supplementation of plant extracts might not affect the total amino acid profile of fish fillets.

Similarly, in the study of Lee et al. (2012), no differences in juvenile sterlet sturgeon (*Acipenser ruthenus*) whole body amino acid composition was observed between the experimental groups supplemented with garlic extracts and the controls. As regards biogenic amines, no histamine was found in fish fillets, whilst putrescine, spermidine and spermine were found in samples from all treatments. The biogenic amine index (sum of concentrations of tyramine, histamine, putrescine and cadaverine) in all samples was well underneath the threshold of 90 mg/kg (Du et al., 2002), which indicates an advanced decomposition of fish.

The supplementation of *P. guajava* at the dose of 5 g/kg feed appeared as the best choice of feed additives in aquaculture to improve the quality of striped catfish fillet during post-harvest ice storage. Both *P. guajava* and *P. amarus* extracts used in a mixture did not enhance the positive impact in comparison with individual plant extracts treatments.

From the findings in immunology by PhD Truong Quynh Nhu (UNamur) obtained in the same *in vivo* experiments, the positive effects of fish dietary supplementation with plant ethanol extracts could improve fish health status, as it is shown by the higher immune response than in fish fed diets without plant extracts. In the validation of the effects of five plant extracts, the results demonstrated that dietary administration of *P. guajava* extract at both doses (2 and 10 g/kg feed) worked very well as good immunostimulant in striped catfish. Results of further *in vivo* feeding trials on the effects of fish supplementation with *P. guajava* and *P. amarus* extracts, individually or in mixture, on immune responses revealed that single *P. guajava* and *P. amarus* extracts at 2 and 5 g/kg feed and their mixture at 0.8 and 5 g/kg feed have the potential to modulate the immune mechanisms and disease resistance of striped catfish, and positively altered the liver proteome profile related to immune system processes. In alive fish, plant-based diets administrations contribute to the improvement of immune responses and antioxidant status, activate phagocytosis and antibody to protect striped catfish against bacterial infection. After slaughtered, the immune system in fish cells is inactive and the degradation of protein is occurring during storage. The demonstration of antioxidant and antimicrobial efficiency to fish flesh may be due to the accumulation of plant extracts in fish tissue after oral administration.

7.1.5 Efficiency of selected plants in this study: was it expected?

From the screening of twenty promising plants commonly used in traditional fish prophylaxis in Vietnamese aquaculture, we found that P. amarus and P. guajava displayed antioxidant and antimicrobial properties due to the presence of various biological compounds（Jamieson et al., 2021; Patel et al., 2011）. These positive outcomes met our expectation. Indeed, the potential use of *P. amarus* was appraised based on its findings of traditional uses and modern bioscientific research. Patel et al. (2011) reviewed that *P. amarus* display therapeutic effects e.g antimicrobial, antiviral activities against hepatitis B, chemoprotective, antimutagenic and act as an hypoglycaemic agent. Ellagitannins (geraniin and corilagin) were indicated to be the most effective mediators of the antiviral HIV activity. The presence of Phyllanthin and hypophyllanthin in *P. amarus* showed antitumor activities against Ehrlich ascites carcinoma in Swiss albino mice, cytotoxic effects on K-562 cells, and hepatoprotective and antioxidant effects.

In addition, various parts of *P. guajava* showed numerous medicinal benefits in folk medicine for the treatment of a variety of illnesses such as gastrointestinal disease, hepatic damage, bacterial and fungal infection, fever, rheumatism, respiratory illness (Gutierrez et al., 2008). Metwally et al. (2010) assigned the antibacterial activity against several bacteria and fungi to five flavonoids derived from the leaves. This effect was also correlated to tannins concentration in the leaves (Mailoa et al., 2014) as well as the content of gallic acid and catechin (de Araújo et al., 2014). *P. guajava* extract showed its capacity to lower the radiolabeling of blood constituent of *Wistar* rats thanks to an antioxidant action and/or due to its alteration on the membrane structures involved in ion transport into cells (Abreu et al., 2006).

On the other hand, several selected plants, especially *A. sativum*, *A. indica*, did not show their bioactive properties as expected. The report of Fei et al. (2015) and European Medicines Agency (2016) demonstrated that solvent extraction, storage duration before being consumed and applied processing could be important factors which contributed to the change of levels of soluble sugar, total polyphenols, organosulfur compounds, including the antioxidant capacities of extracts of garlic cloves. A conclusion from the review of Fernandes et al. (2019) reveals that the identification and quantification of the active compounds in the different parts of *A. indica* are important for a better perception of their properties and potentialities. In addition, these authors

concluded that there is not a specific extraction method for each part of *A. indica* tree, which may lead to dissimilar findings among publications.

7.1.6 Economic feasibility and safety of using plants in aquaculture and seafood products

Plants and plant-derived constituents seem to be promising, alone or complementing traditional drugs, as they are being able to enhance growth, survival, health status, innate and adaptive immune responses as well as disease resistance in fish. Furthermore, unlike chemotherapeutics, plant extracts administration to fish does not seem to be associated with any side effects. Medicinal plants are locally available, inexpensive and eco-friendly for environment. Further investigations are strongly recommended to define the optimal doses as it will need a lot of time and costs to prepare a large quantity of extract if high dose of plant extracts need to be applied in the striped catfish farm.

In addition, study on mechanism of action, stability of plant materials in aquatic environment and digestibility in fish as well as *in vitro* and *in vivo* toxicological screening are required for their safe application. Toxicity of plants has been receiving the concern of scientists and consumers. In the studies of Nhu et al. (2019a, 2020a), other works of our AquaBioactive project, there was no observation of toxicity at any used of plant extracts throughout the experiments in modulating immune responses and liver proteome as well as resistance to bacterial infection in striped catfish (*P. hypophthalmus*). An overdose of plant extracts did not significantly suppress or improve the immune responses as well as the capacity against bacterial infection. In agreement with our results, oral administration of aqueous *P. guajava* extract at 100–500 mg/kg body weight was relatively safe in Wistar rats up to 72 h (Etuk and Francis, 2003). Furthermore, an acute toxicity study of ethanol extract of guava leaves showed no signs of toxicity or cause mortality in albino rats even at doses >2000 mg/kg body weight (Dutta and Das, 2010). Zuco et al. (2002) reported that betulinic acid, one of *P. guajava* - derived constituents, did not display cytotoxicity against human normal dermal fibroblasts or human peripheral blood lymphocytes. *In vivo* studies in mice, rats, and rabbits showed that quercetin extracted from *P. guajava* was not carcinogenic and safe for consumption (Harwood et al., 2007).

Acute and sub-acute toxicity of *P. amarus* extract in Swiss mice and Wistar rats respectively showed insignificant differences observed in body weight gain and blood glucose level between control and treated groups (Adjene and Nwose, 2010; Andrew and Enogieru, 2011). Acute oral administration of *P. amarus* leaf extract in rat liver was not toxic at a dose of 5 g/kg body weight. The chronic toxicity studies of *P. amarus* extracts administration with doses of 100–800 mg/kg body weight showed an absence of cumulative toxicity reflected by the non-significant change in the parameters investigated as well as from the findings of the histological studies (Sirajudeen et al., 2006). However, more experiments need to be conducted for toxicity evaluation of plant extracts on striped catfish before applying them in striped catfish commercial culture.

The stem extract from *E. hirta* was reported to contain phorbol esters, hentriacontane, myricyl alcohol, triterpenes and sterols. Zayed et al. (1998) performed a study on five different *Euphorbia* plant extracts from Egypt and presented them to contain toxic fractions with irritant activity and tumor-promoting activity in mice. Carp (*Cyprinus carpio*) were shown to be highly susceptible to phorbol esters which were present in *Jatropha curcas*. The threshold level of 15 ppm (15 μg/g) of phorbol esters in the diet could cause adverse effects whereby a higher level above 31 μg/g of extract in the diet led to lower average metabolic rate, increase fecal mucus production and rejection of fish feed (Becker and Makkar, 1998). However, besides negative effects, several naturally occurring phorbols act as tumor inhibitors, inhibit human immunodeficiency virus (HIV) replication and possess antileukemic activity (Goel et al., 2007). From their conclusions, detoxification or complete removal of phorbol esters is important before its application in medicine or animal feeds. Another approach to relieve the effects of phorbol esters could be the identification and development of microbial consortia or enzymes that could degrade these compounds in feeds, making them appropriate for animal feeding. Moreover, the potential risks of phorbol esters in water, soil, plants and the potential environmental should be evaluated before using them in agriculture (as biopesticide or insecticide) or health control (antimicrobial or antitumor).

7.1.7 Pros and cons of plant versus traditional treatments (antibiotics)

Plants have been used as herbal medicine as one of the oldest forms of healthcare known to mankind. From a technical viewpoint, medicinal plants are characterized by pharmaceutically active compounds that are able to be used, directly or indirectly, in a therapeutic treatment to

prevent or heal a certain disorder (Briskin, 2000, Djeridane et al., 2006). Characterization of potential medicinal properties in plants, which may involve antioxidant, anti-inflammatory, anti-tumoral and antidiabetic effects, have been studied in several studies (Jung et al., 2006; Watt and Breyer-Brandwijk, 1962; Ekor, 2014; Roleira et al., 2015). Moreover, the presence of different compounds derived from numerous plants such as terpenoids, alkaloids, flavonoids, phenolics, etc. are commonly used today in medicinal pharmaceutics with their beneficial/therapeutic effects. Based on the long-term use by humans (often hundreds or thousands of years), any bioactive compounds obtained from plants might have low human toxicity. Chronic toxic effects would be less likely to indicate that the plant should not be used. Moreover, chemical diversity of secondary plant metabolites resulted from plant evolution may be equal or notable to that found in synthetic combinatorial chemical libraries.

Another significant point of phytotherapy is that it induces less side-effects than chemicals, which is manifested for antibiotics with hypersensitivity, drug interactions, effect on commensal flora, antibacterial resistance, and opportunistic pathogens (Weledji et al., 2017). Singh et al. (2017) presented that herbal antibiotics can work against both gram-negative and gram-positive bacteria. Plant derived antibiotics act mostly by breaking down the cell wall and cell membranes of microorganisms, resulting to the release of cellular content, protein binding domain disruption, enzyme inactivation, and eventually leading to cell death. According to Alvin et al. (2014), natural product derived drugs are called ideal antibiotics. Thus, they may not only effectively kill the microorganism, but also affect cellular events in the pathogenic process. For these reasons, the bacteria, fungi and viruses are unable to develop resistance to botanicals.

In addition, the production of plant extracts for the target extraction of bioactive compounds may be an interesting alternative to waste reduction (particularly at the agro-food level) as these byproducts display numerous advantages such as a low cost, renewable source of biologically relevant compounds and therefore contribute to a more effective management of resources and the development of circular economic patterns (Armendáriz-Barragán et al. 2016, Ross 2014, Sasidharan et al. 2011, Hassan et al. 2009, Ribeiro et al. 2015).

Fabricant and Farnsworth (2001) reviewed that there are still several shortcomings in using plant for treatment. Firstly, plants have inherent potential variability in their chemistry, resulting in alterable biologic activity. Secondly, collecting plants based on their ethnomedical states

demands substantial preliminary planning to determine origin, abundance, threatened or endangered species with extinction, permission of local authorities and the assistance of local botanists. Third, the availability of quality plant species, in particular exotic plants, is restricted to a specific geographical region. Moreover, plant quality considerably depends on environmental conditions, in which pollution is an important variable. Other limitations of phytotherapy as well as for any traditional curing treatment, are the shortage of standardization of the treatment and reproducibility of plant-derived products. This is one of the reasons why the efficiency of medicinal plants has not received highly credibility (Ionescu, 2017).

7.2. CONCLUSIONS

This thesis aimed to investigate the *in vitro* antioxidant and antimicrobial activities of numerous plant extracts based on bibliography review data and a survey conducted in striped catfish farms (the Mekong Delta, Vietnam). These potential plant extracts were evaluated in specific studies regarding the quality of feed and fillets of striped catfish (*Pangasianodon hypophthalmus*) in the preservation period.

In the initial stage of our project, the investigation of drugs, chemicals, plants and plant extract products uses in grow-out farms of striped catfish in An Giang and Dong Thap provinces (the Mekong Delta) provided information on culture practice of farmers. The survey presented that the major pathogens in striped catfish, Bacillary necrosis of Pangasius (BNP) and Motile Aeromonad Septicaemia (MAS), are the ones that farmers must cope with challenges in the fish health management. Farmers used antibiotics in bacterial infection control although several types (enrofloxacin and ciprofloxacin) were forbidden antibiotics according to the Vietnamese Ministry of Agriculture and Rural Development (VMARD). In addition, the commercial products derived from plant and plant extracts were marketed for various purposes, although farmers have no information on their quality and their effectiveness as well as the administrative doses.

Five plant ethanolic extracts were selected from 20 plants and 3 commercial products obtained in the Mekong Delta (Vietnam), for their *in vitro* antioxidant and antimicrobial capacities. Among them, *P. amarus* extract showed the highest antioxidant activity, whereas the other plant extracts were in the following descending order for this antioxidant activity: *P. betle* > *P. guajava* > *E. hirta* > *M. pudica*. Moreover, *P. amarus* extract also exhibited antimicrobial efficiency against two different strains of *A. hydrophila*. Both *P. amarus* and *E. hirta* could be potential plants for further studies as precious natural additives to enhance striped catfish fillets preservation. It was shown that a dip treatment of striped catfish fillets using 0.04% *P. amarus* and 0.06% *E. hirta* extracts by dip treatments on striped catfish fillets enables to prolong the shelf life of fillets up to 8 days as well as to maintain their good quality characteristics, based on the chemical quality analyses regarding their lipid oxidation status as well as their (PV), and sensory organoleptic quality.

Regarding the addition of plant extracts in fish diet preservation, *Mimosa pudica* ethanolic extract (at the concentration of 4 or 20 g/kg) was the most promising candidate to mitigate lipid

oxidation during feed storage at ambient temperature, among the five plant extracts examined. This finding suggests that *M. pudica* would be able to be used as an effective feed preservative.

The supplementation of juvenile striped catfish with *P. guajava* and *P. amarus* extracts individually or in a mixture as well as a single dose of *E. hirta* was shown to prolong the shelf life of fish fillets during ice storage. *P. guajava* extract at 5 g/kg feed was the best choice in reducing TVC, fatty oxidation parameters and improving sensory quality in striped catfish fillets under ice storage as well.

The findings of this work have proved the potential of natural compounds from locally available plants in the Mekong Delta to solve striped catfish fillet quality and aquaculture products issues during storage. *P. amarus* and *E. hirta* extracts in particular can be suggested to be used, because of their antioxidant and antimicrobial activities as well as their effectiveness to improve striped catfish fillets preservation. Ethanolic extract of *M. pudica* can be recommended as the most appropriate natural preservative in feed storage, whereas *P. guajava* extract is the best choice in promoting fish quality due to its positive impact on supplemented juvenile striped catfish.

7.3. PERSPECTIVES

The findings in our study will lead to further consideration of future works. The situation of drugs, chemicals, plant and plant extracts used in striped catfish farming revealed a lack of valuable information about herbal derived commercial products used by farmers. Thus, there is an urgent demand to organize farmers' training courses to update their knowledge on active ingredients, proper use, administrative applied doses of plant extract products.

As a complementary work, the fractions and the pure compounds of the five ethanolic plant extracts selected should be evaluated for their antioxidant and antimicrobial activities. Moreover, it would be interesting to test various bacterial strains known to contaminate fish flesh (*E. coli*, *Pseudomonas*, etc.) for their susceptibility to the five selected plant extracts and their factions/pure compounds as well. In addition, the effects of various factors such as parts of plants harvested, plant physiological stages and geographical conditions also need to be compared.

The application of plant extracts is receiving a lot of attention worldwide. However, before using these plant extracts as food additives, their safety should be completely assessed. As an innovative application, plant extracts could be incorporated into packaging materials such as plastic, coating polymer, edible film, etc. to turn it into an active and intelligent packaging, to improved food preservation.

Plant extracts showed positive effects on protecting fish feed against oxidation. However, any substance used as feed additives must be authorized by the authority in charge of food safety on the basis of their absence of adverse effects on animals or human health or the environment, and considering various applications such as technological additives, sensory additives and zootechnical additives.

Further studies of plant extract on different sizes of striped catfish as well as the determination of the feeding period of these extracts in both laboratory and on-farm conditions should be evaluated. Furthermore, long-term investigation will be essential, to confirm the efficiency of these plant extracts, their safety regarding fish and human health, their positive impacts on the quality of fish products and the environment, and their availability in view of a large-scale regular use.

The reproducibility of the activity of plant-derived active components should be taken into consideration in analytical characterization and authentication of active compounds for establishing quality control procedures.

REFERENCES

Abbas, K.A., A. Mohamed, B. Jamilah, and M. Ebrahimian, 2008. A review on correlations between fish freshness and pH during cold storage. American journal of biochemistry and biotechnology. 4, 416-421.

Abdelhadi, Y., B. Izwan, and B. Safuan, 2012. Chamomile; Matricaria chamomilla; The magic herb in aquaculture. Aquaculture. 1-5.

Abdel-Tawwab, M., I. Adeshina, A. Jenyo-Oni, E.K. Ajani, and B.O. Emikpe, 2018. Growth, physiological, antioxidants, and immune response of African catfish, *Clarias gariepinus* (B.), to dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to *Listeria monocytogenes* infection. Fish & shellfish immunology. 78, 346-354.

Abdel-Tawwab, M., M.H. Ahmad, M.E. Seden, and S.F. Sakr, 2010. Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.), against *Aeromonas hydrophila* infection. Journal of the World Aquaculture Society. 41, 203-213.

Abdollahzadeh, E., M. Rezaei, and H. Hosseini, 2014. Antibacterial activity of plant essential oils and extracts: The role of thyme essential oil, nisin, and their combination to control *Listeria monocytogenes* inoculated in minced fish meat. Food control. 35, 177-183.

Aebi, H., 1984. Catalase *in vitro*, in Methods in enzymology, L. Packer, Editor., Academic: Orlando. p. 121-126.

Abreu, P.R.C., M.C. Almeida, R.M. Bernardo, L.C. Bernardo, L.C. Brito, E.A.C. Garcia, A.S. Fonseca, and M. Bernardo-Filho, 2006. Guava extract (*Psidium guajava*) alters the labelling of blood constituents with technetium-99m. Journal of Zhejiang University Science B. 7, 429-435.

Abutbul, S., A. Golan-Goldhirsh, O. Barazani, and D. Zilberg, 2004. Use of *Rosmarinus officinalis* as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis* sp.). Aquaculture. 238, 97-105.

Adibhatla, R.M. and J. Hatcher, 2006. Phospholipase A2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. Free Radical Biology and Medicine. 40, 376-387.

Adolor, O., I. Onyesom, A. Opajobi, and J. Mordi, 2019. Evaluation of median lethal dose and subchronic oral toxicity assessment of ethanolic leaf extract of *Phyllanthus amarus*. Journal of pharmaceutical research international. 26, 1-8.

Adjene, J.O. and E.U., Nwose, 2010. Histological effects of chronic administration of *Phyllanthus amarus* on the kidney of adult Wistar rat. North American Journal of Medical Sciences. 2, 193–195.

Aghazadeh, M., A.Z. Bialvaei, M. Aghazadeh, F. Kabiri, N. Saliani, M. Yousefi, H. Eslami, and H.S. Kafil, 2016. Survey of the antibiofilm and antimicrobial effects of *Zingiber officinale* (*in vitro* study). Jundishapur journal of microbiology. 9, 1-6.

Agyare, C., E. Baiden, J.A. Apenteng, Y.D. Boakye, and L. Adu-Amoah, 2015. Anti-infective and Anti-inflammatory Properties of *Portulaca oleracea* L. Donnish Journal of Medicinal Plant Research. 2, 1-6.

Ahmad, H., R. Sharma, A. Mansour, and Y.C. Awasthi, 1992. t-Butylated hydroxytoluene enhances intracellular levels of glutathione and related enzymes of rat lens *in vitro* organ culture. Experimental eye research. 54, 41-48.

Ahmad, W., S. Singh, and S. Kumar, 2017. Phytochemical screening and antimicrobial study of *Euphorbia hirta* extracts. Journal of medicinal plants studies. 5, 183-186.

Akiyama, H., K. Fujii, O. Yamasaki, T. Oono, and K. Iwatsuki, 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. Journal of antimicrobial chemotherapy. 48, 487-491.

Akomas, S., S. Ijioma, and C. Emelike, 2015. Effect of *Euphorbia hirta* on haematological and biochemical indices in albino rats. Applied Journal of Hygiene. 4, 1-5.

Akter, M., M.J. Islam, S. Mian, F.H. Shikha, M.H. Rahman, and M. Kamal, 2014. Changes in fillet quality of pangas catfish (*Pangasianodon hypophthalmus*) during frozen storage. World Journal of Fish and Marine Sciences. 6, 146-155.

Alam, M., A.S. Juraimi, M. Rafii, A. Abdul Hamid, F. Aslani, M. Hasan, M. Zainudin, M. Asraf, and M. Uddin, 2014. Evaluation of antioxidant compounds, antioxidant activities, and mineral composition of 13 collected purslane (*Portulaca oleracea* L.) accessions. BioMed Research International. 2014, 1-10.

Aldaly, Z.T., 2010. Antimicrobial activity of piperine purified from *Piper nigrum*. Journal of Basrah Researches (Sciences). 36, 54-61.

Ali, S.S., N. Kasoju, A. Luthra, A. Singh, H. Sharanabasava, A. Sahu, and U. Bora, 2008. Indian medicinal herbs as sources of antioxidants. Food research international. 41, 1-15.

Almalki, M., 2016. In-Vitro Antioxidant properties of the leaf extract of *Mimosa pudica* Linn. Indian J. Sci. Technol. 9, 1-6.

Alsaid, M., H. Daud, S.K. Bejo, and A. Abuseliana, 2010. Antimicrobial activities of some culinary spice extracts against *Streptococcus agalactiae* and its prophylactic uses to prevent streptococcal infection in red hybrid tilapia (*Oreochromis* sp.). World Journal of Fish and Marine Sciences. 2, 532-538.

Al-Snafi, A.E., 2017. Pharmacology and therapeutic potential of *Euphorbia hirta* (Syn: *Euphorbia pilulifera*)-A review. IOSR Journal of Pharmacy. 7, 7-20.

Álvarez, A., B.G. García, M. Jordán, C. Martínez-Conesa, and M. Hernández, 2012. The effect of diets supplemented with thyme essential oils and rosemary extract on the deterioration of farmed gilthead seabream (*Sparus aurata*) during storage on ice. Food Chemistry. 132, 1395-1405.

Alvin, A., K.I. Miller, and B.A. Neilan, 2014. Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. Microbiological research. 169, 483-495.

Amoli, Z.A., T. Mehdizadeh, H. Tajik, and M. Azizkhani, 2019. Shelf life extension of refrigerated, vacuum-packed rainbow trout dipped in an alginate coating containing an ethanolic extract and/or the essential oil of *Mentha Aquatica.* Chemical Papers. 73, 2541-2550.

Andrew, E.O., and A., Enogieru, 2011. Effects of oral administration of *Phyllanthus amarus* leaf extract on the kidneys of adult Wistar rats – a histological study. African Journal of Traditional Complementary and Alternative Medicines. 8, 307–311.

AOAC, 2016. Official methods of Analysis of AOAC International. 20th ed. Maryland, USA: Association of Official Agricultural Chemists.

Apak, R., K. Güçlü, M. Özyürek, and S.E. Karademir, 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of agricultural and food chemistry. 52, 7970-7981.

Arsyad, M.A., T. Akazawa, C. Nozaki, M. Yoshida, K. Oyama, T. Mukai, and M. Ogawa, 2018. Effects of olive leaf powder supplemented to fish feed on muscle protein of red sea bream. Fish physiology and biochemistry. 44, 1299-1308.

Armendáriz-Barragán, B., N. Zafar, W. Badri, S.A. Galindo-Rodríguez, D. Kabbaj, H. Fessi, and A. Elaissari, 2016. Plant extracts: from encapsulation to application. Expert Opinion on Drug Delivery. 13, 1165-1175.

Asha, S., P. Thirunavukkarasu, V.M. Mani, and A.M. Sadiq, 2016. Antioxidant activity of *Euphorbia hirta* Linn leaves extracts. European Journal of Medicinal Plants. 14, 1-14.

Ashie, I., J. Smith, B. Simpson, and N.F. Haard, 1996. Spoilage and shelf-life extension of fresh fish and shellfish. Critical Reviews in Food Science & Nutrition. 36, 87-121.

Ashraf, A., R.A. Sarfraz, M.A. Rashid, A. Mahmood, M. Shahid, and N. Noor, 2016. Chemical composition, antioxidant, antitumor, anticancer and cytotoxic effects of *Psidium guajava* leaf extracts. Pharmaceutical biology. 54, 1971-1981.

Ashraf M.A. and S. Goda, 2008. Effect of dietary *Ginseng herb* (Ginsana® G115) supplementation on growth, feed utilization, and hematological indices of Nile Tilapia, *Oreochromis niloticus* (L.), fingerlings. Journal of the World Aquaculture Society. 39, 205-214.

Asimi, O. and N. Sahu, 2013. Herbs/spices as feed additive in aquaculture. Scientific Journal of Pure and Applied Sciences. 2, 284-292.

Atiya, A., B.N. Sinha, and U. Ranjan Lal, 2018. New chemical constituents from the *Piper betle* Linn.(Piperaceae). Natural product research. 32, 1080-1087.

Aydemir, T. and S. Becerik, 2011. Phenolic content and antioxidant activity of different extracts from *Ocimum basilicum*, *Apium graveolens* and *Lepidium sativum* seeds. Journal of food biochemistry. 35, 62-79.

Azam, K., S. Pervin, S. Naher, Y. Ali, M. Alam, K. Haque, and B. Ahmed, 2005. Quality Changes in Pangus (*Pangasius hypopthalamus*) in Relation to Size and season during storage in ice. Pakistan Journal of Biological Sciences. 8, 636-640.

Azhar, K. and K. Nisa, 2006. Lipids and their oxidation in seafood. Journal of the Chemical Society of Pakistan. 28, 298-305.

Babalola, T. and D. Apata, 2011. Chemical and quality evaluation of some alternative lipid sources for aqua feed production. Agriculture and Biology Journal of North America. 2, 935-943.

Bach, L.T., L.T. Dung, N.T. Tuan, N.T. Phuong, P. Kestemont, J. Quetin-Leclercq, and B.T.B. Hue, 2018. Protective effect of pancreatic β-cells MIN6 by some medicinal plants in the Mekong Delta. Vietnam Journal of Chemistry. 56, 637-641.

Bagge-Ravn, D., Y. Ng, M. Hjelm, J.N. Christiansen, C. Johansen, and L. Gram, 2003. The microbial ecology of processing equipment in different fish industries—analysis of the microflora during processing and following cleaning and disinfection. International journal of food microbiology. 87, 239-250.

Bahmani, M., M. Taherikalani, M. Khaksarian, M. Rafieian-Kopaei, B. Ashrafi, M. Nazer, S. Soroush, N. Abbasi, M. Rashidipour, 2019. The synergistic effect of hydroalcoholic extracts of *Origanum vulgare*, *Hypericum perforatum* and their active components carvacrol and hypericin against *Staphylococcus aureus*. Future science OA. 5, FSO371.

Baixas-Nogueras, S., S. Bover-Cid, M. Vidal-Carou, and M. Veciana-Nogues, 2001. Volatile and nonvolatile amines in Mediterranean hake as function of their storage temperature. Journal of food science. 66, 83-88.

Bakkali, F., S. Averbeck, D. Averbeck, and M. Idaomar, 2008. Biological effects of essential oils–a review. Food and chemical toxicology. 46, 446-475.

Ban, N.T., 2005. Checklist of plant species of Vietnam. Vol. 3. Ha Noi: Agriculture Publishing House. 1248.

Banskota, A.H., Y. Tezuka, Q. Tran, and S. Kadota, 2003. Chemical constituents and biological activities of Vietnamese medicinal plants. Current topics in medicinal chemistry. 3, 227-248.

Bao, H., 2006. QIM Method Scores Quality, Shelf Life of Pangasius Fillets. Global Aquaculture Advocate. 28-30.

Bao, H.N., Y. Shinomiya, H. Ikeda, and T. Ohshima, 2009. Preventing discoloration and lipid oxidation in dark muscle of yellowtail by feeding an extract prepared from mushroom (*Flammulina velutipes*) cultured medium. Aquaculture. 295, 243-249.

Baptista, R.C., C.N. Horita, and A.S. Sant'Ana, 2020. Natural products with preservative properties for enhancing the microbiological safety and extending the shelf-life of seafood: A review. Food Research International. 127, 108762.

Bardócz, S., 1995. Polyamines in food and their consequences for food quality and human health. Trends in Food Science & Technology. 6, 341-346.

Baron, C.P., G.H. Svendsen, I. Lund, A. Jokumsen, H.H. Nielsen, and C. Jacobsen, 2013. Organic plant ingredients in the diet of Rainbow trout (*Oncorhynchus mykiss*): Impact on fish muscle composition and oxidative stability. European Journal of Lipid Science and Technology. 115, 1367-1377.

Barrajón-Catalán, E., S. Fernández-Arroyo, D. Saura, E. Guillén, A. Fernández-Gutiérrez, A. Segura-Carretero, and V. Micol, 2010. Cistaceae aqueous extracts containing ellagitannins show antioxidant and antimicrobial capacity, and cytotoxic activity against human cancer cells. Food and Chemical Toxicology. 48, 2273-2282.

Baska, F., V. Voronin, E. Eszterbauer, L. Müller, S. Marton, and K. Molnár, 2009. Occurrence of two myxosporean species, *Myxobolus hakyi* sp. n. and *Hoferellus pulvinatus* sp. n., in *Pangasianodon hypophthalmus* fry imported from Thailand to Europe as ornamental fish. Parasitology research. 105, 1391-1398.

Bazaka, K., M.V. Jacob, W. Chrzanowski, and K. Ostrikov, 2015. Anti-bacterial surfaces: natural agents, mechanisms of action, and plasma surface modification. Rsc Advances. 5, 48739-48759.

Becker, K., and H.P.S., Makkar, 1998. Effects of phorbolesters in carp (*Cyprinus carpio* L.) Vet. Hum. Toxicol. 40, 82–86.

Begum, M., T. Akter, and M.H. Minar, 2012. Effect of salt and garlic on the quality and microbial content of smoked catfish (*Pangasianodon hypophthalmus*). International journal of Bio-resource and Stress Management. 3, 464-467.

Beltrán, J.M.G., D.G. Silvera, C.E. Ruiz, V. Campo, L. Chupani, C. Faggio, and M.Á. Esteban, 2020. Effects of dietary *Origanum vulgare* on gilthead seabream (*Sparus aurata* L.) immune and antioxidant status. Fish & shellfish immunology. 99, 452-461.

Benkeblia, N., 2004. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). LWT-food science and technology. 37, 263-268.

Bensky, D., A. Gamble, and T. Kaptchuk, 1993. Chinese Herbal Medicine Materia Medica. Seattle, USA: Eastland Press.

Benzie, I.F., and J.J. Strain, 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical biochemistry. 239, 70-76.

Blasi, F., E. Urbani, M.S. Simonetti, C. Chiesi, and L. Cossignani, 2016. Seasonal variations in antioxidant compounds of *Olea europaea* leaves collected from different Italian cultivars. Journal of Applied Botany and Food Quality. 89, 202-207.

Błaszczyk, A., A. Augustyniak, and J. Skolimowski, 2013. Ethoxyquin: an antioxidant used in animal feed. International journal of food science. 2013, 1-12.

Blumenthal, M., A. Goldberg, and J. Brinckmann, 2000. Herbal medicine. Expanded commission E monographs, ed. M. Blumental, A. Goldberg, and J. Brinckmann. Newton, MA: Integrative Medicine Communications. 519.

Bohne, V.J.B., A.-K. Lundebye, and K. Hamre, 2008. Accumulation and depuration of the synthetic antioxidant ethoxyquin in the muscle of Atlantic salmon (*Salmo salar* L.). Food and Chemical Toxicology. 46, 1834-1843.

Borges, A., C. Ferreira, M.J. Saavedra, and M. Simões, 2013. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microbial Drug Resistance. 19, 256-265.

Borkataky, M., B. Kakoty, and L. Saikia, 2013. Proximate analysis and antimicrobial activity of *Eclipta alba* (L.) Hassk.—a traditionally used herb. International Journal of Pharmacy and Pharmaceutical Sciences. 5, 149-154.

Borris, R.P., 1996. Natural products research: perspectives from a major pharmaceutical company. Journal of ethnopharmacology. 51, 29-38.

Botterweck, A.A., H. Verhagen, R.A. Goldbohm, J. Kleinjans, and P.A. Van den Brandt, 2000. Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands cohort study. Food and Chemical Toxicology. 38, 599-605.

Braga, T.V., R.G.R. das Dores, C.S. Ramos, F.C.G. Evangelista, L.M. da Silva Tinoco, F. de Pilla Varotti, M. Carvalho, and A. de Paula Sabino, 2014. Antioxidant, antibacterial and antitumor activity of ethanolic extract of the *Psidium guajava* leaves. American journal of plant sciences. 5, 3492-3500.

Brand-Williams, W., M.E. Cuvelier, and C.L.W.T. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28, 25-30.

Brewer, M., 2011. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Comprehensive reviews in food science and food safety. 10, 221-247.

Brglez Mojzer, E., M. Knez Hrnčič, M. Škerget, Ž. Knez, and U. Bren, 2016. Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. Molecules. 21, 901.

Brillet, A., M.F. Pilet, H. Prevost, A. Bouttefroy, and F. Leroi, 2004. Biodiversity of *Listeria monocytogenes* sensitivity to bacteriocin-producing *Carnobacterium* strains and application in sterile cold-smoked salmon. Journal of Applied Microbiology. 97, 1029-1037.

Briskin, D.P., 2000. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant physiology. 124, 507-514.

Bruno, D. and A. Munro, 1991. The calculation of various treatment dose rates in fish farming. Aquaculture Information Series. Aberdeen, Scotland: Scottish Office Agriculture and Fisheries Department, Marine Laboratory. 10.

Bulfon, C., D. Volpatti, and M. Galeotti, 2014. *In vitro* antibacterial activity of plant ethanolic extracts against fish pathogens. Journal of the world aquaculture society, 45, 545-557.

Bulfon, C., D. Volpatti, and M. Galeotti, 2015. Current research on the use of plant-derived products in farmed fish. Aquaculture Research. 46, 513-551.

Burton, G. and K. Ingold, 1981. Autoxidation of biological molecules. 1. Antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants *in vitro*. Journal of the American Chemical Society. 103, 6472-6477.

Bussmann, R.W., G. Malca-García, A. Glenn, D. Sharon, G. Chait, D. Díaz, K. Pourmand, B. Jonat, S. Somogy, and G. Guardado, 2010. Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. Journal of ethnopharmacology. 132, 101-108.

Cao, G., H.M., Alessio, and R.G. Cutler, 1993. Oxygen-radical absorbance capacity assay for antioxidants. Free radical biology and medicine, 14, 303-311.

Caruso, D., A.M. Lusiastuti, T. Taukhid, J.C. Avarre, M. Yuhana, and S. Sarter, 2017. Ethnobotanical Uses and Antimicrobial Properties of Plants in Small-Scale Tropical Fish Farms: The Case of Indonesian Fish Farmers in Java (Indonesia). Journal of the World Aquaculture Society. 48, 83-92.

Catteau, L., F. Van Bambeke, and J. Quetin-Leclercq, 2015. Preliminary evidences of the direct and indirect antimicrobial activity of 12 plants used in traditional medicine in Africa. Phytochemistry reviews. 14, 975-991.

Čerňáková, M., and D. Košťálová, 2002. Antimicrobial activity of berberine—A constituent of *Mahonia aquifolium*. Folia microbiologica. 47, 375-378.

Chaijan, M., S. Benjakul, W. Visessanguan, and C. Faustman, 2006. Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. Food chemistry. 99, 83-91.

Chakraborty, S.B. and C. Hancz, 2011. Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. Reviews in Aquaculture. 3, 103-119.

Chang, K.L.B., J. Chang, C.-Y. Shiau, and B.S. Pan, 1998. Biochemical, microbiological, and sensory changes of sea bass (*Lateolabrax japonicus*) under partial freezing and refrigerated storage. Journal of Agricultural and Food Chemistry. 46, 682-686.

Chapman, R. A., and W.D. McFarlane, 1943. A Colorimetric Method for the Determination of Fat-Peroxides and Its Application in the Study of the Keeping Quality of Milk Powders. Canadian Journal of Research. 21, 133-139.

Chekki, R.Z., A. Snoussi, I. Hamrouni, and N. Bouzouita, 2014. Chemical composition, antibacterial and antioxidant activities of Tunisian garlic (*Allium sativum*) essential oil and ethanol extract. Mediterranean Journal of Chemistry. 3, 947-956.

Chen, B.-Y., R. Pyla, T.-J. Kim, J.L. Silva, and Y.-S. Jung, 2010. Prevalence and contamination patterns of *Listeria monocytogenes* in catfish processing environment and fresh fillets. Food microbiology. 27, 645-652.

Chevion, S., M.A. Roberts, and M. Chevion, 2000. The use of cyclic voltammetry for the evaluation of antioxidant capacity. Free radical biology and medicine. 28, 860-870.

Chi, T.T.K., J.H. Clausen, P.T. Van, B. Tersbøl, and A. Dalsgaard, 2017. Use practices of antimicrobials and other compounds by shrimp and fish farmers in Northern Vietnam. Aquaculture Reports. 7, 40-47.

Chi, V.V., 1997. Dictionary of medicinal plants in Vietnam, Medical Publishing House: Ha Noi. p. 210-215.

Chi, V.V., 2003. Dictionary of common plants in Vietnam, Science and Technic Publishing House: Ha Noi.

Chi, V.V., 2004. Dictionary of common plants in Vietnam, Science and Technic Publishing House: Ha Noi.

Chopade, A.R., and F.J. Sayyad, 2015. Pain modulation by lignans (phyllanthin and hypophyllanthin) and tannin (corilagin) rich extracts of *Phyllanthus amarus* in carrageenan-induced thermal and mechanical chronic muscle hyperalgesia. Phytotherapy Research. 29, 1202-1210.

Chouliara, E., A. Karatapanis, I. Savvaidis, and M. Kontominas, 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4 C. Food microbiology. 24, 607-617.

Chung, K.-T., T.Y. Wong, C.-I. Wei, Y.-W. Huang, and Y. Lin, 1998. Tannins and human health: a review. Critical reviews in food science and nutrition. 38, 421-464.

Citarasu, T., 2010. Herbal biomedicines: a new opportunity for aquaculture industry. Aquaculture International. 18, 403-414.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical microbiology reviews. 12, 564-582.

Craig, S., L.A. Helfrich, D. Kuhn, and M.H. Schwarz, 2017. Understanding fish nutrition, feeds, and feeding. 1-6

Crespy, V. and G. Williamson, 2004. A review of the health effects of green tea catechins in *in vivo* animal models. The Journal of nutrition. 134, 3431S-3440S.

Crumlish, M., P.C. Thanh, J. Koesling, V.T. Tung, and K. Gravningen, 2010. Experimental challenge studies in Vietnamese catfish, *Pangasianodon hypophthalmus* (Sauvage), exposed to *Edwardsiella ictaluri* and *Aeromonas hydrophila*. Journal of fish diseases. 33, 717-722.

Crumlish, M., T. Dung, J. Turnbull, N. Ngoc, and H. Ferguson, 2002. Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. Journal of Fish Diseases. 25, 733-736.

Cruz Casallas, N.E., P.E. Cruz Casallas, and H. Suárez Mahecha, 2012. Characterization of the nutritional quality of the meat in some species of catfish: a review. Revista Facultad Nacional de Agronomía Medellín. 65, 6799-6809.

Cushnie, T.T. and A.J. Lamb, 2005. Antimicrobial activity of flavonoids. International journal of antimicrobial agents. 26, 343-356.

Daglia, M., 2012. Polyphenols as antimicrobial agents. Current opinion in biotechnology. 23, 174-181.

Dalgaard, P., H. Madsen, N. Samieian, and J. Emborg, 2006. Biogenic amine formation and microbial spoilage in chilled garfish (Belone belone belone)–effect of modified atmosphere packaging and previous frozen storage. Journal of applied microbiology. 101, 80-95.

Dang, T.M.T., T.T.H. Tran, and T.B.H. Bui, 2020. Antifungal activity of extracted herbs on snakehead pathogenic fungal. Can Tho University Journal of Science. 56, 129-136.

Danuwat, P., P. Rimruthai, C. Phattanawan, and D. Peerarat, 2016. Determination of essential amino acids in *Pangasius bocourti.* Journal of Food Processing and Technology. 7, 1-4.

Darah, I., S. Lim, and K. Nithianantham, 2013. Effects of methanol extract of *Wedelia chinensis* osbeck (asteraceae) leaves against pathogenic bacteria with emphasise on *Bacillus cereus.* Indian journal of pharmaceutical sciences. 75, 533-539.

Das, K., M. Yasin, N.U. Mahbub, M.S. Islam, and N. Mahbuba, 2014. Evaluation of antioxidant and cytotoxic activity of methanolic extract of *Mimosa pudica* leaves. The Pharma Innovation. 3, 32.

Dawood, M.A., S. Koshio, and M.Á. Esteban, 2018. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Reviews in Aquaculture. 10, 950-974.

de Araújo, A.A., L.A. L. Soares, M.R.A. Ferreira, M.A. de Souza Neto, G.R. da Silva, R.F. de Araújo Jr, G.C.B. Guerra, and M.C.N. de Melo, 2014. Quantification of polyphenols and evaluation of antimicrobial, analgesic and anti-inflammatory activities of aqueous and acetone–water extracts of *Libidibia ferrea*, *Parapiptadenia rigida* and *Psidium guajava*. Journal of Ethnopharmacology. 156, 88-96.

De Britto, A.J., D.H.S. Gracelin, and S.R. Sebastian, 2011. Antibacterial activity of a few medicinal plants against *Xanthomonas campestris* and *Aeromonas hydrophila.* Journal of Biopesticides. 4, 57.

de Oliveira-Júnior, R.G., C.A.A. Ferraz, M.C. Pontes, N.B. Cavalcante, E.C. da Cruz Araujo, A.P. de Oliveira, L. Picot, L. Araújo Rolim, and J.R.G. da Silva Almeida, 2018. Antibacterial activity of terpenoids isolated from *Cnidoscolus quercifolius* Pohl (Euphorbiaceae), a Brazilian medicinal plant from Caatinga biome. European Journal of Integrative Medicine. 24, 30-34.

De Silva, S.S. and N.T. Phuong, 2011. Striped catfish farming in the Mekong Delta, Vietnam: a tumultuous path to a global success. Reviews in Aquaculture. 3, 45-73.

De Silva, S.S., 2008. Market chains of non-high value cultured aquatic commodities: case studies from Asia. FAO.

Declercq, A.M., F. Haesebrouck, W. Van den Broeck, P. Bossier, and A. Decostere, 2013. Columnaris disease in fish: a review with emphasis on bacterium-host interactions. Veterinary research. 44, 1-17.

Demir, T., 2021. Effects of green tea powder, pomegranate peel powder, epicatechin and punicalagin additives on antimicrobial, antioxidant potential and quality properties of raw meatballs. Molecules. 26, 4052.

Descalzo, A.M. and A. Sancho, 2008. A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. Meat Science. 79, 423-436.

Devi, S., R. Rashid, and M. Kumar, 2017. Phytochemistry and pharmacological properties of *Phyllanthus amarus* Schum: A review. The Pharma Innovation Journal. 6, 169-172.

Dewi, R.T. and F. Maryani, 2015. Antioxidant and α-glucosidase inhibitory compounds of *Centella asiatica.* Procedia Chemistry. 17, 147-152.

Dhiman, A., A. Nanda, S. Ahmad, and B. Narasimhan, 2011. *In vitro* antimicrobial activity of methanolic leaf extract of *Psidium guajava* L. Journal of Pharmacy and Bioallied Sciences. 3, 226.

Dhindsa, R.S., P. Plumb-Dhindsa, and T.A. Thorpe, 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. Journal of Experimental botany. 32, 93-101.

Dillard, C.J., and J.B. German, 2000. Phytochemicals: nutraceuticals and human health. Journal of the Science of Food and Agriculture. 80, 1744-1756.

Directorate of Fisheries, 2020. Reduction of striped catfish export in 2019. Available from: https://tongcucthuysan.gov.vn/vi-vn/th%C6%B0%C6%A1ng-m%E1%BA%A1i-th%E1%BB%A7y-s%E1%BA%A3n/xu%E1%BA%A5t-nh%E1%BA%ADp-kh%E1%BA%A9u/doc-tin/013997/2019-12-05/du-bao-kim-ngach-xuat-khau-ca-tra-nam-2019-giam-manh.

Direkbusarakom, S., 2004. Application of medicinal herbs to aquaculture in Asia. Walailak Journal of Science and Technology (WJST). 1, 7-14.

Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker, and N. Vidal, 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food chemistry. 97, 654-660.

Doan, D.D., N. Nguyen, H. Doan, T. Nguyen, T. Phan, M. Grabe, R. Johansson, G. Lindgren, and N. Stjernström, 1992. Studies on the individual and combined diuretic effects of four

Vietnamese traditional herbal remedies (*Zea mays*, *Imperata cylindrica*, *Plantago major* and *Orthosiphon stamineus*). Journal of ethnopharmacology. 36, 225-231.

Donnelly, C., R. Brackett, S. Doores, W. Lee, and J. Lovett, 1992. *Listeria.* Compendium of methods for the microbiological examination of foods. 3, 637-663.

Doss, A., H.M. Mubarack, and R. Dhanabalan, 2009. Antibacterial activity of tannins from the leaves of *Solanum trilobatum* Linn. Indian Journal of Science and Technology. 2, 41-43.

Douny, C., P. Bayram, F. Brose, G. Degand, and M.L. Scippo, 2016. Development of an LC-MS/MS analytical method for the simultaneous measurement of aldehydes from polyunsaturated fatty acids degradation in animal feed. Drug testing and analysis. 8, 458-464.

Douny, C., R. El Khoury, J. Delmelle, F. Brose, G. Degand, N. Moula, F. Farnir, A. Clinquart, G. Maghuin-Rogister, and M.L. Scippo, 2015. Effect of storage and cooking on the fatty acid profile of omega-3 enriched eggs and pork meat marketed in Belgium. Food Science & Nutrition. 3, 140-152.

Douny, C., S. Benmedjadi, F. Brose, O.H.I. Afé, A. Igout, D.J. Hounhouigan, V.B. Anihouvi, and M.-L. Scippo, 2019. Development of an analytical method for the simultaneous measurement of 10 biogenic amines in meat: application to Beninese grilled pork samples. Food Analytical Methods. 12, 2392-2400.

Drummond, A.J. and R.D. Waigh, 2000. The development of microbiological methods for phytochemical screening. Recent research developments in phytochemistry. 4, 143-152.

Du, W.X., C.M. Lin, A.T. Phu, J. Cornell, M. Marshall, and C.I. Wei, 2002. Development of biogenic amines in yellowfin tuna (*Thunnus albacares*): effect of storage and correlation with decarboxylase-positive bacterial flora. Journal of Food Science. 67, 292-301.

Duc, P., D. Thy, K. Hatai, and Y. Muraosa, 2015. Infection of striped catfish (*Pangasianodon hypophthalmus*) in Viet Nam caused by the fungus *Fusarium incarnatum-equiseti*. Bull. Eur. Ass. Fish Pathol. 35, 208-216.

Dudonne, S., X. Vitrac, P. Coutiere, M. Woillez, and J.-M. Merillon, 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. Journal of agricultural and food chemistry. 57, 1768-1774.

Duffes, F., 1999. Improving the control of *Listeria monocytogenes* in cold smoked salmon. Trends in Food Science & Technology. 10, 211-216.

Dung, T.T., F. Haesebrouck, N.A. Tuan, P. Sorgeloos, M. Baele, and A. Decostere, 2008. Antimicrobial susceptibility pattern of Edwardsiella ictaluri isolates from natural outbreaks of bacillary necrosis of *Pangasianodon hypophthalmus* in Vietnam. Microbial drug resistance. 14, 311-316.

Dutta, S. and S., Das. 2010. A study of the anti-inflammatory effect of the leaves of *Psidium guajava* Linn. on experimental animal models. Pharmacognosy research. 2, 313.

Duy, L.N.D. and N.C. Ha, 2014. Effect of aecetic acid and hot water treatment on *Escherichia coli* and bacterial total of catfish filet (*Pangasius hypophthalmus*). Can Tho University Journal of Science. 1, 1-7.

EC, 2005. European Commission, Commission Regulation 2073/2005/EC of 15th November 2005 on microbiological criteria for foodstuffs. Official Journal. L 338, 1-26.

EFSA Panel on Biological Hazards, 2011. Scientific opinion on risk based control of biogenic amine formation in fermented foods. Efsa Journal. 9, 2393.

Ekaluo, U., E. Ikpeme, E. Ekerette, and C. Chukwu, 2015. *In vitro* antioxidant and free radical activity of some Nigerian medicinal plants: bitter leaf (*Vernonia amygdalina* L.) and guava (*Psidium guajava* Del.). Research Journal of Medicinal Plant. 9, 215-226.

Ekor, M., 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in pharmacology. 4, 177.

Eldeen, I., E. Seow, R. Abdullah, and S. Sulaiman, 2011. *In vitro* antibacterial, antioxidant, total phenolic contents and anti-HIV-1 reverse transcriptase activities of extracts of seven Phyllanthus sp. South African Journal of Botany. 77, 75-79.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Archives of biochemistry and biophysics. 82, 70-77.

Emborg, J., B.G. Laursen, and P. Dalgaard, 2005. Significant histamine formation in tuna (*Thunnus albacares*) at 2°C—effect of vacuum-and modified atmosphere-packaging on psychrotolerant bacteria. International journal of food microbiology. 101, 263-279.

Embuscado, M.E., 2015. Spices and herbs: Natural sources of antioxidants–a mini review. Journal of functional foods. 18, 811-819.

Engel, J.B., C. Heckler, E.C. Tondo, D.J. Daroit, and P. da Silva Malheiros, 2017. Antimicrobial activity of free and liposome-encapsulated thymol and carvacrol against *Salmonella* and

Staphylococcus aureus adhered to stainless steel. International journal of food microbiology. 252, 18-23.

Erickson, M.C., 2002. Lipid oxidation of muscle foods, in Food lipids: Chemistry, nutrition, and biotechnology, C.C. Akoh and D.B. Min, Editors., Marcel Deker, Inc: New York. p. 365-411.

Erkan, N., Ş. Ulusoy, and Ş.Y. Tosun, 2011. Effect of combined application of plant extract and vacuum packaged treatment on the quality of hot smoked rainbow trout. Journal für Verbraucherschutz und Lebensmittelsicherheit. 6, 419-426.

Estevez, M., R. Ramírez, S. Ventanas, and R. Cava, 2007. Sage and rosemary essential oils versus BHT for the inhibition of lipid oxidative reactions in liver pâté. LWT-Food Science and Technology. 40, 58-65.

Etuk, E. and U. Francis, 2003. Acute toxicity and efficacy of *Psidium guajava* leaves water extract on *Salmonella typhi* infected Wistar rats. Pakistan Journal of Biological Sciences. 6, 195-197.

European Commission, 2009. Commission Regulation (EC) No 710/2009 of 5 August 2009 Amending Regulation (EC) No 889/2008 Laying down Detailed Rules for the Implementation of Council Regulation (EC) No 834/2007, as Regards Laying down Detailed Rules on Organic Aquaculture Animal and Seaweed Production. Off. J. Eur. Union. 204, 15-34.

European Medicines Agency, 2017. Assessment report on *Allium sativum* L., bulbus. EMA/HMPC/7686/2013 Committee on Herbal Medicinal Products (HMPC). 90

European Pharmacopoeia Commission, 2018. Tannins in herbal drugs (method 2.8.14), in European Pharmacopoeia. European Directorate for the Quality of Medicines (EDQM): Strasbourg. p. 275-276.

Everse, J. and N. Hsia, 1997. The toxicities of native and modified hemoglobins. Free Radical Biology and Medicine. 22, 1075-1099.

Fabricant, D.S., and N.R. Farnsworth, 2001. The value of plants used in traditional medicine for drug discovery. Environmental health perspectives. 109, 69-75.

Fadhlaoui-Zid, K., J.A. Curiel, G. Landeta, S. Fattouch, I. Reverón, B. de las Rivas, S. Sadok, and R. Muñoz, 2012. Biogenic amine production by bacteria isolated from ice-preserved sardine and mackerel. Food Control. 25, 89-95.

Faleiro, M.L., 2011. The mode of antibacterial action of essential oils. Science against microbial pathogens: communicating current research and technological advances. 2, 1143-1156.

Falguera, V., J.P. Quintero, A. Jiménez, J.A. Muñoz, and A. Ibarz, 2011. Edible films and coatings: Structures, active functions and trends in their use. Trends in Food Science & Technology. 22, 292-303.

Fan, W., J. Sun, Y. Chen, J. Qiu, Y. Zhang, and Y. Chi, 2009. Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food chemistry. 115, 66-70.

Fan, W., Y. Chi, and S. Zhang, 2008. The use of a tea polyphenol dip to extend the shelf life of silver carp (*Hypophthalmicthys molitrix*) during storage in ice. Food chemistry. 108, 148-153.

Farahi, A., M. Kasiri, M. Sudagar, M. Soleimani Iraei, and S. Zorriehzahra, 2012. Effect of dietary supplementation of *Melissa officinalis* and *Aloe vera* on hematological traits, lipid oxidation of carcass and performance in rainbow trout (*Oncorhynchus mykiss*). Online journal of animal and feed research. 1, 1-5.

Farahi, A., M. Kasiri, M. Sudagar, M.S. Iraei, and M.D. Shahkolaei, 2010. Effect of garlic (*Allium sativum*) on growth factors, some hematological parameters and body compositions in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, Aquarium, Conservation & Legislation. 3, 317-323.

Fawole, F.J., N.P. Sahu, A.K. Pal, and A. Ravindran, 2016. Haemato-immunological response of *Labeo rohita* (Hamilton) fingerlings fed leaf extracts and challenged by *Aeromonas hydrophila*. Aquaculture Research. 47, 3788-3799.

FDA, 1995. US Food and Drug Administration, Decomposition and histamine—raw, frozen tuna and mahi-mahi, canned tuna, and related species; revised compliance guide; availability. Federal Register. 149, 39754-39756.

FDA, 2001. US Food and Drug Administration, Scombrotoxin (histamine) formation, in Fish and fishery products hazards and controls guide. Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood: Washington, DC. p. 73-93.

Fei, M.L., L.I. Tong, L.I. Wei, and L. De Yang, 2015. Changes in antioxidant capacity, levels of soluble sugar, total polyphenol, organosulfur compound and constituents in garlic clove during storage. Industrial Crops and Products. 69, 137-142.

Ferguson, H., J. Turnbull, A. Shinn, K. Thompson, T.T. Dung, and M. Crumlish, 2001. Bacillary necrosis in farmed *Pangasius hypophthalmus* (Sauvage) from the Mekong Delta, Vietnam. Journal of Fish Diseases. 24, 509-513.

Fernandes, C.F., G.J. Flick, and T.B. Thomas, 1998. Growth of inoculated psychrotrophic pathogens on refrigerated fillets of aquacultured rainbow trout and channel catfish. Journal of food Protection. 61, 313-317.

Fernandes, S.R., L. Barreiros, R.F. Oliveira, A. Cruz, C. Prudêncio, A.I. Oliveira, C. Pinhoa, N. Santos, and J. Morgado, 2019. Chemistry, bioactivities, extraction and analysis of azadirachtin: State-of-the-art. Fitoterapia. 134, 141-150.

Fernández-Pérez, R., C. Tenorio, and F. Ruiz-Larrea, 2018. Effect of wine polyphenol extracts on the growth of *Escherichia coli*. Exploring Microorganisms: Recent Advances in Applied Microbiology. 226.

Foo, L.Y. and H. Wong, 1992. *Phyllanthusiin* D, an unusual hydrolysable tannin from *Phyllanthus amarus.* Phytochemistry. 31, 711-713.

Foo, L.Y., 1993. Amariin, a di-dehydrohexahydroxydiphenoyl hydrolysable tannin from *Phyllanthus amarus.* Phytochemistry. 33, 487-491.

Foo, L.Y., 1995. Amariinic acid and related ellagitannins from *Phyllanthus amarus.* Phytochemistry. 39, 217-224.

Formanek, Z., J. Kerry, F. Higgins, D. Buckley, P. Morrissey, and J. Farkas, 2001. Addition of synthetic and natural antioxidants to α-tocopheryl acetate supplemented beef patties: effects of antioxidants and packaging on lipid oxidation. Meat science. 58, 337-341.

Francis, G., H.P. Makkar, and K. Becker, 2005. *Quillaja saponins*—a natural growth promoter for fish. Animal feed science and technology. 121, 147-157.

Françoise, L., 2010. Occurrence and role of lactic acid bacteria in seafood products. Food microbiology. 27, 698-709.

Frankel, E., 2005. Lipid oxidation. Bridgewater, United Kingdom: The Oily Press.

FSA. 2022. Food standards agency. Approved additives and E numbers. Available online: https://www.food.gov.uk/business-guidance/approved-additives-and-e-numbers.

García-Soto, B., K. Böhme, J. Barros-Velázquez, and S.P. Aubourg, 2014. Inhibition of quality loss in chilled megrim (*Lepidorhombus whiffiagonis*) by employing citric and lactic acid icing. International journal of food science & technology. 49, 18-26.

Gatlin, D.M., 2007. Dietary supplements for the health and quality of cultured fish, ed. H. Nakagawa, M. Sato, and D.M. Gatlin. Oxon: CABI Publishing. 244.

Gbadamosi, I.T., 2015. Antibacterial attributes of extracts of *Phyllantus amarus* and *Phyllantus niruri* on *Escherichia coli* the causal organism of urinary tract infection. Journal of Pharmacognosy and Phytotherapy. 7, 80-86.

Gennari, M., S. Tomaselli, and V. Cotrona, 1999. The microflora of fresh and spoiled sardines (*Sardina pilchardus*) caught in Adriatic (Mediterranean) Sea and stored in ice. Food microbiology. 16, 15-28.

German, J.B., S.E. Chen, and J.E. Kinsella, 1985. Lipid oxidation in fish tissue. Enzymic initiation via lipoxygenase. Journal of Agricultural and Food Chemistry. 33, 680-683.

Ghaly, A.E., D. Dave, S. Budge, and M. Brooks, 2010. Fish spoilage mechanisms and preservation techniques. American journal of applied sciences. 7, 859.

Ghehdarijani, M.S., A. Hajimoradloo, R. Ghorbani, and Z. Roohi, 2016. The effects of garlic-supplemented diets on skin mucosal immune responses, stress resistance and growth performance of the Caspian roach (*Rutilus rutilus*) fry. Fish & Shellfish Immunology. 49, 79-83.

GHS, 2017. Globally harmonized system of classification and labelling of chemicals, United Nations: New York and Geneva. p. 527.

Gibis, M., 2007. Effect of oil marinades with garlic, onion, and lemon juice on the formation of heterocyclic aromatic amines in fried beef patties. Journal of agricultural and food chemistry. 55, 10240-10247.

Gill, C., 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. Journal of Food Protection. 46, 444-452.

Giménez, B., P. Roncalés, and J.A. Beltrán, 2004. The effects of natural antioxidants and lighting conditions on the quality characteristics of gilt-head sea bream fillets (*Sparus aurata*) packaged in a modified atmosphere. Journal of the Science of Food and Agriculture. 84, 1053-1060.

Giri, S.S., S.S. Sen, C. Chi, H.J. Kim, S. Yun, S.C. Park, and V. Sukumaran, 2015. Effect of guava leaves on the growth performance and cytokine gene expression of *Labeo rohita* and its susceptibility to *Aeromonas hydrophila* infection. Fish & Shellfish Immunology. 46, 217-224.

Gobi, N., C. Ramya, B. Vaseeharan, B. Malaikozhundan, S. Vijayakumar, K. Murugan, and G. Benelli, 2016. *Oreochromis mossambicus* diet supplementation with *Psidium guajava* leaf

extracts enhance growth, immune, antioxidant response and resistance to *Aeromonas hydrophila*. Fish & Shellfish Immunology. 58, 572-583.

Goel, G., H.P., Makkar, G., Francis, and K., Becker, 2007. Phorbol esters: structure, biological activity, and toxicity in animals. International journal of toxicology. 26, 279-288.

Gokoglu, N. and P. Yerlikaya, 2015. Seafood chilling, refrigeration and freezing: science and technology. John Wiley & Sons. 248.

Gonzalez-Burgos, E., and M.P. Gomez-Serranillos, 2012. Terpene compounds in nature: a review of their potential antioxidant activity. Current medicinal chemistry. 19, 5319-5341.

Gram, L. and H.H. Huss, 1996. Microbiological spoilage of fish and fish products. International journal of food microbiology. 33, 121-137.

Gram, L. and H.H. Huss, 2000. Fresh and processed fish and shellfish, in The microbiological safety and quality of food. Aspen Publishers. p. 472-506.

Gram, L. and P. Dalgaard, 2002. Fish spoilage bacteria–problems and solutions. Current opinion in biotechnology. 13, 262-266.

Gram, L., 1993. Inhibitory effect against pathogenic and spoilage bacteria of *Pseudomonas* strains isolated from spoiled and fresh fish. Applied and Environmental Microbiology. 59, 2197-2203.

Gram, L., 2009. Microbiological spoilage of fish and seafood products, in Compendium of the microbiological spoilage of foods and beverages. Springer. p. 87-119.

Gram, L., C. Wedell-Neergaard, and H.H. Huss, 1990. The bacteriology of fresh and spoiling Lake Victorian Nile perch (*Lates niloticus*). International journal of food microbiology. 10, 303-316.

Gram, L., J. Oundo, and J. Bon, 1989. Storage life of Nile perch (*Lates niloticus*) in relation to temperature and intial bacterial load. Tropical science. 29, 221-236.

Greeshma, S., K. Sarika, E. Priya, L. Kumar, T. Joseph, M. Pandey, and M. Prasad, 2019. Shelf life characteristics of *Pangasianodon hypophthalmus* fillets treated with *Moringa oleifera* (Lam) leaf extract. Fishery Technology. 56, 285 – 291.

Grigorakis, K., I. Giogios, A. Vasilaki, and I. Nengas, 2010. Effect of the fish oil, oxidation status and of heat treatment temperature on the volatile compounds of the produced fish feeds. Animal feed science and technology. 158, 73-84.

GSO, 2018. General Statistics Office of Vietnam, Production of fishery. http://www.gso.gov.vn/default_en.aspx?tabid=778.

GSO, 2021. General statistics office. Aquaculture production; Available from: https://www.gso.gov.vn/px-web-2/?pxid=V0655&theme=N%C3%B4ng%2C%20l%C3%A2m%20nghi%E1%BB%87p%20v%C3%A0%20th%E1%BB%A7y%20s%E1%BA%A3n.

Gu, H.-F., C.-M. Li, Y.-j. Xu, W.-f. Hu, M.-h. Chen, and Q.-h. Wan, 2008. Structural features and antioxidant activity of tannin from persimmon pulp. Food Research International. 41, 208-217.

Guardone, L., L. Tinacci, A. Armani, and M. Trevisani, 2022. Residues of veterinary drugs in fish and fish products: An analysis of RASFF data over the last 20 years. Food Control. 135, 108780.

Guha, G., V. Rajkumar, R.A. Kumar, and L. Mathew, 2010. Aqueous extract of *Phyllanthus amarus* inhibits chromium (VI)-induced toxicity in MDA-MB-435S cells. Food and chemical toxicology. 48, 396-401.

Guimarães, C.F.M., E.T. Mársico, M.L.G. Monteiro, M. Lemos, S.B. Mano, and C.A. Conte Junior, 2016. The chemical quality of frozen Vietnamese *Pangasius hypophthalmus* fillets. Food science & nutrition. 4, 398-408.

Gupta, D., M. Kumar, and V. Gupta, 2018. An *in vitro* investigation of antimicrobial efficacy of *Euphorbia hirta* and *Murraya koenigii* against selected pathogenic microorganisms. Asian journal of pharmaceutical and clinical research. 11, 359-363.

Gurusamy, K. and P. Saranya, 2010. *In vitro* antioxidant potential of ethanolic contents of *Eclipta alba* and *Wedelia chinensis.* Journal of Pharmacy Research. 3, 2825-2827.

Gutierrez, R.M., S. Mitchell, and R.V. Solis, 2008. *Psidium guajava*: A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology. 117, 1–27.

Gyawali, R., S. Hayek, and S.A. Ibrahim, 2015. Plant extracts as antimicrobials in food products: Mechanisms of action, extraction methods, and applications. Handbook of natural antimicrobials for food safety and quality. 1, 49-68.

Ha, N.C., L.T.B. Hiep, L.V. Toan, L.T.H. Nho, N.T.N. Tuyet, and N.T.L. Ngoc, 2021. Ergothioneine accumulated in the catfish muscle by supplementation of *Flammulina velutipes*

extract prevents lipid oxidation, improves color stability, and limits soft structure of the fillet during frozen storage. Journal of Food Processing and Preservation. 45, e15340.

Hai, V.D., S.M.T. Hoang, N.T.Q. Hung, N.M. Ky, B. Gwi-Nam, P-h. Park, S.W. Chang, Q-V. Bach, T.N.T. Tran, and D.D. Nguyen, 2019. Characteristics of airborne bacteria and fungi in the atmosphere in Ho Chi Minh city, Vietnam-A case study over three years. International biodeterioration & biodegradation. 145, 104819.

Hammer, K.A., C.F. Carson, and T.V. Riley, 1999. Antimicrobial activity of essential oils and other plant extracts. Journal of applied microbiology. 86, 985-990.

Hamre, K., K. Kolås, and K. Sandnes, 2010. Protection of fish feed, made directly from marine raw materials, with natural antioxidants. Food Chemistry. 119, 270-278.

Hanh, D., 2005. Discussion on the use of antibiotics and chemicals in seafood production and processing. Journal of Fishery. 2, 16-20.

Harborne, J.B., 1999. Classes and functions of secondary products from plants. Chemicals from plants. 26, 1-25.

Harikrishnan, R., C. Balasundaram, and M.-S. Heo, 2011. Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture. 317, 1-15.

Harwood, M., B. Danielewska-Nikiel, J.F. Borzelleca, G.W. Flamm, G.M. Williams, and T.C. Lines. 2007. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association. 45, 2179–2205.

Haslam, E., 1989. Plant polyphenols: vegetable tannins revisited. Cambridge, UK: Cambridge University Press. 230.

Hassan, A., S. Rahman, F. Deeba, and S. Mahmud, 2009. Antimicrobial activity of some plant extracts having hepatoprotective effects. Journal of Medicinal Plants Research. 3, 020-023.

Hawke, J.P., A.C. McWhorter, A.G. Steigerwalt, and D.J. Brenner, 1981. *Edwardsiella ictaluri* sp. nov., the causative agent of enteric septicemia of catfish. International Journal of Systematic and Evolutionary Microbiology. 31, 396-400.

Health Canada, 2021. List of Permitted Preservatives. Available from: https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/food-additives/lists-permitted/11-preservatives.html.

Henderson, R.J. and D.R. Tocher, 1987. The lipid composition and biochemistry of freshwater fish. Progress in lipid research. 26, 281-347.

Hernández, A., B.G. García, M. Jordán, and M. Hernández, 2014a. Improved conservation of gilthead seabream (*Sparus aurata*) in ice storage. The influence of doses of rosemary extract added to feed. Aquaculture. 426, 31-40.

Hernández, A., B.G. García, M. Jordán, and M. Hernández, 2014b. Natural antioxidants in extruded fish feed: Protection at different storage temperatures. Animal Feed Science and Technology. 195, 112-119.

Ho, P.H., 2003a. An illustrated flora of Vietnam. Vol. 3. Ho Chi Minh City: Tre Publishing House. 1020.

Ho, P.H., 2003b. An illustrated flora of Vietnam. Vol. 2. Ho Chi Minh City: Tre Publishing House. 951.

Ho, P.H., 2003c. An illustrated flora of Vietnam. Vol. 1. Ho Chi Minh City: Tre Publishing House. 991.

Ho, Y.-L., S.-S. Huang, J.-S. Deng, Y.-H. Lin, Y.-S. Chang, and G.-J. Huang, 2012. *In vitro* antioxidant properties and total phenolic contents of wetland medicinal plants in Taiwan. Botanical studies. 53, 55-66.

Hoa, N.P., 2012. The use of Yucca extract in aquaculture, in Principals and technique in aquaculture, T.Q. Phu, Editor., Agriculture Publisher: HCMC. p. 170.

Hoe, T.D., N.T.N. Thuy, T.T.V. Ha, L.T.B. Ngoc, and P.K. Thu, 2016. Report on Vietnam Seafood exports Q.III/2016, in Vietnam Association of Seafood Exporters and Producers, H. L., Editor.

Holley, R., and D. Patel, 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food microbiology. 22, 273-292.

Hornero-Méndez, D., A. Pérez-Gálvez, and M.I. Mínguez-Mosquera, 2001. A rapid spectrophotometric method for the determination of peroxide value in food lipids with high carotenoid content. Journal of the American Oil Chemists' Society. 78, 1151-1155.

Hoseinifar, S.H., A. Sohrabi, H. Paknejad, V. Jafari, M. Paolucci, and H. Van Doan, 2019. Enrichment of common carp (*Cyprinus carpio*) fingerlings diet with *Psidium guajava*: the effects on cutaneous mucosal and serum immune parameters and immune related genes expression. Fish & shellfish immunology. 86, 688-694.

Hoseinifar, S.H., M. Khalili, R. Rufchaei, M. Raeisi, M. Attar, H. Cordero, and M.Á. Esteban, 2015. Effects of date palm fruit extracts on skin mucosal immunity, immune related genes expression and growth performance of common carp (*Cyprinus carpio*) fry. Fish & shellfish immunology. 47, 706-711.

Hossain, M., M. Islam, F. Shikha, M. Kamal, and M. Islam, 2005. Physicochemical changes in thai pangas (*Pangasius sutchi*) muscle during ice-storage in an insulated box. Pakistan Journal of Biological Sciences. 8, 798-804.

Hossain, M.A., M. Kabir, S. Salehuddin, S.M. Rahman, A. Das, S.K. Singha, M.K. Alam, and A. Rahman, 2010. Antibacterial properties of essential oils and methanol extracts of sweet basil *Ocimum basilicum* occurring in Bangladesh. Pharmaceutical biology. 48, 504-511.

Houghton, P. and A. Raman, 1998. Methods for extraction and sample clean-up, in Laboratory handbook for the fractionation of natural extracts. Chapman and Hall: London, UK. p. 199.

Houicher, A., A. Bensid, J.M. Regenstein, and F. Özogul, 2021. Control of biogenic amine production and bacterial growth in fish and seafood products using phytochemicals as biopreservatives: A review. Food Bioscience. 39, 100807.

Hraš, A.R., M. Hadolin, Ž. Knez, and D. Bauman, 2000. Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food chemistry. 71, 229-233.

Hsieh, R. and J. Kinsella, 1989. Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. Advances in food and nutrition research. 33, 233-341.

Hue Ngan, D., H.T.C. Hoai, L. Mai Huong, P.E. Hansen, and O. Vang, 2008. Bioactivities and chemical constituents of a Vietnamese medicinal plant Che Vang, *Jasminum subtriplinerve* Blume (Oleaceae). Natural Product Research. 22, 942-949.

Hultmann, L., T.M. Phu, T. Tobiassen, Ø. Aas-Hansen, and T. Rustad, 2012. Effects of pre-slaughter stress on proteolytic enzyme activities and muscle quality of farmed Atlantic cod (*Gadus morhua*). Food chemistry. 134, 1399-1408.

Huss, H.H., 1988. Fresh fish--quality and quality changes: a training manual prepared for the FAO/DANIDA Training Programme on Fish Technology and Quality Control. Food & Agriculture Org.

Huss, H.H., 1995. Quality and quality changes in fresh fish. Vol. 348. FAO Rome.

Huss, H.H., A. Reilly, and P.K.B. Embarek, 2000. Prevention and control of hazards in seafood. Food control. 11, 149-156.

ICMSF, 1986. Sampling plans for fish and shellfish. International Commission on Microbiological Specifications for Foods, Microorganisms in Foods. Sampling for Microbiological Analysis: Principles and Scientific Applications. 2, 181-196.

ICMSF, 2005. Microbiological Ecology of Food Commodities, in Micro-organisms in Food 6, J.-L.C. T. A. Roberts, L. GramR. B. TompkinJ. I. Pitt, L. G. M. GorrisK. M. J. Swanson, Editor., Kluwer Academic/Plenum Publishers: New York. p. 174–249.

Igwe, C.U., L.A. Nwaogu, and C.O. Ujuwondu, 2007. Assessment of the hepatic effects, phytochemical and proximate compositions of *Phyllanthus amarus.* African Journal of Biotechnology. 6, 728-731.

Ilangkovan, M., I. Jantan, M.A. Mesaik, and S.N.A. Bukhari, 2015. Immunosuppressive effects of the standardized extract of *Phyllanthus amarus* on cellular immune responses in Wistar-Kyoto rats. Drug design, development and therapy. 9, 4917.

Ionescu, M.I., 2017. Are herbal products an alternative to antibiotics?, in Bacterial Pathogenesis and Antibacterial Control, Sahra Kırmusaoğlu, Editor, IntechOpen. P. 3-22.

Ingold, K.V., 1962. Metal catalysis, in Symposium on Foods: Lipids and their Oxidation, H.W. Schultz, E.A. Day, and R.O. Sinnhuber, Editors., Avi Pub. Co.: Westport, CT. p. 93.

International IDF standards, 1991. Section 74A: 1991. International Dairy Federation, IDF-square Vergot 41.

Israel, G.D, 1992. Determining sample size. PEOD6. University of Florida. p. 1-5.

Ito, N., S. Fukushima, and H. Tsuda, 1985. Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. CRC Critical reviews in Toxicology. 15, 109-150.

Jamieson, S., C.E. Wallace, N. Das, P. Bhattacharyya, and A. Bishayee, 2021. Guava (*Psidium guajava* L.): a glorious plant with cancer preventive and therapeutic potential. Critical Reviews in Food Science and Nutrition 1-32.

Jamkhande, P.G., A.S. Wattamwar, A.D. Kankudte, P.S. Tidke, and M.G. Kalaskar, 2016. Assessment of *Annona reticulata* Linn. leaves fractions for invitro antioxidative effect and antimicrobial potential against standard human pathogenic strains. Alexandria Journal of Medicine. 52, 19-25.

Jayalakshmi, B., K. Raveesha, M. Murali, and K. Amruthesh, 2015. Phytochemical, antibacterial and antioxidant studies on leaf extracts of *Piper betle* L. International Journal of Pharmacy and Pharmaceutical Sciences. 7, 23-29.

Jeyakumari, A., G. Ninan, C. Joshy, U. Parvathy, A. Zynudheen, and K. Lalitha, 2016. Effect of chitosan on shelf life of restructured fish products from pangasius (*Pangasianodon hypophthalmus*) surimi during chilled storage. Journal of Food science and Technology. 53, 2099-2107.

Jeyakumari, A., R. Roy, G. Ninan, and V. Renuka, 2017. Effect of Spice Extracts on the Biochemical, Textural and Sensory Attributes of Pangasius (*Pangasianodon hypophthalmus*) Chunks During Ice Storage. The Indian Journal of Nutrition and Dietetics. 54, 149-160.

Ji, H.F., H.Y. Zhang, and L. Shen, 2006. Proton dissociation is important to understanding structure–activity relationships of gallic acid antioxidants. Bioorganic & Medicinal Chemistry Letters. 16, 4095-4098.

Jung, M., M. Park, H.C. Lee, Y.H. Kang, E.S. Kang, and S.K. Kim, 2006. Antidiabetic agents from medicinal plants. Current medicinal chemistry. 13, 1203-1218.

Kalac, P. and P. Krausová, 2005. A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods. Food Chemistry. 90, 219-230.

Kaleeswaran, B., S. Ilavenil, and S. Ravikumar, 2011a. Dietary supplementation with *Cynodon dactylon* (L.) enhances innate immunity and disease resistance of Indian major carp, *Catla catla* (Ham.). Fish & shellfish immunology. 31, 953-962.

Kaleeswaran, B., S. Ilavenil, and S. Ravikumar, 2011b. Growth response, feed conversion ratio and antiprotease activity of *Cynodon dactylon* (L.) mixed diet in *Catla catla* (Ham.). Journal of Animal and Veterinary Advances 10, 511-517.

Kalita, D., R. Kar, and J.G. Handique, 2012. A theoretical study on the antioxidant property of gallic acid and its derivatives. Journal of Theoretical and Computational Chemistry. 11, 391-402.

Kamel, C., 2001. Tracing modes of action and the roles of plant extracts in non-ruminants. Recent advances in animal nutrition: 2001. 135-150.

Karl, H., I. Lehmann, H. Rehbein, and R. Schubring, 2010. Composition and quality attributes of conventionally and organically farmed Pangasius fillets (*Pangasius hypophthalmus*) on the German market. International journal of food science & technology. 45, 56-66.

Karou, D., A. Savadogo, A. Canini, S. Yameogo, C. Montesano, J. Simpore, V. Colizzi, and A.S. Traore, 2006. Antibacterial activity of alkaloids from *Sida acuta.* African journal of biotechnology. 5, 195-200.

Kaveti, B., L. Tan, K.T. Sarnnia, and M. Baig, 2011. Antibacterial activity of *Piper betle* leaves. International Journal of Pharmacy Teaching and Practices. 2, 129-132.

Khalili Tilami, S. and S. Sampels, 2018. Nutritional value of fish: lipids, proteins, vitamins, and minerals. Reviews in Fisheries Science & Aquaculture. 26, 243-253.

Khan, S., F. Al-Qurainy, M. Ram, S. Ahmad, and M.Z. Abdin, 2010. Phyllanthin biosynthesis in *Phyllanthus amarus*: Schum and Thonn growing at different altitudes. Journal of Medicinal Plants Research. 4, 041-048.

Khayat, A. and D. Schwall, 1983. Lipid oxidation in seafood. Food Technology (USA).

Kim, S.-J., S.C. Min, H.-J. Shin, Y.-J. Lee, A.R. Cho, S.Y. Kim, and J. Han, 2013. Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. Meat Science. 93, 715-722.

Kirubakaran, C.J.W., C.P. Alexander, and R.D. Michael, 2010. Enhancement of non-specific immune responses and disease resistance on oral administration of *Nyctanthes arbortristis* seed extract in *Oreochromis mossambicus* (Peters). Aquaculture Research. 41, 1630-1639.

Kiyama, R., 2017. Estrogenic terpenes and terpenoids: Pathways, functions and applications. European Journal of Pharmacology. 815, 405-415.

Koleckar, V., K. Kubikova, Z. Rehakova, K. Kuca, D. Jun, L. Jahodar, and L. Opletal, 2008. Condensed and hydrolysable tannins as antioxidants influencing the health. Mini reviews in medicinal chemistry. 8, 436-447.

Koltover, V., 2010. Antioxidant biomedicine: from free radical chemistry to systems biology mechanisms. Russian Chemical Bulletin. 59, 37-42.

Kostyra, E. and N. Baryłko-Pikielna, 2006. Volatiles composition and flavour profile identity of smoke flavourings. Food Quality and Preference. 17, 85-95.

Kuete, V., 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta medica. 76, 1479-1491.

Kumar Sarangi, M. and S. Padhi, 2017. Dengue and its phytotherapy: A review. International Journal of Pharmaceutical and Phytopharmacological Research. 4, 37-46.

Kumar, S., R. Malhotra, and D. Kumar, 2010. *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. Pharmacognosy reviews. 4, 58.

Kumaran, A. and R.J. Karunakaran, 2007. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT-Food Science and Technology. 40, 344-352.

Labuza, T.P. and J.L. Dugan, 1971. Kinetics of lipid oxidation in foods. Critical Reviews in Food Science & Nutrition. 2, 355-405.

Lahlou, M., 2004. Methods to study the phytochemistry and bioactivity of essential oils. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 18, 435-448.

Lakshmanan, R., J.R. Piggott, and A. Paterson, 2003. Potential applications of high pressure for improvement in salmon quality. Trends in Food Science & Technology. 14, 354-363.

Lanigan, R.S., and T.A. Yamarik, 2002. Final report on the safety assessment of BHT (1). International Journal of Toxicology. 21, 19-94.

Le, Q.U., H.L. Lay and M.C. Wu, 2018. Antioxidant activities and HepG2 cells growth inhibitory capacity of whole plant ethanol extracts (*Eclipta alba* Hassk and *Mesona procumbens* Hemsl). Journal of food biochemistry. 42, e12454.

Lee, D.-H., S.-R. Lim, C.-S. Ra, and J.-D. Kim, 2012. Effects of dietary garlic extracts on whole body amino acid and fatty acid composition, muscle free amino acid profiles and blood plasma changes in juvenile sterlet sturgeon, Acipenser ruthenus. Asian-Australasian journal of animal sciences. 25, 1419.

Li, C., Q. Xu, H. Xu, and T. Zhang, 2008. Effects of different feed additives on immunity and antioxidation on rainbow trout (*Oncrhynchus mykiss* Walbaum). Journal of Anhui Agricultural University. 35, 456-461.

Li, H., D. Yang, Z. Li, M. He, F. Li, J. Jiang, S. Tang, P. Peng, W. Du, and Y. Ma, 2019. Effects of Angelica sinensis extracts on lipid oxidation in fish feeds and growth performance of juvenile Jian carp (*Cyprinus carpio* var. Jian). Animal Nutrition. 5, 109-114.

Li, H., X. Zhou, P. Gao, Q. Li, H. Li, R. Huang, and M. Wu, 2016. Inhibition of lipid oxidation in foods and feeds and hydroxyl radical-treated fish erythrocytes: a comparative study of *Ginkgo biloba* leaves extracts and synthetic antioxidants. Animal Nutrition. 2, 234-241.

Li, T., W. Hu, J. Li, X. Zhang, J. Zhu, and X. Li, 2012a. Coating effects of tea polyphenol and rosemary extract combined with chitosan on the storage quality of large yellow croaker (*Pseudosciaena crocea*). Food Control. 25, 101-106.

Li, T., J. Li, W. Hu, X. Zhang, X. Li, and J. Zhao, 2012b. Shelf-life extension of crucian carp (*Carassius auratus*) using natural preservatives during chilled storage. Food Chemistry. 135, 140-145.

Limpisophon, K., and G. Schleining, 2017. Use of gallic acid to enhance the antioxidant and mechanical properties of active fish gelatin film. Journal of Food Science. 82, 80-89.

Lin, E.-S., H.-J. Chou, P.-L. Kuo, and Y.-C. Huang, 2010. Antioxidant and antiproliferative activities of methanolic extracts of *Perilla frutescens.* Journal of Medicinal Plants Research. 4, 477-483.

Lin, Y., R. Labbe, and K. Shetty, 2004. Inhibition of *Listeria monocytogenes* in fish and meat systems by use of oregano and cranberry phytochemical synergies. Applied and environmental microbiology. 70, 5672-5678.

Linhartová, Z., R. Lunda, P. Dvořák, J. Bárta, V. Bártová, J. Kadlec, E. Samková, J. Bedrníček, M. Pešek, and I. Laknerová, 2019. Influence of rosemary extract (*Rosmarinus officinalis*) Inolens to extend the shelf life of vacuum-packed rainbow trout (*Oncorhynchus mykiss*) fillets stored under refrigerated conditions. Aquaculture International. 27, 833-847.

Liston, J. Microbiology in fishery science. in Advances in fish science and technology: papers presented at the Jubilee conference of the Torry Research Station, Aberdeen, Scotland, 23-27 July 1979, edited by JJ Connell and staff of Torry Research Station. 1980. Farnham, Surrey, England, Fishing News Books, 1980.

Liu, R., and S.A. Mabury, 2019. Synthetic phenolic antioxidants in personal care products in Toronto, Canada: Occurrence, human exposure, and discharge via greywater. Environmental Science & Technology. 53, 13440-13448.

Liu, R. and S.A. Mabury, 2020. Synthetic phenolic antioxidants: A review of environmental occurrence, fate, human exposure, and toxicity. Environmental science & technology. 54, 11706-11719.

Liu, X., M. Zhao, J. Wang, B. Yang, and Y. Jiang, 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. Journal of food composition and Analysis. 21, 219-228.

Liu, X., Z. Wei, L. Fan, D.e. Ge, F. Wang, and J. Zhao, 2018. Effect of Chinese rice wine on proteolytic characterization originating from endogenous proteases in topmouth culter (*Culter alburnus*). PeerJ Preprints. 6, e26568v1.

Lobo, V., A. Patil, A. Phatak, and N. Chandra, 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews. 4, 118.

Logambal, S., S. Venkatalakshmi, and R. Dinakaran Michael, 2000. Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). Hydrobiologia. 430, 113-120.

Lombardi, G., L. Cossignani, L. Giua, M.S. Simonetti, A. Maurizi, G. Burini, R. Coli, and F. Blasi, 2017. Phenol composition and antioxidant capacity of red wines produced in Central Italy changes after one-year storage. J. Appl. Bot. Food Qual. 90, 197-204.

Lone, G. and H. Hans, 1996. Microbiological spoilage of fish and fish products. Int. J. Fd. Microbiol. 33, 121-137.

Lovkova, M.Y., G. Buzuk, S. Sokolova, and N. Kliment'eva, 2001. Chemical features of medicinal plants. Applied Biochemistry and Microbiology. 37, 229-237.

Low Dog, T. 2009. Smart Talk on Supplements and Botanicals: Flor de Jamaica-Herbal Diuretics-Hawthorn. Alternative and Complimentary Therapies. 15, 214-215.

Luc, N.T., L. Du, and N. Dzung, 2013. Optimization of the smoking process of pangasius fish fillet to increase the product quality. Advance Journal of Food Science and Technology. 5, 206-212.

Luna, A., R. Lema-Alba, J.S. Dambolena, J.A. Zygadlo, M.C. Lábaque, and R.H. Marin, 2017. Thymol as natural antioxidant additive for poultry feed: oxidative stability improvement. Poultry science. 96, 3214-3220.

Lundebye, A.-K., H. Hove, A. Måge, V. Bohne, and K. Hamre, 2010. Levels of synthetic antioxidants (ethoxyquin, butylated hydroxytoluene and butylated hydroxyanisole) in fish feed and commercially farmed fish. Food Additives & Contaminants: Part A. 27, 1652-1657.

Ly, L.T.T., D.N. Nguyen, P.H. Vo, and C.V. Doan, 2009. Hemorrhage disease of cultured tra catfish (*Pangasianodon hypophthalmus*) in Mekong Delta (Vietnam). The Israeli Journal of Aquaculture – Bamidgeh. 61, 215-224.

Madsen, H.L. and G. Bertelsen, 1995. Spices as antioxidants. Trends in food science & technology. 6, 271-277.

Magnadottir, B., 2010. Immunological control of fish diseases. Marine biotechnology. 12, 361-379.

Mahmoud, B.S., K. Yamazaki, K. Miyashita, S. Il-Shik, C. Dong-Suk, and T. Suzuki, 2004. Bacterial microflora of carp (*Cyprinus carpio*) and its shelf-life extension by essential oil compounds. Food Microbiology. 21, 657-666.

Mai, V.T., 2012. Use of veterinary medicines in Vietnamese aquaculture: current status. FAO Fisheries and Aquaculture Technical Paper. 91-98.

Mailoa, M.N., M. Mahendradatta, A. Laga, and N. Djide, 2014. Antimicrobial activities of tannins extract from guava leaves (*Psidium guajava* L.) on pathogens microbial. International Journal of Scientific & Technology Research. 3, 236-241.

Maqsood, S. and S. Benjakul, 2010. Synergistic effect of tannic acid and modified atmospheric packaging on the prevention of lipid oxidation and quality losses of refrigerated striped catfish slices. Food Chemistry. 121, 29-38.

Maqsood, S. and S. Benjakul, 2011. Effect of bleeding on lipid oxidation and quality changes of Asian seabass (*Lates calcarifer*) muscle during iced storage. Food chemistry. 124, 459-467.

Maqsood, S., S. Benjakul, and A. Kamal-Eldin, 2012. Haemoglobin-mediated lipid oxidation in the fish muscle: A review. Trends in Food Science & Technology. 28, 33-43.

Marissiaux, L., M.-L. Scippo, G. Daube, N. Botteldoorn, S. Gobert, A. Ghuysen, S. Denayer, and B. Scholtes, 2018. An awkward fishing expedition. Acta Anaesthesiologica Belgica. 69, 1-4.

Marwah, R.G., M.O. Fatope, R. Al Mahrooqi, G.B. Varma, H. Al Abadi, and S.K.S. Al-Burtamani, 2007. Antioxidant capacity of some edible and wound healing plants in Oman. Food chemistry. 101, 465-470.

Mazumder, A., A. Mahato, and R. Mazumder, 2006. Antimicrobial potentiality of *Phyllanthus amarus* against drug resistant pathogens. Natural product research. 20, 323-326.

McCord, J.M., and I. Fridovich, 1969. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). Journal of Biological chemistry. 244, 6049-6055.

Meriga, B., R. Mopuri, and T. MuraliKrishna, 2012. Insecticidal, antimicrobial and antioxidant activities of bulb extracts of *Allium sativum.* Asian Pacific journal of tropical medicine. 5, 391-395.

Metwally, M., 2009. Effects of garlic (*Allium sativum*) on some antioxidant activities in tilapia nilotica (*Oreochromis niloticus*). World Journal of fish and marine sciences. 1, 56-64.

Metwally, A.M., A.A. Omar, F.M. Harraz, and S.M. El Sohafy, 2010. Phytochemical investigation and antimicrobial activity of *Psidium guajava* L. leaves. Pharmacognosy magazine. 6, 212.

Mikage, M. and C. Mouri, 1999. Pharmacognostical studies of berberis plants (Berberidaceae) from Nepal (1): altitudinal, interspecific, and partial variations of berberine content in the bark. Natural medicines. 53, 249-254.

Milardović, S., D. Iveković, and B.S. Grabarić, 2006. A novel amperometric method for antioxidant activity determination using DPPH free radical. Bioelectrochemistry. 68, 175-180.

Minisci, F., 2013. Free radicals in biology and environment. Dordrecht, Netherlands: Springer Science Business Media, B.V. 500.

Mitra, R. and S. Jain, 1985. Concept of *Phyllanthus niruri* (Euphorbiaceae) in Indian floras. Nelumbo-The Bulletin of the Botanical Survey of India. 27, 161-176.

Mizushima, Y., K. Takama, and K. Zama, 1977. Effect of copper, iron and hemin on lipid oxidation in fish flesh homogenate. Bulletin of the Faculty of Fisheries Hokkaido University. 28, 207-211.

Mohan, C.O., C.N. Ravishankar, and T.K. Srinivasagopal, 2008. Effect of O_2 scavenger on the shelf-life of catfish (*Pangasius sutchi*) steaks during chilled storage. Journal of the Science of Food and Agriculture. 88, 442-448.

Mohebbi, A., A. Nematollahi, E.E. Dorcheh, and F.G. Asad, 2012. Influence of dietary garlic (*Allium sativ*um) on the antioxidative status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research. 43, 1184-1193.

Mojani, M., A. Ghasemzadeh, A. Rahmat, S. Loh, and R. Ramasamy, 2014. Assessment of bioactive compounds, nutritional composition and antioxidant activity of Malaysian young ginger (*Zingiber officinale* Roscoe). International Food Research Journal. 21, 1931.

Mošovská, S., D. Nováková, and M. Kaliňák, 2015. Antioxidant activity of ginger extract and identification of its active components. Acta Chimica Slovaca. 8, 115-119.

Muroi, H., and I. Kubo, 1996. Antibacterial activity of anacardic acid and totarol, alone and in combination with methicillin, against methicillinresistant *Staphylococcus aureus*. Journal of Applied Bacteriology. 80, 387-394.

NAFIQAD, 2021. National Agro-Forestry-Fisheries Quality Assurance Department. Monitoring Program for Certain Harmful Substances Residues in aquaculture fish and products thereof

in 2019 and Plan for 2020; Available from: http://www.nafiqad.gov.vn/Portals/0/DOCUMENTS/Report%20on%20Residues%202019-2020%20En.pdf.

Namdeo, A.G., 2018. Cultivation of medicinal and aromatic plants, in Natural products and drug discovery, S.C. Mandal, V. Mandal, and T. Konishi, Editors., Elsevier. p. 525-553.

Namhui, Y. and K. Bae, 2010. Screening of Vietnamese medicinal plants for cytotoxic activity. Natural product sciences. 16, 1-7.

Na-Phatthalung, P., M. Teles, S.P. Voravuthikunchai, L. Tort, and C. Fierro-Castro, 2018. Immunomodulatory effects of *Rhodomyrtus tomentosa* leaf extract and its derivative compound, rhodomyrtone, on head kidney macrophages of rainbow trout (*Oncorhynchus mykiss*). Fish physiology and biochemistry. 44, 543-555.

Narendhirakannan, R.T., J.G. Nirmala, A. Caroline, S. Lincy, M. Saj, and D. Durai, 2012. Evaluation of antibacterial, antioxidant and wound healing properties of seven traditional medicinal plants from India in experimental animals. Asian Pacific Journal of Tropical Biomedicine. 2, S1245-S1253.

Nawar, W.W., 1996. Lipids, in Food chemistry, O.R. Fennema, Editor., Marcel Dekker: New York. p. 225–320.

Neelabh, C., A. Nahid and N. Kumar, 2017. Study on methanolic extract of *Ageratum conyzoides* for its ability to act as an antioxidant and to suppress the microbial growth. The Pharma Innovation. 6, 170.

Nettleton, J.A. and J. Exler, 1992. Nutrients in wild and farmed fish and shellfish. Journal of Food Science. 57, 257-260.

Newman, S.G., 1993. Bacterial vaccines for fish. Annual Review of Fish Diseases. 3, 145-185.

Nguyen, A.L., M.H. Truong, J.A. Verreth, R. Leemans, R.H. Bosma, and S.S. De Silva, 2015. Exploring the climate change concerns of striped catfish producers in the Mekong Delta, Vietnam. SpringerPlus. 4, 1-8.

Nguyen, A.T., J. Fontaine, H. Malonne, M. Vanhaelen, J. Dubois, T. Ky Pham, and P. Duez, 2005. Cytotoxicity of five plants used as anticancer remedies in Vietnamese traditional medicine, in Recent Progress in Medicinal Plants, V.K. Singh, Singh, Arunachalam V.K.; Govil, J.N., Editor., Studium-Press: New Delhi, India. p. 145-155.

Nguyen, L.A.D., M.P. Tran, C. Douny, J. Quetin-Leclercq, T.B.H. Bui, T.B. Le, Q.N. Truong, T.B.H. Bui, T.T.H. Do, T.P. Nguyen, P. Kestemont, and M.-L. Scippo, 2020. Screening and comparative study of *in vitro* antioxidant and antimicrobial activities of ethanolic extracts of selected Vietnamese plants. International Journal of Food Properties. 23, 481-496.

Nguyen, Q.T., M.P. Tran, S.N. Huynh, S. Quennery, T.T.H. Do, T.P. Nguyen, P. Kestemont, and M.L. Scippo, 2014. Situation of chemicals used in rice-fish, stripped catfish cultured in pond and red tilapia cultured in cage in Mekong Delta. Can Tho University Journal of Science. 2, 278-283.

Nguyen, V., J. Sakoff, and C. Scarlett, 2017. Physicochemical properties, antioxidant and cytotoxic activities of crude extracts and fractions from *Phyllanthus amarus.* Medicines. 4, 42.

Nguyen, V.T., H.N.T. Pham, M.C. Bowyer, I.A. van Altena, and C.J. Scarlett, 2016. Influence of solvents and novel extraction methods on bioactive compounds and antioxidant capacity of *Phyllanthus amarus*. Chemical Papers. 70, 556-566.

Nguyen, V.T., Q. Van Vuong, M.C. Bowyer, I.A. Van Altena, and C.J. Scarlett, 2015. Effects of different drying methods on bioactive compound yield and antioxidant capacity of *Phyllanthus amarus.* Drying Technology. 33, 1006-1017.

Nhu, T.Q., B.T.B. Hang, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019a. Plant extract-based diets differently modulate immune responses and resistance to bacterial infection in striped catfish (*Pangasianodon hypophthalmus*). Fish & shellfish immunology. 92, 913-924.

Nhu, T.Q., B.T.B. Hang, A. Vinikas, B.T.B. Hue, J. Quetin-Leclercq, M.-L. Scippo, N.T. Phuong, and P. Kestemont, 2019b. Screening of immuno-modulatory potential of different herbal plant extracts using striped catfish (*Pangasianodon hypophthalmus*) leukocyte-based *in vitro* tests. Fish & shellfish immunology. 93, 296-307.

Nhu, T.Q., B.T.B. Hang, V. Cornet, M. Oger, L.T. Bach, N.L. Anh Dao, D.T. Thanh Huong, J. Quetin-Leclercq, M.-L. Scippo, and N.T. Phuong, 2020a. Single or combined dietary supply of *Psidium guajava* and *Phyllanthus amarus* extracts differentially modulate immune responses and liver proteome in striped catfish (*Pangasianodon hyphophthalmus*). Frontiers in immunology. 11, 797.

Nhu, T.Q., N.P. Dam, B.T.B. Hang, B.T.B. Hue, M.-L. Scippo, N.T. Phuong, J. Quetin-Leclercq, and P. Kestemont, 2020b. Immunomodulatory potential of extracts, fractions and pure compounds from *Phyllanthus amarus* and *Psidium guajava* on striped catfish (*Pangasianodon hypophthalmus*) head kidney leukocytes. Fish & Shellfish Immunology. 104, 289-303.

Nieva-Echevarría, B., M.J. Manzanos, E. Goicoechea, and M.D. Guillén, 2015. 2, 6-Di-tert-butyl-hydroxytoluene and its metabolites in foods. Comprehensive Reviews in Food Science and Food Safety. 14, 67-80.

Ninomiya M., H. Hatta, M. Fujiki, M. Kim, T.R. Yamamoto, 1995. Enhancement of chemotactic activity of yellowtail (*Seriola quinqueradiata*) leucocytes by oral administration of *Quillaja saponin*. Fish & Shellfish Immunology. 5, 325–328.

Nordic Committee on Food Analysis, 2006. Aerobic Plate Count in Foods. NMKL method 86, Nordic Committee on Food Analysis: Oslo, Norway.

Noseda, B., M.T. Islam, M. Eriksson, M. Heyndrickx, K. De Reu, H. Van Langenhove, and F. Devlieghere, 2012. Microbiological spoilage of vacuum and modified atmosphere packaged Vietnamese *Pangasius hypophthalmus* fillets. Food Microbiology. 30, 408-419.

Novoslavskij, A., M. Terentjeva, I. Eizenberga, O. Valciņa, V. Bartkevičs, and A. Bērziņš, 2016. Major foodborne pathogens in fish and fish products: a review. Annals of microbiology. 66, 1-15.

Nusbaum, K. and E. Morrison, 2002. Edwardsiella ictaluri bacteraemia elicits shedding of *Aeromonas hydrophila* complex in latently infected channel catfish, *Ictalurus punctatus* (Rafinesque). Journal of Fish Diseases. 25, 343-350.

Ocheng, F., F. Bwanga, M. Joloba, A.-K. Borg-Karlson, A. Gustafsson, and C. Obua, 2014. Antibacterial activities of extracts from Ugandan medicinal plants used for oral care. Journal of ethnopharmacology. 155, 852-855.

Ojagh, S.M., M.A. Sahari, and M. Rezaei, 2004. Effect of natural antioxidants on quality of common kilka (clupeonella cultriventris caspia) during storage with ice. Journal of marine sciences and technology. 3, 1-7.

Olafsdottir, G., E. Martinsdóttir, J. Oehlenschläger, P. Dalgaard, B. Jensen, I. Undeland, I. Mackie, G. Henehan, J. Nielsen, and H. Nilsen, 1997. Methods to evaluate fish freshness in research and industry. Trends in food science & technology. 8, 258-265.

Olatunde, O.O. and S. Benjakul, 2018. Natural preservatives for extending the shelf-life of seafood: A revisit. Comprehensive Reviews in Food Science and Food Safety. 17, 1595-1612.

Olowosulu, A.K., and Y.K.E. Ibrahim, 2006. Studies on the antimicrobial screening of aqueous extracts of five plants used in Folk medicine in Nigeria. West African Journal of Biological Science. 3, 21-6.

Oluboyo, B., A. Oluboyo, and S. Kalu, 2016. Inhibitory effects of *Phyllanthus amarus* extracts on the growth of some pathogenic microorganisms. African Journal of Clinical and Experimental Microbiology. 17, 166-172.

Omaye, S.T., J.D. Turnbull, and H.E. Sauberlich, 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids, in Methods in enzymology, J. Abelson, M. Simon, G. Verdine and A. Pyle, Editors., Academic press: London, p. 3-11.

Orban, E., T. Nevigato, G. Di Lena, M. Masci, I. Casini, L. Gambelli, and R. Caproni, 2008. New trends in the seafood market. Sutchi catfish (*Pangasius hypophthalmus*) fillets from Vietnam: Nutritional quality and safety aspects. Food Chemistry. 110, 383-389.

Ortega-Ramirez, L.A., I. Rodriguez-Garcia, J.M. Leyva, M.R. Cruz-Valenzuela, B.A. Silva-Espinoza, G.A. Gonzalez-Aguilar, M.W. Siddiqui, and J.F. Ayala-Zavala, 2014. Potential of medicinal plants as antimicrobial and antioxidant agents in food industry: a hypothesis. Journal of food science. 79, R129-R137.

Othman, A., A. Ismail, F.A. Hassan, B.N.M. Yusof and A. Khatib, 2016. Comparative evaluation of nutritional compositions, antioxidant capacities, and phenolic compounds of red and green sessile joyweed (Alternanthera *sessilis*). Journal of Functional Foods. 21, 263-271.

Ou, B., M. Hampsch-Woodill, J. Flanagan, E.K. Deemer, R.L. Prior, and D. Huang, 2002. Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. Journal of agricultural and food chemistry. 50, 2772-2777.

Oyaizu, M., 1986. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese journal of nutrition and dietetics. 44, 307-315.

Ozkan, A. and A. Erdogan, 2012. A comparative study of the antioxidant/prooxidant effects of carvacrol and thymol at various concentrations on membrane and DNA of parental and drug resistant H1299 cells. Natural product communications. 7, 1934578X1200701201.

Pachanawan, A., P. Phumkhachorn, and P. Rattanachaikunsopon, 2008. Potential of *Psidium guajava* supplemented fish diets in controlling *Aeromonas hydrophila* infection in tilapia (*Oreochromis niloticus*). Journal of bioscience and bioengineering. 106, 419-424.

Panangala, V.S., C.A. Shoemaker, V.L. Van Santen, K. Dybvig, and P.H. Klesius, 2007. Multiplex-PCR for simultaneous detection of 3 bacterial fish pathogens, *Flavobacterium columnare*, *Edwardsiella ictaluri*, and *Aeromonas hydrophila*. Diseases of aquatic organisms. 74, 199-208.

Parashar, A., C. Gupta, S.K. Gupta, and A. Kumar, 2009. Antimicrobial ellagitannin from pomegranate (*Punica granatum*) fruits. International journal of fruit Science. 9, 226-231.

Parlapani, F.F., K.A. Kormas, and I.S. Boziaris, 2015. Microbiological changes, shelf life and identification of initial and spoilage microbiota of sea bream fillets stored under various conditions using 16S rRNA gene analysis. Journal of the Science of Food and Agriculture. 95, 2386-2394.

Patel, J.R., P. Tripathi, V. Sharma, N.S. Chauhan, and V.K. Dixit, 2011. *Phyllanthus amarus*: ethnomedicinal uses, phytochemistry and pharmacology: a review. Journal of Ethnopharmacology. 138, 286-313.

Paul, A., M. Frederich, R. Uyttenbroeck, P. Malik, S. Filocco, A. Richel, S. Heuskin, T. Alabi, R.C. Megido, and T. Franck, 2016. Nutritional composition and rearing potential of the meadow grasshopper (*Chorthippus parallelus* Zetterstedt). Journal of Asia-Pacific Entomology. 19, 1111-1116.

Pearson, A., J.D. Love, and F. Shorland, 1977. "Warmed-over" flavor in meat, poultry, and fish, in Advances in food research. Elsevier. p. 1-74.

Peres, M.T., F. Delle Monache, A.B. Cruz, M.G. Pizzolatti, and R.A. Yunes, 1997. Chemical composition and antimicrobial activity of *Croton urucurana* Baillon (Euphorbiaceae). Journal of ethnopharmacology. 56, 223-226.

Perumal, P.C., D. Sophia, C.A. Raj, P. Ragavendran, T. Starlin, and V.K. Gopalakrishnan, 2012. *In vitro* antioxidant activities and HPTLC analysis of ethanolic extract of *Cayratia trifolia* (L.). Asian Pacific Journal of tropical disease. 2, S952-S956.

Perumal, S., R. Mahmud, S. Pillai, W.C. Lee, and S. Ramanathan, 2012. Antimicrobial activity and cytotoxicity evaluation of *Euphorbia hirta* (L.) extracts from Malaysia. APCBEE Procedia. 2, 80-85.

Perviz, S., H. Khan, and A. Pervaiz, 2016. Plant alkaloids as an emerging therapeutic alternative for the treatment of depression. Frontiers in Pharmacology. 7, 28.

Pezeshk, S., M. Rezaei, and H. Hosseini, 2011. Effects of turmeric, shallot extracts, and their combination on quality characteristics of vacuum-packaged rainbow trout stored at 4±1 C. Journal of food science. 76, M387-M391.

Phan, L.T., T.M. Bui, T.T. Nguyen, G.J. Gooley, B.A. Ingram, H.V. Nguyen, P.T. Nguyen, and S.S. De Silva, 2009. Current status of farming practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta, Vietnam. Aquaculture. 296, 227-236.

Phu, T.M., N.T. Phuong, T.T. Dung, D.M. Hai, V.N. Son, A. Rico, J.H. Clausen, H. Madsen, F. Murray, and A. Dalsgaard, 2016. An evaluation of fish health-management practices and occupational health hazards associated with Pangasius catfish (*Pangasianodon hypophthalmus*) aquaculture in the Mekong Delta, Vietnam. Aquaculture Research. 47, 2778-2794.

Phuong, N. and D. Oanh, 2009. Striped catfish (*Pangasianodon hypophthalmus*) aquaculture in Viet Nam: an unprecedented development within a decade, in Success Stories in Asian Aquaculture, S.S. De Silva and F.B. Davy, Editors., Springer, NACA and IDRC: Dordrecht, Bangkok and Ottawa. p. 133-149.

Phuong, N.T. and D.T.H. Oanh, 2010. Striped catfish aquaculture in Vietnam: a decade of unprecedented development, in Success stories in Asian aquaculture. Springer. p. 131-147.

Pinto, T., A. Aires, F. Cosme, E. Bacelar, M.C. Morais, I. Oliveira, J. Ferreira-Cardoso, R. Anjos, A. Vilela, and B. Gonçalves, 2021. Bioactive (Poly) phenols, Volatile Compounds from Vegetables, Medicinal and Aromatic Plants. Foods. 10, 106.

Prajapati, R., S. Roy, S. Mishra, S. Raza, and L. Thakur, 2014. Formulation development, standardization and antimicrobial activity of *Ageratum conyzoides* extracts and their formulation. International Journal of Pharmacy and Pharmaceutical Sciences. 6, 369-374.

Pratt, D.E. and B.J. Hudson, 1990. Natural antioxidants not exploited commercially, in Food antioxidants. Springer. p. 171-191.

Prester, L., 2011. Biogenic amines in fish, fish products and shellfish: a review. Food Additives & Contaminants: Part A. 28, 1547-1560.

Pu, H., X. Li, Q. Du, H. Cui, and Y. Xu, 2017. Research progress in the application of Chinese herbal medicines in aquaculture: a review. Engineering. 3, 731-737.

Pyrzynska, K., and A. Pękal, 2013. Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. Analytical Methods. 5, 4288-4295.

Rabeta, M. and S. Lin, 2015. Effects of different drying methods on the antioxidant activities of leaves and berries of *Cayratia trifolia.* Sains Malaysiana. 44, 275-280.

Raharjo, S., J.N. Sofos, and G.R. Schmidt, 1992. Improved speed, specificity, and limit of determination of an aqueous acid extraction thiobarbituric acid-C18 method for measuring lipid peroxidation in beef. Journal of Agricultural and Food Chemistry. 40, 2182-2185.

Rahman, M.M., M.S.B. Sayeed, M.A. Haque, M.M. Hassan, and S. Islam, 2012. Phytochemical screening, antioxidant, anti-Alzheimer and anti-diabetic activities of *Centella asiatica*. Journal of natural product and plant resources. 2, 504-511.

Rainboth, W.J., 1996. Fishes of the Cambodian Mekong. Quebec City, QC, Canada: Food & Agriculture Organization. 303.

Rajeshkumar, N., K. Joy, G. Kuttan, R. Ramsewak, M.G. Nair, and R. Kuttan, 2002. Antitumour and anticarcinogenic activity of *Phyllanthus amarus* extract. Journal of Ethnopharmacology. 81, 17-22.

Ramji, N., N. Ramji, R. Iyer, and S. Chandrasekaran, 2002. Phenolic antibacterials from *Piper betle* in the prevention of halitosis. Journal of ethnopharmacology. 83, 149-152.

Rao, B.M., D. Jesmi, and P. Viji, 2017. Chilled storage of *Pangasianodon hypophthalmus* fillets coated with plant oil incorporated alginate gels: Effect of clove leaf, clove bud, rosemary and thyme oils. Journal of Aquatic Food Product Technology. 26, 744-755.

Rao, B.M., L.N. Murthy, and M. Prasad, 2013. Shelf life of chill stored panagasius (*Pangasianodon hypophthalmus*) fish fillets: effect of vacuum and polyphosphate. Indian Journal of Fisheries. 60, 93-98.

RASFF, 2011. The rapid alert system for food and feed - 2011 Annual Report. Available from: https://op.europa.eu/en/publication-detail/-/publication/79564291-a792-4c3b-aeb8-53455d22c9a8/language-en/format-PDF/source-200407129.

Rattanachaikunsopon, P. and P. Phumkhachorn, 2009. Prophylactic effect of *Andrographis paniculata* extracts against *Streptococcus agalactiae* infection in Nile tilapia (*Oreochromis niloticus*). Journal of Bioscience and Bioengineering. 107, 579-582.

Rattanachaikunsopon, P. and P. Phumkhachorn, 2010a. Effect of *Cratoxylum formosum* on innate immune response and disease resistance against *Streptococcus agalactiae* in tilapia *Oreochromis niloticus*. Fisheries Science. 76, 653-659.

Rattanachaikunsopon, P. and P. Phumkhachorn, 2010b. Potential of cinnamon (*Cinnamomum verum*) oil to control Streptococcus iniae infection in tilapia (*Oreochromis niloticus*). Fisheries Science. 76, 287-293.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine. 26, 1231-1237.

Regi Raphael, K., T. Ajith, S. Joseph, and R. Kuttan, 2002. Anti-mutagenic activity of *Phyllanthus amarus* Schum & Thonn *in vitro* as well as *in vivo.* Teratogenesis, carcinogenesis, and mutagenesis. 22, 285-291.

Reverter, M., N. Bontemps, D. Lecchini, B. Banaigs, and P. Sasal, 2014. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture. 433, 50-61.

Rezaei, M., N. Montazeri, H.E. Langrudi, B. Mokhayer, M. Parviz, and A. Nazarinia, 2007. The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. Food Chemistry. 103, 150-154.

Rezaeizadeh, A., A. Zuki, M. Abdollahi, Y. Goh, M. Noordin, M. Hamid, and T. Azmi, 2011. Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia.* African Journal of Biotechnology. 10, 4932-4940.

Ribeiro, A.S., M. Estanqueiro, M.B. Oliveira, and J.M. Sousa Lobo, 2015. Main benefits and applicability of plant extracts in skin care products. Cosmetics. 2, 48-65.

Richards, M.P., and H.O. Hultin, 2000. Effect of pH on lipid oxidation using trout hemolysate as a catalyst: a possible role for deoxyhemoglobin. Journal of Agricultural and Food Chemistry. 48, 3141-3147.

Richards, M.P. and H.O. Hultin, 2002. Contributions of blood and blood components to lipid oxidation in fish muscle. Journal of Agricultural and Food chemistry. 50, 555-564.

Richheimer, S.L., M.W. Bernart, G.A. King, M.C. Kent, and D.T. Beiley, 1996. Antioxidant activity of lipid-soluble phenolic diterpenes from rosemary. Journal of the American Oil Chemists' Society. 73, 507-514.

Rico, A., T.M. Phu, K. Satapornvanit, J. Min, A. Shahabuddin, P.J. Henriksson, F.J. Murray, D.C. Little, A. Dalsgaard, and P.J. Van den Brink, 2013. Use of veterinary medicines, feed additives and probiotics in four major internationally traded aquaculture species farmed in Asia. Aquaculture. 412, 231-243.

Rifkind, J.M., L. Zhang, J.M. Heim, and A. Levy, 1988. The role of hemoglobin in generating oxyradicals, in Oxygen radicals in biology and medicine, M.G. Simic, K.A. Taylor, J.F. Ward and C. Sonntag. Editors., Springer: Boston. p. 157-162.

Roberts, R.J., 1993. Motile aeromonad septicemia, in Bacterial diseases of fish, V. Inglis, R.J. Roberts, and N.R. Bromage, Editors., John Wiley and Sons: New York. p. 143–156.

Roberts, T., J. Cordier, L. Gram, R. Tompkin, J. Pitt, L. Gorris, and K. Swanson, 2005. Micro-Organisms in Foods 6: Microbial Ecology of Food Commodities. New York: Springer US. 764.

Roberts, T.R. and C. Vidthayanon, 1991. Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. Proceedings of the Academy of Natural Sciences of Philadelphia. 97-143.

Rocchetti, G., J.P. Pagnossa, F. Blasi, L. Cossignani, R.H. Piccoli, G. Zengin, D. Montesano, P.S. Cocconcelli, and L. Lucini, 2020. Phenolic profiling and in vitro bioactivity of *Moringa oleifera* leaves as affected by different extraction solvents. Food Research International. 127, 108712.

Rodgers, C. and M. Furones, 2009. Antimicrobial agents in aquaculture: practice, needs and issues. Options Méditerranéennes. 86, 41-59.

Rodrigues, B.L., T.S. Alvares, M.d. Costa, G.S.L. Sampaio, C. Lázaro De La Torre, E.T. Mársico, and C.A. Conte Júnior, 2013. Concentration of biogenic amines in rainbow trout (*Oncorhynchus mykiss*) preserved in ice and its relationship with physicochemical parameters of quality. Journal of Aquaculture Research & Development. 4, 174-177.

Rodriguez, M.B.R., C. da Silva Carneiro, M.B. da Silva Feijó, C.A.C. Júnior, and S.B. Mano, 2014. Bioactive amines: aspects of quality and safety in food. Food and Nutrition Sciences. 5, 138-146.

Rokey, G.J., J. Krehbiel, K. Matson, and G. Huber, 1985. Production of pet food, in Feed manufacturing technology III, R.R. McEllhiney, Editor., American Feed Industry Association: Arlington, VA. p. 222-237.

Roleira, F.M., E.J. Tavares-da-Silva, C.L. Varela, S.C. Costa, T. Silva, J. Garrido and F. Borges, 2015. Plant derived and dietary phenolic antioxidants: Anticancer properties. Food Chemistry. 183, 235-258.

Ross, K.A., 2014. Concepts important in understanding the health benefits of phenolics in fruits and vegetables: Extractable & non-extractable phenolics and the influence of cell wall polysaccharides on bioaccessibility & bioavailability. Research in Health and Nutrition. 2, 29-43.

Rota, M.C., A. Herrera, R.M. Martínez, J.A. Sotomayor, and M.J. Jordán, 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food control. 19, 681-687.

Rounds, L., C.M. Havens, Y. Feinstein, M. Friedman, and S. Ravishankar, 2012. Plant extracts, spices, and essential oils inactivate Escherichia coli O157: H7 and reduce formation of potentially carcinogenic heterocyclic amines in cooked beef patties. Journal of agricultural and food chemistry. 60, 3792-3799.

Royes, J.-A.B. and F.A. Chapman, 2003. Preparing your own fish feeds. EDIS. 1-7.

Ruiz-Capillas, C. and A. Moral, 2001. Residual effect of CO_2 on hake (*Merluccius merluccius* L.) stored in modified and controlled atmospheres. European Food Research and Technology. 212, 413-420.

Saeed, N., M.R. Khan, and M. Shabbir, 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complementary and alternative medicine. 12, 1-12.

Salehi, B., C.F. Rodrigues, A.C. Karaca, G. Gülseren, E. Åženol, E. Demircan, B. Özçelik, S. Tutuncu, F. Sharopov, J. Sharifi-Rad, A. Maroyi, and N. Martins, 2020. Plant-food-derived bioactives: Key health benefits and current nanosystems as a strategy to enhance their bioavailability. Cellular and Molecular Biology. 66, 232-242.

Sanjuás-Rey, M., B. García-Soto, J. Barros-Velázquez, J.R. Fuertes-Gamundi, and S.P. Aubourg, 2011. Effect of a two-step natural organic acid treatment on microbial activity and lipid damage during blue whiting (*Micromesistius poutassou*) chilling. International Journal of Food Science & Technology. 46, 1021-1030.

Sanjuás-Rey, M., J.M. Gallardo, J. Barros-Velázquez, and S.P. Aubourg, 2012. Microbial activity inhibition in chilled mackerel (*Scomber scombrus*) by employment of an organic acid-icing system. Journal of food science. 77, M264-M269.

Saranraj, P. and P. Sivasakthivelan, 2012. Screening of antibacterial activity of the medicinal plant *Phyllanthus amarus* against urinary tract infection causing bacterial pathogens. Applied journal of hygiene. 1, 19-24.

Sarker, S.D., L. Nahar, and Y. Kumarasamy, 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 42, 321-324.

Sasidharan, S., Y. Chen, D. Saravanan, K.M. Sundram, and L.Y. Latha, 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African journal of traditional, complementary and alternative medicines. 8, 1-10.

Saxena, M., J. Saxena, R. Nema, D. Singh, and A. Gupta, 2013. Phytochemistry of medicinal plants. Journal of pharmacognosy and phytochemistry. 1, 6.

Schormüller, J., 1968. Handbuch der Lebensmittelchemie. Vol. Band III/2. New York: Springer Verlag, Berlin, Heidelberg. p. 880-1641.

Sebranek, J., V. Sewalt, K. Robbins, and T. Houser, 2005. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat science. 69, 289-296.

Sen, A. and A. Batra, 2013. The study of *in vitro* and *in vivo* antioxidant activity and total phenolic content of *Phyllanthus amarus* Schum. & Thonn.: A medicinally important plant. International Journal of Pharmacy and Pharmaceutical Sciences. 5, 942-947.

Senjobi, C., A. Ettu, and C. Otujo, 2017. Antibacterial and antifungal activities of leaf extracts of *Phyllanthus amarus* Schum and Thonn. Journal of Pharmacognosy and Phytotherapy. 9, 6-10.

Shahriar, M., S. Akhter, M.I. Hossain, M.A. Haque, and M.A. Bhuiyan, 2012. Evaluation of *in vitro* antioxidant activity of bark extracts of *Terminalia arjuna.* Journal of Medicinal Plants Research. 6, 5286-5298.

Shahsavani, D., H.R. Kazerani, S. Kaveh, and H. Gholipour-Kanani, 2010. Determination of some normal serum parameters in starry sturgeon (*Acipenser stellatus* Pallas, 1771) during spring season. Comparative Clinical Pathology. 19, 57-61.

Shalaby, E.A. and S.M. Shanab, 2013. Antioxidant compounds, assays of determination and mode of action. African journal of pharmacy and pharmacology. 7, 528-539.

Sharma, A., R.T. Singh and S.S. Handa, 1993. Estimation of phyllanthin and hypophyllanthin by high performance liquid chromatography in *Phyllanthus amarus.* Phytochemical analysis. 4, 226-229.

Sharma, S., I.A. Khan, I. Ali, F. Ali, M. Kumar, A. Kumar, R.K. Johri, S.T. Abdullah, S. Bani, A. Pandey, K.A. Suri, B.D. Gupta, N.K. Satti, P. Dutt, and G.N. Qazi, 2009. Evaluation of the antimicrobial, antioxidant, and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. Antimicrobial agents and chemotherapy. 53, 216-222.

Sheikhlar, A., G.Y. Meng, R. Alimon, N. Romano, and M. Ebrahimi, 2017. Dietary *Euphorbia hirta* extract improved the resistance of sharptooth catfish *Clarias gariepinus* to *Aeromonas hydrophila*. Journal of aquatic animal health. 29, 225-235.

Sherwin, E., 1978. Oxidation and antioxidants in fat and oil processing. Journal of the American Oil Chemists' Society. 55, 809-814.

Shetty, M., B. Maiti, M. Venugopal, I. Karunasagar, and I. Karunasagar, 2014. First isolation and characterization of Edwardsiella tarda from diseased striped catfish, *Pangasianodon hypophthalmus* (Sauvage). Journal of Fish Diseases. 37, 265-271.

Shewan, J., 1962. The bacteriology of fresh and spoiling fish and some related chemical changes. Recent advances in food science. 1, 167-193.

Shoemaker, C.A., P.H. Klesius, and J.J. Evans, 2007. Immunization of eyed channel catfish, Ictalurus punctatus, eggs with monovalent *Flavobacterium columnare* vaccine and bivalent *F. columnare* and *Edwardsiella ictaluri* vaccine. Vaccine. 25, 1126-1131.

Shyur, L.-F., J.-H. Tsung, J.-H. Chen, C.-Y. Chiu, and C.-P. Lo, 2005. Antioxidant properties of extracts from medicinal plants popularly used in Taiwan. International Journal of Applied Science and Engineering. 3, 195-202.

Simamora, A., L. Paramita, N. Azreen, A.W. Santoso, and K.H. Timotius, 2018. *In vitro* Antidiabetic and Antioxidant Activities of Aqueous Extract from the Leaf and Fruit of *Psidium guajava* L. The Indonesian Biomedical Journal. 10, 156-64.

Simeonidou, S., A. Govaris, and K. Vareltzis, 1997. Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and Mediterranean hake

(*Merluccius mediterraneus*). Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 204, 405-410.

Simeonidou, S., A. Govaris, and K. Vareltzis, 1997. Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and Mediterranean hake (*Merluccius mediterraneus*). Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 204, 405-410.

Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 16, 144-158.

Singh, D., S. Narayanamoorthy, S. Gamre, A.G. Majumdar, M. Goswami, U. Gami, S. Cherian, and Subramanian, M., 2018. Hydroxychavicol, a key ingredient of *Piper betle* induces bacterial cell death by DNA damage and inhibition of cell division. Free Radical Biology and Medicine. 120, 62-71.

Singh, S.B., K. Young, and L.L. Silver, 2017. What is an "ideal" antibiotic? Discovery challenges and path forward. Biochemical pharmacology. 133, 63-73.

Sirajudeen, K.N.S., S.A., Sulaiman, M., Madhavan, Z., Ismail, M., Swamy, M.L., Ismail, M., Yaacob, 2006. Safety evaluation of aqueous extract of leaves of a plant *Phyllanthus amarus*, in rat liver. African Journal of Traditional Complementary and Alternative Medicines. 3, 78–93.

Škrovánková, S., L. Mišurcová, and L. Machů, 2012. Antioxidant activity and protecting health effects of common medicinal plants. Advances in food and nutrition research. 67, 75-139.

Slabbert, N.P., 1977. Ionisation of some flavanols and dihydroflavonols. Tetrahedron. 33, 821-824.

Smith, J., F. Ameri, and P. Gadgil, 2008. Effect of marinades on the formation of heterocyclic amines in grilled beef steaks. Journal of food science. 73, T100-T105.

Soumaya, K., F. Chaouachi, R. Ksouri, and M. El Gazzah, 2013. Polyphenolic composition in different organs of Tunisia populations of *Cynara cardunculus* L. and their antioxidant activity. Journal of Food and Nutrition Research. 1, 1-6.

Steinberg, M.H., B.G. Forget, D.R. Higgs, and D.J. Weatherall, 2009. Disorders of hemoglobin: genetics, pathophysiology, and clinical management. Cambridge University Press. 846.

Stohs, S.J. and D. Bagchi, 1995. Oxidative mechanisms in the toxicity of metal ions. Free radical biology and medicine. 18, 321-336.

Ström, G.H., H. Björklund, A.C. Barnes, C.T. Da, N.H.Y. Nhi, T.T. Lan, U. Magnusson, A. Norman Haldén, and S. Boqvist, 2019. Antibiotic use by small-scale farmers for freshwater aquaculture in the upper Mekong Delta, Vietnam. Journal of Aquatic Animal Health. 31, 290-298.

Subagja, J., J. Slembrouck, and M. Legendre, 1999. Larval rearing of an Asian catfish *Pangasius hypophthalmus* (Siluroidei, Pangasiidae): Analysis of precocious mortality and proposition of appropriate treatments. Aquatic Living Resources. 12, 37-44.

Subba, B. and P. Aryal, 2016. Study of Biological Activity and Chemical Constituent of *Annona reticulata.* Journal of Institute of Science and Technology. 21, 157-163.

Sukumaran, V., S.C. Park, and S.S. Giri, 2016. Role of dietary ginger *Zingiber officinale* in improving growth performances and immune functions of *Labeo rohita* fingerlings. Fish & shellfish immunology. 57, 362-370.

Suh, H.J., M.S. Chung, Y.H. Cho, J.W. Kim, D.H. Kim, K.W. Han, and C.J. Kim, 2005. Estimated daily intakes of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyl hydroquinone (TBHQ) antioxidants in Korea. Food additives and contaminants. 22, 1176-1188.

Sultanbawa, Y., 2011. Plant antimicrobials in food applications: Minireview. Science against microbial pathogens: Communicating current research and technological advances. 2, 1084-99.

Sun, D., J.G. Hurdle, R. Lee, R. Lee, M. Cushman, and J.M. Pezzuto, 2012. Evaluation of flavonoid and resveratrol chemical libraries reveals abyssinone II as a promising antibacterial lead. ChemMedChem. 7, 1541-1545.

Sun, M., Z. Chang, P.J. Van den Brink, J. Li, F. Zhao, and A. Rico, 2016. Environmental and human health risks of antimicrobials used in *Fenneropenaeus chinensis* aquaculture production in China. Environmental Science and Pollution Research. 23, 15689-15702.

Sutton, J., S. Balfry, D. Higgs, C.-H. Huang, and B. Skura, 2006. Impact of iron-catalyzed dietary lipid peroxidation on growth performance, general health and flesh proximate and fatty acid composition of Atlantic salmon (*Salmo salar* L.) reared in seawater. Aquaculture. 257, 534-557.

Svanevik, C.S. and B.T. Lunestad, 2011. Characterisation of the microbiota of Atlantic mackerel (*Scomber scombrus*). International journal of food microbiology. 151, 164-170.

Sveinsdottir, K., G. Hyldig, E. Martinsdottir, B. Jørgensen, and K. Kristbergsson, 2003. Quality Index Method (QIM) scheme developed for farmed Atlantic salmon (*Salmo salar*). Food Quality and Preference. 14, 237-245.

Svobodova, B., L. Barros, R.C. Calhelha, S. Heleno, M.J. Alves, S. Walcott, M. Bittova, V. Kuban, and I.C. Ferreira, 2017. Bioactive properties and phenolic profile of *Momordica charantia* L. medicinal plant growing wild in Trinidad and Tobago. Industrial crops and products. 95, 365-373.

Syahidah, A., C. Saad, and H. Daud, 2012. Potential antibacterial activity of local herb extracts on fish pathogenic bacteria. in 31st Symposium of the Malaysian Society for Microbiology. Microbiology Research in the Omics Era. p. 13-15

Syahidah, A., C. Saad, H. Daud, and Y. Abdelhadi, 2015. Status and potential of herbal applications in aquaculture: A review. Iranian Journal of Fisheries Science. 14, 27-44.

Székely, C., F. Shaharom-Harrison, G. Cech, K. Mohamed, and K. Molnár, 2009. Myxozoan pathogens of Malaysian fishes cultured in ponds and net-cages. Diseases of Aquatic Organisms. 83, 49-57.

Taemchuay, D., T. Rukkwamsuk, T. Sakpuaram, and N. Ruangwises, 2009. Antibacterial activity of crude extracts of *Centella asiatica* against *Staphylococcus aureus* in bovine mastitis. Kasetsart Veterinarians. 19, 119-128.

Tan, W.C., I.B. Jaganath, R. Manikam, and S.D. Sekaran, 2013. Evaluation of antiviral activities of four local Malaysian *Phyllanthus* species against herpes simplex viruses and possible antiviral target. International journal of medical sciences. 10, 1817-1829.

Taniguchi, M., and I. Kubo, 1993. Ethnobotanical drug discovery based on medicine men's trials in the African savanna: screening of east African plants for antimicrobial activity II. Journal of Natural Products. 56, 1539-1546.

Tanimoto, S.-y., K. Koike, and S. Takahashi, 1993. Improvement in broiled meat texture of cultured eel by feeding of tochu leaf powder. Bioscience, biotechnology, and biochemistry. 57, 325-327.

Tao, Z., M. Sato, H. Zhang, T. Yamaguchi, and T. Nakano, 2011. A survey of histamine content in seafood sold in markets of nine countries. Food Control. 22, 430-432.

Tappel, A.D., 1955. Unsaturated lipide oxidation catalyzed by hematin compounds. Journal of Biological Chemistry. 217, 721-733.

Taukoorah, U., N. Lall, and F. Mahomoodally, 2016. *Piper betle* L. (betel quid) shows bacteriostatic, additive, and synergistic antimicrobial action when combined with conventional antibiotics. South African journal of botany. 105, 133-140.

Taylor, A., 1987. Effect of water quality on lipid oxidation. Food Science and Technology Today. 1, 158-159.

Teeli, R.A., S.A. Ganie, M.S. Dar, W. Raja, and S.S. Yadav, 2018. Antioxidant activity of *Euphorbia hirta* L. leaf extracts. Research Journal of Pharmacy and Technology. 11, 199-202.

Tekwu, E.M., A.C. Pieme, and V.P. Beng, 2012. Investigations of antimicrobial activity of some Cameroonian medicinal plant extracts against bacteria and yeast with gastrointestinal relevance. Journal of ethnopharmacology. 142, 265-273.

Thanh, L.H. and P.H. Chuong, 2010. Case Study #10-12, Vietnam's Aquaculture Trade: Food Safety and Sanitation Issues, in Food Policy for Developing Countries: Case Studies, P. Pinstrup-Andersen and F. Cheng, Editors, CUL Initiatives in Publishing: Cornell University. p. 1-13.

Thiangthum, S., B. Dejaegher, M. Goodarzi, C. Tistaert, A. Gordien, N.N. Hoai, M.C. Van, J. Quetin-Leclercq, L. Suntornsuk, and Y. Vander Heyden, 2012. Potentially antioxidant compounds indicated from *Mallotus* and *Phyllanthus* species fingerprints. Journal of Chromatography B. 910, 114-121.

Thiansilakul, Y., S. Benjakul, and M.P. Richards, 2010. Changes in heme proteins and lipids associated with off-odour of seabass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus*× *O. niloticus*) during iced storage. Food Chemistry. 121, 1109-1119.

Thinh, N.Q. and T.M. Phu, 2021. The use of chemicals and drugs in aquaculture in Vietnam. First ed, ed. N.T. Phuong and T.L.H. Nguyen. Can Tho: Can Tho University Publishing House 84.

Thu, N.B., T.N. Trung, D.T. Ha, N.M. Khoi, T.V. Hung, T.T. Hien, N.-H. Yim, and K.-H. Bae, 2010. Screening of Vietnamese medicinal plants for cytotoxic activity. Natural Product Sciences. 16, 43-49.

Thuong, P.T., M.-K. Na, N.H. Dang, T.M. Hung, P.T. Ky, T.V. Thanh, N.H. Nam, N.D. Thuan, D.-E. Sok, and K.-H. Bae, 2006. Antioxidant activities of Vietnamese medicinal plants. Natural Product Sciences. 12, 29-37.

Thuy, L.T. and T.T.M. Thu, 2011. The use of chitosan as a preservative in Tra fish fillet frozen (*Pangasianodon hypophthalmus*). Scientific journal of Can Tho University. 17, 77-85.

Thuy, L.T.M., D.T.T. Huong, B.C. Linh, and N.D. Quynh, 2015. Effect of gelatin coating incorporated with gallic acid on the quality of Tra catfish paste (*Pangasianodon hypophthalmus*) in cold storage. Scientific journal of Can Tho University. 1, 39-46.

Tien, N.T., T.T. Dung, N.A. Tuan, and M. Crumlish, 2012. First identification of Flavobacterium columnare infection in farmed freshwater striped catfish *Pangasianodon hypophthalmus*. Diseases of Aquatic Organisms. 100, 83-88.

Tomar, R.S., V. Shrivastava, and S. Kaushik, 2014. *In vitro* efficacy of methanolic extract of *Mimosa pudica* against selected micro-organisms for its broad spectrum antimicrobial activity. International Journal of Current Microbiology and Applied Sciences. 3, 780-784.

Tong Thi, A.N., B. Noseda, S. Samapundo, B.L. Nguyen, K. Broekaert, G. Rasschaert, M. Heyndrickx, and F. Devlieghere, 2013. Microbial ecology of Vietnamese Tra fish (*Pangasius hypophthalmus*) fillets during processing. International journal of food microbiology. 167, 144-152.

Tong Thi, A.N., L. Jacxsens, B. Noseda, S. Samapundo, B.L. Nguyen, M. Heyndrickx, and F. Devlieghere, 2014. Evaluation of the microbiological safety and quality of Vietnamese *Pangasius hypophthalmus* during processing by a microbial assessment scheme in combination with a self-assessment questionnaire. Fisheries Science. 80, 1117-1128.

Tortosa, V., V. Pietropaolo, V. Brandi, G. Macari, A. Pasquadibisceglie, and F. Polticelli, 2020. Computational methods for the identification of molecular targets of toxic Food Additives. Butylated Hydroxytoluene as a case study. Molecules. 25, 2229.

Tougas, T.P., J.M. Jannetti, and W.G. Collier, 1985. Theoretical and experimental response of a biamperometric detector for flow injection analysis. Analytical Chemistry. 57, 1377-1381.

Tran, M.P., T.K.D. Huynh, L.A.D. Nguyen, T.N.H. Nguyen, Q.T. Nguyen, and H. Tomoaki, 2021. The effect of guava (*Psidium guajava*) leaf extract on the quality of cobia (*Rachycentron canadum*) fillets during ice storage. Can Tho University Journal of Science. 13, 52-63.

Trang Thi Huong Tran, Son Minh Ta, Dang Thu Nguyen, D.V.E. Rafe Dent, M. Durleva, 2016. The Interface Between Access and Benefit-sharing Rules and BioTrade in Viet Nam, United Nations Conference on Trade and Development.

Trong, T.Q., N.V. Hao, and D. Griffiths, 2002. Status of Pangasiid aquaculture in Viet Nam. MRC Technical Paper. No. 2, 16p.

Tsikas, D., 2017. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Analytical biochemistry. 524, 13-30.

Ushie, O., P. Neji, E. Etim, and G. Nsor, 2013. Phytochemical screening and antimicrobial activities of *Phyllanthus amarus* stem bark extracts. International Journal of Modern Biology and Medicines. 3, 101-112.

Van, K.V., 2012. The use of garlic in treatment of bacterial disease, in Principles and technique in aquaculture, T.Q. Phu, Editor., Agriculture Publisher: HCMC. p. 170.

Van Doan H., S.H., Hoseinifar, K. Sringarm, S. Jaturasitha, B. Yuangsoi, M.A.O. Dawood, M.A. Esteban, E. Ringø, C. Faggio, 2019. Effects of Assam tea extract on growth, skin mucus, serum immunity and disease resistance of Nile tilapia (*Oreochromis niloticus*) against *Streptococcus agalactiae*. Fish & Shellfish Immunology. 93, 428–435.

VASEP, 2019. Vietnam Association of Seafood Exporters and Producers. Fishery profile; Available from: http://vasep.com.vn/1192/OneContent/tong-quan-nganh.htm.

VASEP, 2019. Vietnam Association of Seafood Exporters and Producers. Sector profiles; Available from: http://seafood.vasep.com.vn/673/onecontent/sector-profiles.htm.

VASEP, 2020. Vietnam Association of Seafood Exporters and Producers. Fishery Profile; Available from: http://seafood.vasep.com.vn/why-buy-seafood/fishery-profile.

VASEP, 2021. Production of raw pangasius in H1. Vietnam Association of Seafood Exporters and Producers Available from: http://seafood.vasep.com.vn/key-seafood-sectors/pangasius/news/production-of-raw-pangasius-in-h1-16369.html.

VASEP, 2021. Vietnam Association of Seafood Exporters and Producer. Characteristics of the sector; Available from: https://vasep.com.vn/san-pham-xuat-khau/ca-tra/tong-quan-nganh-ca-tra.

Veiga, M., E.M. Costa, S. Silva, and M. Pintado, 2020. Impact of plant extracts upon human health: A review. Critical reviews in food science and nutrition. 60, 873-886.

Venkatramalingam, K., J.G. Christopher, and T. Citarasu, 2007. *Zingiber officinalis* an herbal appetizer in the tiger shrimp *Penaeus monodon* (Fabricius) larviculture. Aquaculture Nutrition. 13, 439-443.

Viegas, O., L.F. Amaro, I.M. Ferreira, and O. Pinho, 2012. Inhibitory effect of antioxidant-rich marinades on the formation of heterocyclic aromatic amines in pan-fried beef. Journal of agricultural and food chemistry. 60, 6235-6240.

Vietnam Ministry of Public Health, 2012. National technical regulation of Microbiological contaminants in food (QCVN 8-3:2012/BYT). Available from: http://www.fsi.org.vn/pic/files/qcvn-8-3_2011-byt-ve-o-nhiem-vi-sinh-vattrong-tp_bia_merged.pdf.

Viji, P., S. Tanuja, G. Ninan, K. Lalitha, A. Zynudheen, P. Binsi, and T. Srinivasagopal, 2015. Biochemical, textural, microbiological and sensory attributes of gutted and ungutted sutchi catfish (*Pangasianodon hypophthalmus*) stored in ice. Journal of food science and technology. 52, 3312-3321.

Viji, P., S. Tanuja, N. George, and A.A.Z.K.V. Lalitha, 2014. Quality characteristics and shelf life of Sutchi cat fish (*Pangasianodon hypophthalmus*) steaks during refrigerated storage. International Journal of Agriculture and Food Science Technology. 5, 105-116.

Visciano, P., M. Schirone, and A. Paparella, 2020. An overview of histamine and other biogenic amines in fish and fish products. Foods. 9, 1795.

VMARD, 2012. Vietnamese Ministry of Agriculture and Rural Development. https://vanban.chinhphu.vn/default.aspx?pageid=27160&docid=156490.

VMARD, 2015. Vietnamese Ministry of Agriculture and Rural Development. https://www.mard.gov.vn/Pages/tang-cuong-kiem-soat-du-luong-khang-sinh-trong-nuoi-trong-thuy-san-28436.aspx.

VMARD, 2016. Vietnamese Ministry of Agriculture and Rural Development. http://vanban.chinhphu.vn/portal/page/portal/chinhphu/hethongvanban?class_id=1&mode=detail&document_id=186403.

Wang, H., B. Che, A. Duan, J. Mao, R.A. Dahlgren, M. Zhang, H. Zhang, A. Zeng, X. Wang, 2014. Toxicity evaluation of beta-diketone antibiotics on the development of embryo-larval zebrafish (*Danio rerio*). Environmental toxicology. 29, 1134–1146.

Wang, K.-H., R.-D. Lin, F.-L. Hsu, Y.-H. Huang, H.-C. Chang, C.-Y. Huang, and M.-H. Lee, 2006. Cosmetic applications of selected traditional Chinese herbal medicines. Journal of ethnopharmacology. 106, 353-359.

Watt, J.M., and M.G. Breyer-Brandwijk, 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa being an Account of their Medicinal and other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. 2nd ed. Edinburgh: E & S Livingstone.1457.

Wayner, D.D.M., G.W. Burton, K.U. Ingold, and S. Locke, 1985. Quantitative measurement of the total, peroxyl radical-trapping antioxidant capability of human blood plasma by controlled peroxidation: The important contribution made by plasma proteins. FEBS letters. 187, 33-37.

Weledji, E.P., E.K. Weledji, J.C. Assob, and D.S. Nsagha, 2017. Pros, cons and future of antibiotics. New Horizons in Translational Medicine. 4, 9-14.

WHO, 2011. World Health Organization. Media centre: traditional medicine fact sheet N◦ 134; Available from: http://www.who.int/mediacentre/factsheets/fs134/en/.

Wink M., 2016. Alkaloids: Toxicology and health effects, in Encyclopedia of Food and Health, B. Caballero, P. Finglas, and F. Toldrá, Editors, Academic Press: USA. p. 106-114.

Wolfe, K.L., and R.H. Liu, 2007. Cellular antioxidant activity (CAA) assay for assessing antioxidants, foods, and dietary supplements. Journal of agricultural and food chemistry. 55, 8896-8907.

Wolfson, J.S., and D.C. Hooper, 1985. The fluoroquinolones: structures, mechanisms of action and resistance, and spectra of activity in vitro. Antimicrobial agents and Chemotherapy. 28, 581-586.

Wright, J.S., E.R. Johnson, and G.A. DiLabio, 2001. Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. Journal of the American Chemical Society. 123, 1173-1183.

Wu, G., C. Yuan, M. Shen, J. Tang, Y. Gong, D. Li, F. Sun, C. Huang, and X. Han, 2007. Immunological and biochemical parameters in carp (*Cyprinus carpio*) after Qompsell feed ingredients for long-term administration. Aquaculture Research. 38, 246-255.

Wu, T., M. He, X. Zang, Y. Zhou, T. Qiu, S. Pan, and X. Xu, 2013. A structure–activity relationship study of flavonoids as inhibitors of *E. coli* by membrane interaction effect. Biochimica et Biophysica Acta (BBA)-Biomembranes. 1828, 2751-2756.

Xie, J., B. Liu, Q. Zhou, Y. Su, Y. He, L. Pan, X. Ge, and P. Xu, 2008. Effects of anthraquinone extract from rhubarb *Rheum officinale* Bail on the crowding stress response and growth of common carp *Cyprinus carpio* var. Jian. Aquaculture. 281, 5-11.

Yang, X., W. Song, N. Liu, Z. Sun, R. Liu, Q.S. Liu, Q. Zhou, and G. Jiang, 2017. Synthetic phenolic antioxidants cause perturbation in steroidogenesis *in vitro* and *in vivo*. Environmental science & technology. 52, 850-858.

Yanishlieva, N.V., E. Marinova, and J. Pokorný, 2006. Natural antioxidants from herbs and spices. European Journal of lipid science and Technology. 108, 776-793.

Yashin, A., Y. Yashin, X. Xia, and B. Nemzer, 2017. Antioxidant activity of spices and their impact on human health: A review. Antioxidants. 6, 70.

Yehye, W.A., N.A. Rahman, A. Ariffin, S.B. Abd Hamid, A.A. Alhadi, F.A. Kadir, and M. Yaeghoobi, 2015. Understanding the chemistry behind the antioxidant activities of butylated hydroxytoluene (BHT): A review. European journal of medicinal chemistry. 101, 295-312.

Yin, X., H. Wang, Y. Zhang, R.A. Dahlgren, H. Zhang, M. Shi, M. Gao, X. Wang, 2014. Toxicological assessment of trace beta-diketone antibiotic mixtures on zebrafish (*Danio rerio*) by proteomic analysis. PLoS One 9, e102731.

Yoden, K. and T. Iio, 1989. Determination of thiobarbituric acid-reactive substances in oxidized lipids by high-performance liquid chromatography with a postcolumn reaction system. Analytical biochemistry. 182, 116-120.

Yong, S.H. and M. Karel, 1978. Reaction of histidine with methyl linoleate: characterization of the histidine degradation products. Journal of the American Oil Chemists' Society. 55, 352-357.

Yoshida, T., L. Chen, T. Shingu, and T. Okuda, 1988. Tannins and Related Polyphenols of Euphorbiaceous Plants. IV.: Euphorbins A and B, Novel Dimeric Dehydroellagitannins from *Euphorbia hirta* L. Chemical and Pharmaceutical bulletin. 36, 2940-2949.

Yoshida, T., O. Namba, L. Chen, and T. Okuda, 1990a. Euphorbin E, a hydrolyzable tannin dimer of highly oxidized structure, from *Euphorbia hirta.* Chemical and Pharmaceutical Bulletin. 38, 1113-1115.

Yoshida, T., O. Namba, L. Chen, and T. Okuda, 1990b. Tannins and Related Polyphenols of Euphorbiaceous Plants. V.: Euphorbin C, an Equilibrated Dimeric Dehydroellagitannin Having a New Tetrameric Galloyl Group. Chemical and Pharmaceutical Bulletin. 38, 86-93.

Yuet Ping, K., I. Darah, Y. Chen, S. Sreeramanan, and S. Sasidharan, 2013. Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. BioMed research international. 2013, 1-14.

Zayed, S.M., M. Farghaly, H. Taha, H. Gotta, and E. Hecker, 1998. Dietary cancer risk conditional carcinogens in produce of livestock fed on species of spurge (Euphorbiaceae). I. Skin irritant and tumor-promoting ingenane-type diterpene esters in *E. peplus*, one of several herbaceous *Euphorbia* species contaminating fodder of livestock. Journal of Cancer Research and Clinical Oncology. 124, 131–140.

Zeng, M., Z. He, Z. Zheng, F. Qin, G. Tao, S. Zhang, Y. Gao, and J. Chen, 2014. Effect of six Chinese spices on heterocyclic amine profiles in roast beef patties by ultra performance liquid chromatography-tandem mass spectrometry and principal component analysis. Journal of agricultural and food chemistry. 62, 9908-9915.

Zhao, Y., Y. Wu, and M. Wang, 2015. Bioactive Substances of Plant Origin 30. Handbook of food chemistry. 967, 967-1008.

Zheng, Z., J.Y. Tan, H. Liu, X. Zhou, X. Xiang, and K. Wang, 2009. Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). Aquaculture. 292, 214-218.

Zhou, G., H. Yu, and Y. Yu, 2006. Study on bacteriostatic action in different parts *of Houttuynia cordata.* Hunan Forestry Science & Technology 33, 38-40.

Zhu, F., 2020. A review on the application of herbal medicines in the disease control of aquatic animals. Aquaculture. 526, 735422.

Zhuang, S., Y. Li, S. Jia, H. Hong, Y. Liu, and Y. Luo, 2019. Effects of pomegranate peel extract on quality and microbiota composition of bighead carp (*Aristichthys nobilis*) fillets during chilled storage. Food Microbiology. 82, 445-454.

Zilberg, D., A. Tal, N. Froyman, S. Abutbul, N. Dudai, and A. Golan-Goldhirsh, 2010. Dried leaves of *Rosmarinus officinalis* as a treatment for streptococcosis in tilapia. Journal of Fish Diseases. 33, 361-369.

Zuco, V., R. Supino, S.C. Righetti, L. Cleris, E. Marchesi, C. Gambacorti-Passerini, and F. Formelli, 2002. Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. Cancer Letters. 175, 17–25.

APPENDIX 1

Scientific and botanical names of plants (from World Flora Online, https://wfoplantlist.org/plant-list/)

Scientific name	Botanical name
Ageratum conyzoides	*Ageratum conyzoides* L.
Allium sativum	*Allium sativum* L.
Aloe vera	*Aloe vera* (L.) Burm.f.
Alternanthera sessilis	*Alternanthera sessilis* (L.) DC.
Andrographis paniculata	*Andrographis paniculata* (Burm.f.) Wall.
Angelica sinensis	*Angelica sinensis* (Oliv.) Diels
Annona reticulata	*Annona reticulata* L.
Areca catechu	*Areca catechu* L.
Astragalus membranaceus	*Astragalus membranaceus* Fisch. ex Bunge
Azadirachta indica	*Azadirachta indica* A.Juss.
Blumea balsamifera	*Blumea balsamifera* DC.
Camellia sinensis	*Camellia sinensis* (L.) Kuntze
Cayratia trifolia	*Cayratia trifolia (*L.) Mabb. & J.Wen
Centella asiatica	*Centella asiatica* (L.) Urb.
Cinnamomum verum	*Cinnamomum verum* J.Presl
Citrus limon	*Citrus limon* (L.) Osbeck
Cleome chelidonii	*Cleome chelidonii* L.f.
Combretum quadrangulare	*Combretum quadrangulare* Kurz
Cratoxylum formosum	*Cratoxylum formosum* (Jack) Benth. & Hook.f. ex Dyer
Curcuma longa	*Curcuma longa* L.
Cynara cardunculus	*Cynara cardunculus* L.
Cynodon dactylon	*Cynodon dactylon* (L.) Pers.
Eclipta alba	*Eclipta alba* (L.) Hassk.
Euphorbia hirta	*Euphorbia hirta* L.
Flammulina velutipes	-
Ginkgo biloba	*Ginkgo biloba* L.
Houttuynia cordata	*Houttuynia cordata* Thunb.
Melissa officinalis	*Melissa officinalis* L.
Mentha aquatica	*Mentha aquatica* L.
Mimosa pudica	*Mimosa pudica* L.
Momordica charantia	*Momordica charantia* L.
Moringa oleifera	*Moringa oleifera* Lam.
Ocimum basilicum	*Ocimum basilicum* L.
Origanum vulgare	*Origanum vulgare* L.
Perilla frutescens	*Perilla frutescens* (L.) Britton
Phoenix dactylifera	*Phoenix dactylifera* L.
Phyllanthus amarus	*Phyllanthus amarus* Schumach. & Thonn.

Phyllanthus emblica	*Phyllanthus emblica* L.
Phyllanthus urinaria	*Phyllanthus urinaria* L.
Piper betle	*Piper betle* L.
Portulaca oleracea	*Portulaca oleracea* L.
Psidium guajava	*Psidium guajava* L.
Rheum officinale	*Rheum officinale* Baill.
Rosmarinus officinalis	*Rosmarinus officinalis* L.
Sophora flavescens	*Sophora flavescens* Aiton
Terminalia arjuna	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.
Trigonella foenum-graecum	*Trigonella foenum-graecum* L.
Vaccinium macrocarpon	*Vaccinium macrocarpon* Aiton
Wedelia chinensis	*Wedelia chinensis* (Osbeck) Merr.
Yucca schidigera	*Yucca schidigera* Roezl ex Ortgies
Zingiber officinale	*Zingiber officinale* Roscoe

APPENDIX 2: QUESTIONNAIRE FOR STRIPED CATFISH FARMS

I. General information of farm

1. Farmer name:……..... Gender: ……
2. Address: Village:……. Commune:…….…….. District:……...
3. Phone number:……
4. Kind of cooperation: (*1= Company; 2= Family; 3= Cooperative; 4= Doing outwork; 5= Other*): …….…….....
5. Education *(0 = No education; 1 = Primary school; 2 = Secondary school; 3 = high school; 4 = Intermediate education; 5= Bachelor/Higher education; 6 = Post graduate)*
6. Number of year of farming: ……. year(s).
7. Training: Yes/No:.....……Trainer:.....Main content:.....
8. Number of grow-out ponds: ……. Total area: .....
9. Number of input-water ponds: ……. Total area: ……
10. Number of output-water ponds: ..... Total area: ……..
11. Number of sediments/mud storage ponds:..... Total area: ……
12. Stocking density (fish/m^2): …….....…….....
13. Size of fingerling (cm, g or number of fish/kg): …….....
14. Fingerling cost: …….....(VND/fish).
15. What types of feed use?

    Trash fish: ..... kg

    Fish pellets:..... kg

    Feed name: ..... Avg FCR……..
16. What is the approximated annual yield?.....tons/ha per crop
17. Size of harvested fish: Type 1…….…….…….....g/fish;

    Type 2…….....g/fish
18. Selling cost: Type 1……..……VND/kg,

    Type 2……..……VND/kg.
19. Selling fish to: traders, retail in markets, company, Cambodia?
20. Checking antibiotic before selling? Yes.....No.....
21. The farm is following any kind of standards/practices or certification scheme?

    BMP, GlobalGAP, BAP-GAA/ACC, PAD/ASC, others/NO…….....

Fish diseases

List down the most common disease you deal with in the last crop?

Bacillary Necrosis of Pangasius: …….. period(s)/crop, time of occurrence …….....

Motile Aeromonad Septicaemia..... period(s)/crop, time of occurrence …….

Pale gill and liver syndrome …… period(s)/crop, time of occurrence ….....

Yellow fillet syndrome ……. period(s)/crop, time of occurrence ….....

Parasite infection ……... period(s)/crop, time of occurrence …….....

Other diseases…….....

Performing antimicrobial susceptible test? Yes/No.....

Record book of drugs/chemicals use during operation: Yes/No.....

Record book of feed use: Yes/No.....

II. Uses of drugs, chemicals and plants

Names of active element, products and company

……
……
……

1. Antibiotics

Enrofloxacin…….. Doses and application times/crop

Ciprofloxacin Doses and application times/crop

Flumequine .Doses and application times/crop

Amoxicillin .Doses and application times/crop

Florfenicol .Doses and application times/crop

Sulfonamides +trimethoprime .Doses and application times/crop

Doxycyline Doses and application times/crop

Oxytetracycline .Doses and application times/crop

Cefalexin.....Doses and application times/crop

Ampicillin.....Doses and application times/crop

Cefotaxime.....Doses and application times/crop

Levofloxacin.....Doses and application times/crop

Gentamicin.....Doses and application times/crop

Others: ……..

2. Disinfectants:

Iodine…… Doses and application times/crop

Benzalkonium chloride……..Doses and application times/crop

$KMnO_4$ ……..Doses and application times/crop

Coper sulfate……...Doses and application times/crop

Chlorine powder…….Doses and application times/crop

Lime……...Doses and application times/crop

Salt……. Doses and application times/crop

Yucca……..Doses and application times/crop

Others: ……

3. Internal parasite control:

Praziquantel…… Doses and application times/crop

Ivermectin.....Doses and application times/crop

Others: ……..

4. Mixture of minerals and vitamins:

Minerals……..Doses and application times/crop

Vitamins……..Doses and application times/crop

Others: ..... ……..……

5. Probiotics……Doses and application times/crop

6. Plants and plant extract products

Brand name: …….....

Mix into feed or apply into ponds: ……

Doses:……..

Properties: treating diseases, disinfecting water or improving digestion:……

Price:……..

Effeciency: Good……., Not good……

How much does the drugs/chemicals cost for 1 pond? ……

III. Health aspect

1. Do the farmer keep record book of drugs/chemicals/feed/fingerling? Yes/No.

2. Who instructed farmer to use drugs/chemicals and diagnose the disease (multiple choices):

a. Safety instructions described on the package b. By veterinarian/technicians

c. Extensionist. d. Experiences of farmers.

e. Others: ……

3. Do you buy drugs/chemicals and use it directly or buy it and store it to use later? ***observe the place and ask***?

4. Do you separate the place of chemical storage and living/cooking place? ***Record by observe the place, do not ask them.***

5. Is the any direct contact between the skin of the workers and antibiotics, disinfectants and probiotics?.....……..…….

6. Do farm workers use any protection during handling of antibiotics or disinfectants? And which one? ……..……

7. Do workers regularly clean their hands/take a shower after handling of chemicals or contact with water/feed containing chemicals? ……

8. Do you know about the banned antibiotic? Who showed you? Say some types if you know?

……..…….

9. Do some workers have common signs of illness/poisons? List down here? Skin lesion, cough?

……..

BENEFITS OF STRIPED CATFISH FARMING

.....
.....
.....
.....

DISADVANTAGES OF STRIPED CATFISH FARMING

.....
.....
.....
.....

RECOMMENDATIONS IN SOLVING ISSUES IN STRIPED CATFISH FARMING

.....
.....
.....
.....

Presses de la Faculté de Médecine vétérinaire de l'Université de Liège

4000 Liège (Belgique)

D/2022/0480/7

ISBN 978-2-87543-191-2