Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-022-01629-2

ORIGINAL PAPER

Prunus amygdalus var. amara (bitter almond) seed oil: fatty acid composition, physicochemical parameters, enzyme inhibitory activity, antioxidant and anti-inflammatory potential

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Received: 25 March 2022 / Accepted: 16 September 2022

Abstract
Oil seeds are a natural source of phytochemicals with high nutritional value. The present study aims to evaluate for the first time the antioxidant, enzyme inhibitory, and anti-inflammatory activities in vitro and in vivo, as well as the physicochemical properties, fatty acid compositions, total polyphenol, flavonoid, and tocopherol contents of Algerian *Prunus amygdalus var. amara* cold-pressed oil. Bitter almond produces seeds that yield 35.5% of oil. It was characterized by a high saponification index of 215.94 mg KOH g^{-1} of oil, a peroxide value of 7.74 meq O_2kg of oil, a K_{232} of 2.612, and a K_{270} of 0.39. This oil represented a valuable source of healthy fatty acids. GC–MS analysis revealed oleic (68.27%) and linoleic (16.14%) as the main fatty acids. α-Tocopherol (85.77 mg/kg) was found to be the major component. The total phenolic and flavonoid contents were 21.94 ± 0.29 and 21.52 ± 0.14 µg/mL, respectively. The oil showed good antioxidant activity ($A_{0.50}$ = 34 ± 0.44 µg/mL) using the CUPRAC assay and modest activity with DPPH, ABTS, FRAP, and β-carotene assays, respectively. BAO (bitter almond oil) displayed the most promising α-glucosidase and α-amylase activities compared to acarbose as a reference molecule and moderate acetylcholinesterase (AChE) inhibitory activity. Moreover, BAO demonstrated in vitro and in vivo anti-inflammatory efficacy in a dose-dependent manner, comparable to that of diclofenac sodium. These effects were confirmed by histological examinations. Overall, our results showed that BAO has a strong potential to design new industrial preparations with nutritional, pharmaceutical, and cosmeceutical applications.

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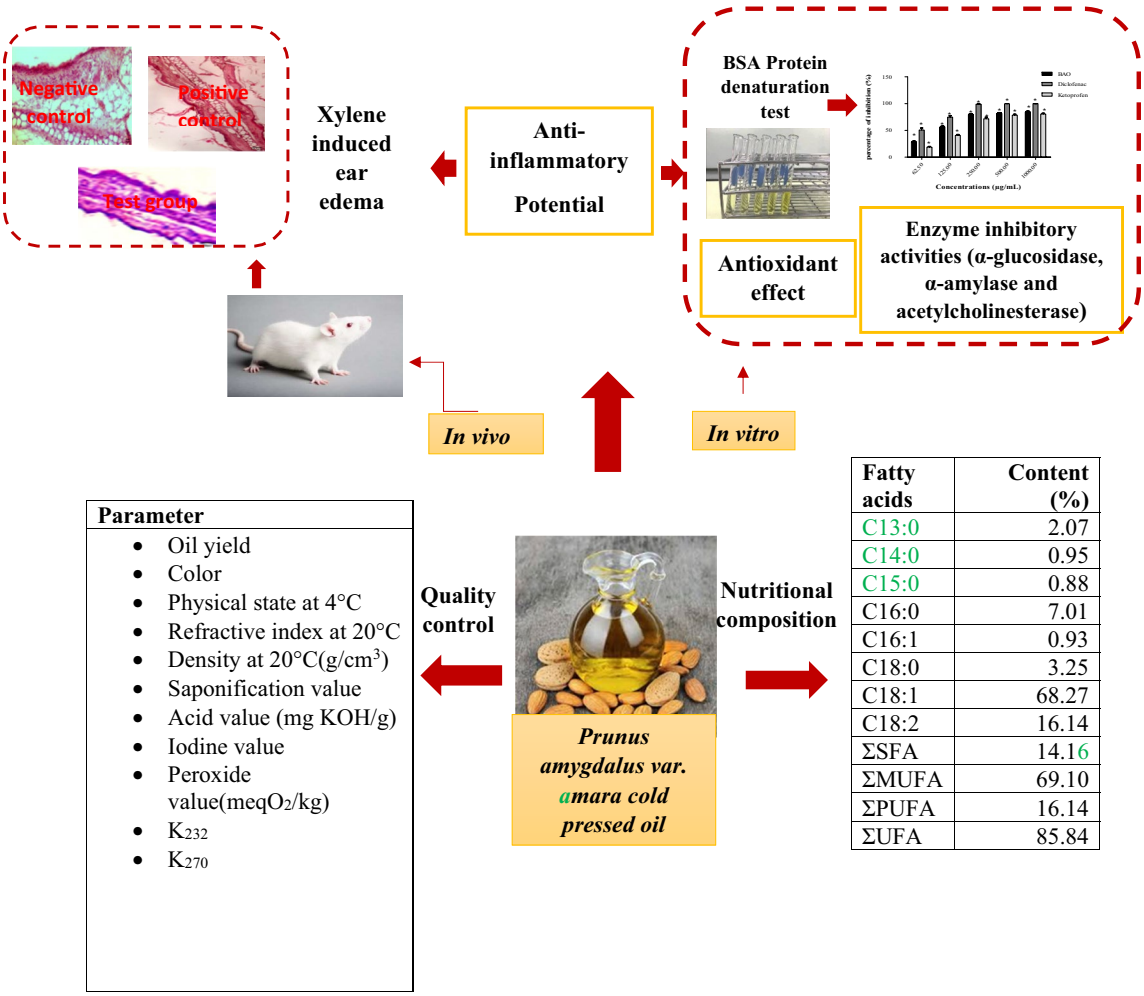
Published online: 26 September 2022

Graphical abstract



Keyword *Prunus amygdalus var. amara* oil · Physicochemical parameters · Fatty acids · Antioxidant · Enzyme inhibitory · Anti-inflammatory

Introduction

Quite recently, considerable attention has been paid to oilseed species from the genus *Prunus,* for example*, P. armeniaca L.* [1], *P. avium* [2], and *P. persica* [3],due to their favorable fatty acid composition and biologically active compounds [4]. These oils play an increasingly important role not only in food factories but also in the cosmetic, chemical, and pharmaceutical industries [5]. Seeds from *Prunus* species represent a valuable source of vitamin-E active compounds, high-unsaturated fatty acid content with a dominance of oleic acid, a moderate amount of linoleic acid, and low concentrations of saturated fatty acids. For that reason, they are considered good substitutes for olive and sunflower oils. [6].

P. amygdalus seeds belong to the Rosacea family. It includes about 430 species, naturally widespread throughout temperate regions [7, 8]. Based on taste, two major varieties were identified: the bitter almond (*P. amygdalus var.amara*) and the sweet almond (*P. amygdalus var.* dulcis) [9]. Bitter almond is the fruit of *P. amygdalus var. amara,* which contains higher amounts of amygdalin in the kernel than any other Rosacea species, which is by enzymatic hydrolysis degraded to glucose, benzaldehyde, and hydrogen cyanide (HCN) (a toxic compound) [10, 11]. The kernels and cold-pressed oil derived from them have a distinct "almond" flavor due to the presence of amygdalin.

Despite their toxic effects, pharmacological activities have been documented in a number of articles [12].

Several reports and clinical studies have demonstrated that bitter almond is highly valuable in traditional and modern medicine. Bitter almond has long been thought to provide a variety of biological activities in oral and topical applications, including wound healing, burns, acne, arthritis, hemorrhoids, digestion, and gastroprotection. However, there have been no reports of these effects being investigated clinically. Prevention of stretch marks in pregnancy, tumor growth inhibition in various types of cancer, antioxidant activities, and the reduction of modifiable cardiovascular and diabetes risks, including glucose homeostasis and inflammation, have been investigated for pharmacological purposes[13, 14].

Increasing interest has been focused on cold-pressed oils since the cold-pressing system involves no heat, no chemical treatments, and no refining process [15]. Therefore, this procedure allows all bioactive compounds to be preserved in the oil, and above all, the oil keeps the flavor and taste of the raw material from which it is obtained, which could make the nutritional and health properties of oilseeds more efficiently maintained. [3, 16]. BAO is rich in oleic and linoleic acids, with monounsaturated and polyunsaturated fatty acids, as well as naturally occurring vitamins, such as A, B1, B2, B6, and E [14].

Almond is regarded as one of the most polymorphic-cultivated fruit species in the world, with significant genetic diversity [9]. According to the Food and Agriculture Organization (FAO), the United States, Spain, and Iran are the top areas of almond production in the world. In the Maghreb, Morocco and Tunisia were the dominant [17]. However, in Algeria, despite its large surface and its biodiversity, bitter almond cultivation has not received enough attention. Moreover, the existing area dates back to the French colonial period. The Algerian Ministry of Agriculture recently set aside more than 300,000 hectares for the growth of almond, pistachio, and walnut trees to ensure the almond sector's long-term viability. The bitter almond was mostly grown in Medea, Ain Defla, Setif, Sidi-Bel Abbes, and Tlemcen. And it was primarily used in cosmetics. To the best of our knowledge, there have been no reports available in the literature that discuss the enzyme inhibition and anti-inflammatory activities of BAO.

Due to the numerous traditional uses of *P. amygdalus var. amara* and the lack of comprehensive studies investigating their pharmacological effects, we attempt to evaluate the chemical composition of the Algerian cold-pressed oil for the first time, with particular emphasis on the physicochemical properties, fatty acid profile using the GC–MS method, tocopherols, total polyphenols, and total flavonoids. Moreover, we explored the in vitro antioxidant activities using five methods and compared them with three antioxidant standards molecules. Furthermore, the enzyme inhibition activity, including α-glucosidase, α-amylase, and anticholinesterase inhibition activity as well as its anti-inflammatory potential, were assessed in vitro using the BSA (bovine serum albumin) protein denaturation method and in vivo using a xylene-induced ear edema model followed by histological examination.

Materials and methods

Chemical and reagents

Different reagents and chemicals were used in the present study including:Folin-ciocalteu's reagent (FCR),1,1-diphenyl-2icrylhydrazyl(DPPH),2,2′′-azinobis(3-ethylbenz othiazoline-6-sulfonic acid) diammonium salt (ABTS), β-carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), Trichloroacetic acid (TCA), butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), α-tocopherol, Neocupronine, Potassium ferricyanide, 3-(2-Pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5,5′'-disulfonic acid disodium salt (Ferrene), Diméthyl sulfoxide (DMSO), Acetylcholinesterase from electriceel (AChE, Type-VI-S, EC 3.1.1.7, 827,84 U/mg), Acetylthiocholine iodide, 5,5′-Dithiobis (2-nitrobenzoic) acid (DTNB), Galantamine, 4-Nitrophenyl-α-D-glucopyranoside(≥ 99%),α-Glucosidase from Saccharomyces cerevisiae (Type I, ≥ 10 units/mg protein),α-amylase (1U), starch, Iodine reagent, Hydrogen Chloride (HCl) Acarbose (≥ 95%) were provided by Sigma Chemical Company (Sigma-Aldrich GmbH, Stern-Heim, Germany).Sodium Carbonate, Aluminum Nitrate, Iron (III) chloride ($FeCl_3$), Iron (II) chloride($FeCl_2$), Sodium bicarbonate, Copper (II) chloride, Potassium Iodide (KI), Potassium persulfate, Potassium acetate, were obtained from Biochem Chemopharma. All other chemicals and solvents were of analytical grade.

Plant material

Bitter almond nut samples were collected in August 2018 from the cultivated area in TABLET (NE: 36° 15′ 51 ″ N; 2° 45′ 14 ″ E) Medea, Algeria. The collected seeds (~ 5 kg) were handpicked to avoid damages, cleaned, air-dried at ambient temperature, identified by "Ali Ferradji, professor at National Higher School of Agronomy, El Harrach, Algiers, ALGERIA", conserved under a voucher specimen (PAA/2018/LBEH), and stored at 4 °C until analysis.

Seed oil extraction

After broken or damaged nuts and other impurities were removed, whole seeds were extracted with the cold press

(4–6 kg/h capacity) at 40 °C. The oil was purified from solid impurities by sedimentation for one week, followed by filtration. The purified oil was kept in a hermetically closed, colored bottle at 4 °C.

Physicochemical characteristics

The ISO standard methods [18–22] were employed to determine the physicochemical parameters (acid value, density, iodine value, peroxide value, refractive index saponification value, and specific extinction at UV) of BAO.

Tocopherols analysis

Amounts of tocopherols (α,β and γ tocopherols) were determined with an Agilent 1100 Series HPLC system chromatograph. 0.2 g of BAO was dissolved in 4 mL of hexane and vortexed to form a homogenous phase. Vortexed oil samples were injected into a Hypersil gold (C18) reverse phase column (150 × 4.6 mm). Standard solutions of tocopherols are also prepared in n-hexane and used for calibration curve construction. A 10 µL sample volume was injected, whereas, elution was made using a mobile phase consisting of a mixture of methanol and water (95:5 V/V) at a flow rate of 1.5 mL/min at 30 °C. A DAD detector was used to detect tocopherols at the wavelength of 210, 254, and 292 nm. To identify tocopherols, the retention times (RT) of the unknown samples were compared against absolute/pure tocopherol compounds (α-, β- and γ-tocopherols). The injection volume was 20 µL. The analyses were carried out at room temperature. Results were given as mg per kg of oil [23].

Fatty acid composition

The BAO was analyzed as methyl esters to determine the fatty acid composition according to The ISO 12966–2:2011 [24] standard method. The fatty acid methyl esters (FAMEs) were prepared by adding 1 mL of isooctane to 50 mg of oil. The mixture was heated in a water bath at 50 °C for a few seconds and then added to 200 µL of HCl (2 N). The top layer (1 µL) was injected into a capillary column gas chromatography (GC) model coupled with a mass spectrometer by Perkin Elmer (Clarus SQ8S GC/MS) and a polar capillary column (Rt-2560) (diameter 100 m, 0.25 mm internal diameter, 30 min length, and 0.25 µm film in thickness), helium was used as carrier gas (1 mL/min). The detector temperature was 225 °C, and the column temperature was 100 °C for 4 min, then increased at a rate of 4 °C/min to 240 °C and held for 20 min. The FAMEs were identified by comparing their retention times to pure standard FAMEs purchased from Sigma and analyzed under the same conditions. BAO FAMEs were quantified according to their area percentages obtained by integrating the peaks. The results were expressed as a percentage of individual fatty acids in the lipid fraction.

Total phenolic (TPC) and total flavonoid content (TFC)

The TPC of BAO was evaluated by using the Folin–Ciocalteu method [25] and the results were given as micrograms of Gallic acid equivalents per milligrams of extract (µg GAE/mL). Moreover, for TFC of BAO was evaluated by spectrophotometry method according to Tel et al. [26] and the results were given as micrograms quercetin equivalents per milligram of extract (µg QE/mL).

Antioxidant activity

DPPH radical scavenging activity

The DPPH free antiradical activity was evaluated by using the spectrophotometry method according to Blois [27]. The results were expressed as IC_{50} (µg/mL) corresponding to the concentration of 50% inhibition, defined as the quantity of an antioxidant essential to decrease DPPH radical absorbance by 50%. Antioxidant standards α-tocopherol, BHT, and BHA, were used to compare the antioxidant activity.

ABTS scavenging activity

The ABTS scavenging activity was evaluated by spectrophotometric analysis according to Re et al. [28] with slight modifications. The results expressed as IC_{50} (µg/mL) corresponding to the concentration of 50% inhibition, defined as the quantity of an antioxidant essential to decrease ABTS radical absorbance by 50%. Antioxidant standards BHA and BHT were used to compare samples scavenging activity.

Ferric reducing power assay

The ferric reducing power of BAO was evaluated according to the method described by Oyaizu [29] with slight modifications. The results were expressed as absorbance and compared with antioxidant standards BHA, BHT, and α-tocopherol. Results were expressed as $A_{0.50,}$ corresponding to the concentration indicating 0.500 absorbances.

Cupric ion reducing antioxidant capacity assay

The copper reducing activity was evaluated according to the method described by Apak et al. [30]. The results expressed as $A_{0.50}$ (µg/mL) corresponding to the concentration indicating 0.500 absorbances. Antioxidant standards BHA and

BHT were used to compare the reducing capacity of the extracts.

β-carotene linoleic acid bleaching assay

β-carotene linoleic acid bleaching assay was evaluated according to Marco [31] with slight modifications. Antioxidant activity results expressed as IC_{50} (µg/mL) corresponding to the concentration of 50% inhibition. Antioxidant standards BHA and BHT were used, for comparison of samples scavenging activity**.**

Enzyme inhibition assays

α-Glucosidase inhibitory activity

The inhibitory effect of BAO on the α-glucosidase enzyme from Saccharomyces cerevisiae was investigated using the 4-nitrophenyl-α-D-glycopyranoside as a substrate for the enzymatic reaction, as described by Sinéad Lordan [32] with minor modifications. Absorbance was measured spectrophotometrically at 405 nm. Acarbose is used as a positive control. All experiments were performed in triplicate, and the results are given as 50% inhibition concentration (IC_{50}).

α-Amylase inhibitory activity

The inhibitory effect of BAO on α-amylase enzyme was determined, by measuring starch hydrolysis with iodine-potassium iodide as a coloring reagent, as described by Zengin et al. [33]. The absorbance was measured spectrophotometrically at 405 nm. Acarbose was used as a positive control. The results expressed as 50% inhibition concentration (IC_{50}).

AChE inhibition assay

The inhibitory activity of BAO on AChE activity was determined according to the spectrophotometric procedure of Ellman et al. [34]. Acetylthiocholine iodide (AChI) is used as a substrate for enzymatic reactions. The hydrolysis of AChI substrates was determined with maximum absorption at a wavelength of 412 nm. Galanthamine was used as a reference compound. The results expressed as 50% inhibition concentration (IC_{50}).

For all the bioassays, the measurements of activity results were performed using a 96-well microplate reader, the PerkinElmer Multimode Plate Reader EnSpire at the National Center for Biotechnology Research.

In vitro anti-inflammatory activity

The in vitro anti-inflammatory activity of BAO was evaluated using the inhibition BSA denaturation method, according to Kandikattu et al. [35]. 1 mL of sample extract was added to 1 mL of BSA solution at 0.2%, prepared in a 0.05 M Tris–HCl buffer at pH 6.6. The reaction mixture was allowed to stand for 15 min at 37 °C. The samples were then heated to 72 °C for 5 min and cooled to room temperature. The absorbance was calculated at 660 nm using a UV spectrophotometer. The experiments were performed in triplicate. Diclofenac sodium and ketoprofen were used as positive controls. The inhibition percentage of denaturation of BSA is calculated by the following formula:

$$I\,(\%) = AC - AS/AC * 100.$$

I: the inhibition percentage, AS: absorbance of the sample, AC: absorbance of the control.

In vivo anti-inflammatory effect

Animal experimentation

Twenty male NMRI mice (6-week-old, 20–25 g), were provided by the Pasteur Institute Algiers, Algeria. The animals were kept in laboratory condition for seven days, as an adaptation period at the animal factory of the experimental station of the Faculty of Natural and Life Sciences at Saad Dahleb University, Blida, Algeria. Mice were housed in cages with free access to water and food under the condition of a 12-h light–dark cycle. The room temperature and humidity were maintained at 25 °C and 35–60%, respectively. All animal procedures performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the European Union guidelines (2010/63/EU), approved by the committee of the Algerian Association of Experimental Animal Sciences (88–08/1988).

In vivo anti-inflammatory-xylene induced ear edema

The topical anti-inflammatory activity of BAO was determined using a xylene-induced ear edema assay on mice, according to the method previously described by Wang et al. [36] with a few modifications. The animals were divided into four groups of five mice each. Group I, received topically pure BAO at 100% (20µL). Group II received BAO at 50% (20µL) dissolved in Vaseline oil. Group III received the reference drug, diclofenac sodium 50 mg/kg, and served as a positive control. Group IV received Vaseline oil, which was used as a vehicle, and

served as a control. One hour after the topical administration of the different substances, 20 µL of xylene was applied to the inner and outer surfaces of the right ear of the animals. Five hours after the induction of inflammation, the animals were sacrificed by cervical dislocation. Ear biopsies (8 mm in diameter) were cut using a metallic punch. Percentages of edema inhibition were obtained for each group by measuring the difference in weight of the right and left ears of the same animal, and the percentage of inhibition (I%) was calculated using the following formula:

$$I\ \% = [\text{Increase in ear edema (control)} - \text{increase in edema (treated)} / (\text{increase in ear edema})] * 100.$$

Histological examination Histological examination The mouse ear biopsies were fixed in 10% formaldehyde and then washed and dehydrated using an ascending grade of alcohol. They were then washed with xylene, embedded in paraffin, and cut into 5 µm slices before being stained with hematoxylin and eosin (H&E) and examined under a light microscope.

Statistical analysis

Data means and standard deviation (SD) were calculated, from independent experiments. Data analysis was carried out using one-way ANOVA followed by multirange post hoc Dunnett's test (comparison of the control group with other groups). Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA), and ($p < 0.05$) was considered significant.

Table 1 Physico-chemical properties of BAO

Parameter	value
Oil yield	35.5% ± 0.33
Color	Golden yellow
Physical state at 4 °C	liquid
Refractive index at 20 °C	1.466 ± 0.00
Density at 20 °C(g/cm^3)	0.912 ± 0.00
Saponification value	215.88 ± 0.04
Acid value (mg KOH/g)	0.49 ± 0.03
Iodine value (g of I_2 per 100 g of oil)	88.93 ± 0.60
Peroxide value(meqo_2/kg)	7.74 ± 0.02
K_{232}	2.612 ± 0.02
K_{270}	0.390 ± 0.03

Values were expressed as means ± SD (n = 3). K_{232} and K_{270}: specific extinction coefficients at 232 and 270 nm, respectively

Results and discussion

Physicochemical characteristics

The current study is the first to report on Algerian BAO. The results of the physicochemical properties of this oil are summarized in Table 1. The oil content was found to be relatively high (35.5%). It indicates that bitter almond seeds are a potent natural source of oil. These results were comparably similar to those previously reported in several papers. For example, Hanine et al. [37] found that the oil content did not exceed 35% of Moroccan almond cultivars. The physical properties revealed that the oil has a visible yellow color, and it remained liquid at room temperature. The density of BAO was 0.912 g/cm^3, which was within the limit of the previously reported studies on this oil [38]. The refractive index was 1.466 at 20 °C and was similar to certain almond oils reported for other genotypes from Turkey by the same extraction method, such as *P.amygdalus var dulcis* (Ferragnes, 1.466), (Cristomorto 1.463), and (Tuono 1.470) [38].

Additionally, a high saponification index of BAO was found (215.94 mg KOH/g of oil), and may indicate a high content of low molecular weight triacylglycerol [39]. However, a considerable variation was noticed in almond oilseeds obtained from Turkey, when the saponification value did not exceed 103.6 mg KOH/g of oil for all the genotypes tested [38]. Based on the high saponification index of BAO obtained in the present study, it could be considered as an important ingredient in soap and shampoo fabrication [39].

The acidity gives an estimation of the quantity of free fatty acids. The acid value of BAO was low (0.44) and within the acceptable limits for virgin and cold-pressed oils according to the codex standard for named vegetable oils [40].

On the other hand, the iodine value, which refers to the unsaturated fatty acid present in an oil, is used as an indicator to predict the oil shelf life [41]. This value was found relatively low (88.93 g of I_2 per 100 g of oil) in BAO, which, therefore, indicates its suitability for consumption, after removing the toxic compounds. This index is in good agreement with previous results reported for almond oil [41]. In contrast with other works on other edible oils such as argan, grapefruit, and peanut oils, our bitter almond derived oil was highly unsaturated.

Moreover, it was noticed that the peroxide value was 7.78-eq O_2 kg of oil, revealing that the tested oil was fresh and of high quality by taking into account its oxidative stability. It is generally considered that oil, with a peroxide value higher than 9 meq O_2/kg causes undesirable health problems [41]. Since this parameter is used to evaluate the

stability of oil from oxidation and to detect its rancidity, it is suggested that BAO may have a longer shelf life.

Furthermore, the initial value of K_{232} remained relatively low (2.612). This low index of specific extinction at 232 nm reflected the absence of any primary oxidation products. For K_{270}, the value was 0.39. However, the increase in the absorption at 270 nm can be related to the secondary oxidation products [42].

To summarize, some variations affecting the physicochemical characteristics of BAO might be explained by various genetic, agronomic, climatic factors, and storage conditions.

Total phenolic and flavonoids contents.

Polyphenols are naturally occurring compounds and secondary metabolites of plants, generally produced to protect plants against biotic and abiotic stressors, acting as antioxidant, anti-inflammatory, antimicrobial, anti-diabetic, and anticancer molecules [43, 44]. Total phenolic and flavonoids content of BAO were measured; the results are summarized in (Table 2). The total phenolic content was 21.94 µg gallic acid equivalent/mL. On the other hand, total flavonoids do not exceed 21.52 µg quercetin equivalent/mL. In terms of flavonoids, these findings are consistent with those of Keser et al. [45], who found total flavonoid (22.98 mg catechin/g) in methanolic extract of a bitter almond Turkish cultivar. In terms of phenolic content, our findings are higher than those of Özcan et al. [38], who found that total polyphenol content in bitter almonds ranged from 15.80 ± 0.01 to 17.60 ± 0.01 µg/mL cultivated in Turkey using ultrasound-assisted extraction method. In comparison with previous studies, it is noticed that some bioactive compounds were higher, and others were lower. These variations from one oil to another could be linked to many factors influencing their concentration in the oil. The cultivar, extraction system, and the conditions of processing and storage are critical factors for the final content of polyphenols. [46].

Moreover, it was demonstrated that the total phenolic content of oils extracted with different organic solvents from *Prunus* kernels was low due to the limited transfer of phenolic compounds to the oil [15]. The low content of polyphenols and flavonoids, may affect the oxidative stability and the antioxidant activity of BAO.

Table 2 Fatty acid composition of BAO

Fatty acids	Content (%)
C13:0	2.07
C14:0	0.95
C15:0	0.88
C16:0	7.01
C16:1	0.93
C18:0	3.25
C18:1	68.27
C18:2	16.14
ΣSFA	14.16
ΣMUFA	69.10
ΣPUFA	16.14
ΣUFA	85.84

C13: tridecanoic acid; C14:myristic acid; C15:pentadecyclic acid;C16:0 hexadecanoic or palmitic acid; *C16:1* Cis-9-hexadecenoic or palmitoleic acid; *C18:0* octadecanoic or stearic acid; *C18:1n − 9* Cis-9-octadecenoic or oleic acid; *C18:2* Cis-9,Cis-12octadecadienoic or linoleic acid;

Fatty acid composition

The fatty acid composition is important from several perspectives, including nutritional quality and health benefits [47]. Eight different fatty acids (Table 3) were found in the BAO, with a majority of two fatty acids, oleic (C18:1, 68.27%) and linoleic (C18:2, 16.14%), followed by palmitic acid (C16:0, 7.01%). Altogether, they represent more than 91.42% of the total fatty acids. Thus, this oil could be considered an oleic-linoleic acid one. Tridecanoic, myristic, stearic, pentadecyclic, and palmitoleic acids constituted a further 8.08%. The polyunsaturated/saturated (P/S) ratio from BAO was 5.30.Generally; oils with an index of more than 1 are regarded as edible oils with high nutritional value for humans [48]. Moreover, total unsaturated fatty acids reached 85.84%. Our results are comparably similar to some oils from the genus *Prunus,* previously studied [49–51].

Furthermore, oleic acid is an important fatty acid, which participates in nervous cell construction, and it has a fundamental role in cardiovascular disease prevention, obesity-induced insulin resistance, and type 2 diabetes [39]. On the other hand, linoleic acid (C18:2; ω6) is an essential fatty acid that cannot be synthesized by animal cells, for which it should be imported exogenously [52]. In this regard, the

Table 3 Total phenolic and flavonoids contents

Extract	Phenolic content (µg GAE/mL)	Flavonoids content (µg QE/mL)
BAO	21.94 ± 0.29	21.52 ± 0.14

Values were expressed as means ± SD (n = 3)

Total phenolic compounds were expressed as µg gallic acid equivalent/ml (µg GAE/mL)

Flavonoids contents were expressed as µg quercetin equivalent /ml (µg QE/mL)

tested oil could be regarded as a good source of this acid. Potential health benefits attributed to linoleic acid include cardioprotective effects, modulation of the inflammatory response, and a positive effect on both central nervous system function and behavior [53]. Considering the valuable fatty acid content found in BAO, it could be suggested as a dietary agent for providing these fatty acids to the human body.

Tocopherol content

Tocopherols are classified into four homologs, namely, α, β, γ, and δ. Vegetable oils are naturally rich in these compounds. Table 4 reveals the tocopherol content of BAO. A significant value (85.515 mg/kg) of total tocopherol compounds was detected in BAO. α-tocopherol was found to be dominant, with a concentration of 81.86 mg/kg. It represents more than 95% of the total content. On the other side, γ -tocopherol was the minor homolog with a value of 3.64 mg/kg. It represents only 4% of the total content of tocopherols. The present results are in concordance with those obtained by Fernandes et al. [10] that reported 97.3 mg/kg for α-tocopherol and 2.8 mg/kg for γ- tocopherol from bitter almond seeds cultivated in Brazil. In another study, Çelik et al. [54] reported that the total tocopherol content of oils from 71 different almond varieties obtained from Turkish cultivars ranged from 0.00 to 119.18%. Besides, Ozcan, Matthaus, et al. [51] reported a total tocopherol content that varied from 47.42 to 80.15 mg/kg from 31 genotypes of almond oil.

Table 4 Tocopherols composition from BAO

Compound	(mg/kg oil)	(%)
α-Tocopherol	81.869 ± 0.15	95.73
β-Tocopherol	nd	nd
γ -Tocopherol	3.645 ± 0.01	4.26
Total Tocopherols	85.515 ± 0.02	100

Values were expressed as means ± SD (n = 3), nd:not detected

Furthermore, previous research has investigated the tocopherol content variation from different almond genotypes and found it to be highly variety-dependent [55]. Tocopherols in oilseeds are strongly influenced by extraction techniques, climatic factors, and analytical conditions [39]. For example, El Bernoussi et al. [41] studied the influence of the storage conditions on the oil quality of bitter and sweet almonds. They found that tocopherols from bitter almonds are the most influenced; their concentration decreases with time. This decrease could affect mainly the quality of the oil from the point of view of its biological properties. Regarding the tocopherol content, our results confirm that BAO is a promising natural source of α-tocopherol compared to other varieties from different cultivars.

Antioxidant activity

There is currently a great interest in developing novel antioxidants for applications in the pharmaceutical, cosmetic, and food industries. Plant extracts with antioxidant capacity may act through different mechanisms, including oxygen-radical absorption, reducing power, and free radical scavenging [56]. Therefore, using five methods based on multiple chemical reactions is necessary to determine the antioxidant activity [57]. DPPH, ABTS, FRAP, CUPRAC, and β-carotene/linoleic acid bleaching assays, were used for screening the antioxidant activity of BAO. The results are presented in Table 5 and expressed in terms of IC_{50} and $A_{0.50.}$

DPPH and ABTS assays were used to measure the radical scavenging capacity of BAO. The results showed that the activity was moderate; the IC_{50} values were 594 ± 5.72 µg/mL and 639.39 ± 3.29 µg/mL, respectively, and were relatively far from the standard used. Previous studies [38] indicate good antioxidant potential, with IC_{50} ranging from 17.34 to 84.84 µg/mL in different almond genotypes from Turkish cultivars. Keser et al. [45] demonstrated that IC_{50} = 83.49 at 1000 µg/mL using the ABTS method from bitter almond methanolic extract cultivated in Turkey.

Moreover, reducing abilities were evaluated using two tests, CUPRAC and FRAP. In the case of the CUPRAC

Table 5 Antioxidants activity of *P.amygdalus var. amara* extract by DPPH, ABTS, FRAP, CUPRAC, and β-carotene assays

	DPPH IC_{50}(µg/mL)	ABTS IC_{50}(µg/mL)	FRAP $A_{0.5}$ (µg/mL)	CUPRAC $A_{0.5}$ (µg/mL)	β-Carotene IC_{50} (µg/mL)
BAO	595.35 ± 5.72	637.39 ± 3.29	> 800	34.77 ± 0.44	165.73 ± 34.96
BHA	6.14 ± 0.41	1.29 ± 0.30	7.99 ± 0.87	5.35 ± 0.71	0.91 ± 0.01
BHT	12.99 ± 0.41	1.81 ± 0.10	61.67 ± 0.40	8.97 ± 3.94	1.05 ± 0.03
α-Tocopherol	13.02 ± 5.17	7.59 ± 0.53	34.93 ± 2.38	19.92 ± 1.46	1.79 ± 0.03

$_{IC50}$ and $A_{0.50}$ values are defined as the concentration of 50% inhibition percentages and the concentration at 0.50 absorbance respectively. IC_{50} and $A_{0.50}$ were calculated by linear regression analysis and expressed as Mean ± SD (n = 3)

BHA butylated hydroxyanisole, *BHT* butylated hydroxytoluene

assay, BAO recorded the best and the highest activity, $A_{0.50} = 34.77 \pm 0.44$ µg/mL relatively close to α-tocopherol ($A_{0.50} = 19.92 \pm 1.46$ µg/mL. However, the ferric reducing power assay of BAO does not show any activity at 800 µg/mL.

The total antioxidant activity of this oil was determined by using β-carotene bleaching activity. As seen in Table 5, BAO showed medium efficiency ($IC_{50} = 165.73 \pm 34.96$ µg/mL) toward this molecule and was far from BHA, BHT, and α -tocopherol when IC_{50} was 0.90, 1.05, and 1.79 µg/mL, respectively. These findings supported previous studies that have demonstrated good antioxidant activity using DPPH, ABTS, FRAP, and β-carotene methods from six Portuguese almond cultivars when the percentage of inhibition using β-carotene assay ranged from 64.28 ± 1.65 to $90.58 \pm 2.1\%$. [58].

Regarding the present results and the chemical composition of this oil, it can be noticed that BAO has a moderate scavenging capacity, good reducing power in the CUPRAC assay, and medium total antioxidant activity. The antioxidant and reducing power activity of BAO has been attributed to the presence of phenolic and flavonoid compounds. As it can be seen in Table 3, The low content of these compounds leads to moderate antioxidant capacity. Concerning the fatty acid profile of this oil, Fratianni et al. [3] suggested that the high content of oleic acid could negatively affect the antioxidant capacity of the tested cold-pressed oils.

Generally, using different complementary antioxidant assays could make the comparison difficult. For example, some phenolic compounds require a longer time to react. Additionally, the synergy between the antioxidants in the mixture depends on many factors, such as the structure and concentration [59–61].To conclude, with the low antioxidant activity found in this experiment, the oxidative stability and shelf life of this oil could be affected.

Enzyme inhibition bioassays

α-Glucosidase and α-amylase inhibitory activity

The anti-diabetic effect of this plant was determined by the potential of BAO to inhibit the α-glucosidase and α-amylase enzymes, which are frequently used for in vitro screening based on the inhibition of carbohydrate hydrolysis [62]. The inhibition of these enzymes, could play a fundamental role in the management of diabetes mellitus and control the glucose concentration in the blood [63]. Therefore, we assessed the inhibitory potential of BAO toward these enzymes. The results are presented in Table 6.

The inhibitory activities of α-glucosidase from BAO revealed a relatively strong effect ($IC_{50} = 0.290$ mg/mL) closer to the reference compound (acarbose, $IC_{50} = 0.275$ mg/mL). The results showed that BAO exhibited more α-amylase inhibitory activity, with an IC_{50} value of 0.115 mg/mL than acarbose, ($IC_{50} = 3.65$ mg/mL) used as standard. Referring to the literature, our findings are consistent with several studies that have investigated the in vitro anti-diabetic potential of using α-glucosidase and α-amylase inhibition assays from different species belonging to the genus *Prunus* [61, 64]. Additionally, Nowicka et al. [65] have demonstrated that cultivars of *P. persica* possess high activity against α-amylase and α-glucosidase due to their high content of anthocyanin.

Our results confirmed previous studies that have proved that some vegetable oils like *Olea europaea* oil, Cuminum *cyminum L.* oil, and *Argania spinosa* oil showed strong inhibitory α-glucosidase and α-amylase activities [66]. It was reported that the liposoluble compounds found in these oils could have a crucial part in the inhibition of enzymes associated with diabetes type 2 [67]. Regarding the GC–MS analysis of BAO, Su et al. [68] suggested that unsaturated fatty acids, particularly oleic, linoleic, and α-linolenic acids, might contribute the most to the inhibition of the α-glucosidase pathway, and they have less impact on α-amylase activity. In addition, according to Teng and

Table 6 Enzyme inhibitory and in vitro anti-inflammatory activities of BAO

Extracts	ACHE IC_{50} (µg/mL)	α-Glucosidase IC_{50} (mg/mL)	α-Amylase IC_{50} (mg/mL)	Anti-inflammatory IC_{50} (µg/mL)
BAO	126.40 ± 4.67	0.290 ± 0.39	0.115 ± 5.94	127.32 ± 0.73
Acarbose	–	0.275 ± 1.59	3.650 ± 10.70	–
Galantamine	6.27 ± 1.15	–	–	–
Dicolfenac sodium	–	–	–	41.89 ± 5.02
Ketoprofen	–	–	–	166.12 ± 6.06

IC_{50} values is defined as the concentration of 50% inhibition percentages and calculated by linerar regression analysis and expressed as Mean ± SD (n = 3)

"–"not tested

Chen [67], the inhibitory effect of oilseed increased, with the increase of double bonds. Thus, for fatty acids, the existence of double bonds was an important factor of inhibition on α-glucosidase and α-amylase. Thus, it could be explained by the strong effect of BAO on these enzymes. In general, the α-amylase and α-glucosidase inhibitory activities of BAO presented here were found to be interesting and have not previously been reported elsewhere. Therefore, the data presented for this oil could be assumed as the first report on these enzymes.

Anticholinesterase inhibitory activity

The acetylcholinesterase inhibitors drugs could play a critical part in the treatment of neurodegenerative disorders like Alzheimer's diseases. Today, one of the best approaches to the management of these diseases is the inhibition of acetylcholinesterase. The research community is always looking for natural alternatives to synthetic inhibitors to reduce their serious side effects. Table 6, showed the inhibitory activity of BAO against this enzyme.

The results showed that BAO had a low inhibition effect on the AChE *(*$IC_{50} = 126.4 \pm$ µg/mL) compared with galantamine ($IC_{50} = 6.27 \pm 1.15$ µg/mL), which has been used as a standard molecule. Similar results were found by Bonesi et al.[69]when they investigated the potential inhibitors of cholinesterase from two *prunus* species cultivated in Italy, and the IC_{50} results ranged from (97.60 ± 1.94 to 171.80 ± 3.63 µg/mL). Regarding the chemical composition of these plants, several reports attributed the neuroprotective effects of plants to their phenolic and flavonoid compounds. Mihaylova et al. [70] have concluded that even low-containing polyphenolic fractions exhibit an effect on AChE, which is in concordance with our finding and can be explained by the positive effect of BAO on the inhibitory activity of acetylcholinesterase despite their low content of polyphenols and flavonoids. In the present study, concerning the GC–MS content analysis, it might be considered that oleic, linoleic, palmitoleic, and other fatty acids identified in BAO could have an inhibitory effect on AChE [53].

Based on the results found in this research and referring to the literature, BAO seemed to be an efficient alternative solution to synthetic drugs to manage related diseases, such as diabetes mellitus (α-glucosidase and α-amylase) and Alzheimer's diseases (acetylcholinesterase). However, it has not been effectively exploited because of the presence of amygdalin. Nevertheless, its toxic effect, amygdalin, can have a beneficial impact on human health, such as anti-tumor, anti-inflammatory, and analgesic, reducing neurodegeneration, as well as blood glucose [71].

Furthermore, several studies confirmed that cold-pressed oils extracted from the Rosacea family seeds are generally less toxic compared to their kernels. For example, Pavlovic et al. [72] found a low concentration of amygdalin in cold-pressed apricot kernel oil (0.4 mg/g oil), while amygdalin content in apricot kernels was 5.0 mg/g. Besides that, other studies observed no amygdalin in oils from peach, plum, and apricot kernels. In contrast, the apricots and plum kernels had an amygdalin content of between 3 and 24 mg/kg. Therefore, it could be concluded that these oils are suitable for food industry applications since they are free from amygdalin [15].

In vitro anti-inflammatory activity

Protein denaturation occurs when the tertiary and secondary structures of the protein are lost. When proteins are denatured, they lose their biological function. One of the well-known causes of inflammation is the denaturation of tissue proteins. As a result, determining protein denaturation can be used as a simple screening test for anti-inflammatory drugs before using animals [73]. Consequently, we assessed for the first time the effect of BAO on protein denaturation to determine their potential in vitro anti-inflammatory activity. Regarding the results in Table 6, the quantity of oil needed for 50% inhibition was relatively moderate, ($IC_{50} = 127.32 \pm 0.73$ µg/mL). Compared to the standards used, the BAO is the most effective, and it showed stronger anti-inflammatory activity than ketoprofen, which was used as a positive control.

The anti-inflammatory activity of BAO showed no significant differences between diclofenac and ketoprofen ($p < 0.05$). As shown in Fig. 1 and Table 6, and compared to the reference molecules (diclofenac and ketoprofen), BAO showed an anti-inflammatory effect in a dose-dependent manner against the protein denaturation induced by the high temperature. At 500 µg/mL, a significant anti-inflammatory effect was obtained with a percentage of inhibition of 81.8%, which was relatively close to the inhibition scores of diclofenac (99.87%) and higher than that of the standard molecule ketoprofen (78.55%). As it can be seen in Table 6, the IC_{50} (127.32 ± 0.73 µg/mL) of the extract

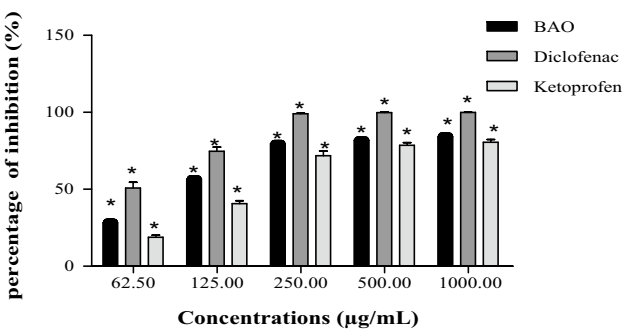


Fig. 1 In vitro anti-inflammatory effect of BAO and the reference compounds. *values are significantly different (Tukey test, $p < 0.05$)

tested was lower than the ketoprofen standard molecule (166.12 ± 6.06 µg/mL), and relatively higher than the IC_{50} of the diclofenac reference component (41.89 ± 5.02 µg/mL). Similar to our results, Fratianni et al. [3] reported that the in vitro anti-inflammatory potential of five cold pressed *prunus* seed oils using BSA bioassays, recorded the best activity superior to diclofenac, which is used as a reference compound, and the IC_{50} values ranged from (3.29 ± 0.206 to 4.339 ± 0.403 µg/mL).

In general, our findings are in agreement with previous studies that exploited the anti-inflammatory potential of oilseeds [74, 75]. To our knowledge, this is the first study to deal with the protein inhibition potential of BAO. Moreover, based on the fatty acid profile found in this oil, it has been reported that unsaturated fatty acids play a major part in the anti-inflammatory mechanism of oilseeds. Several studies indicate that oleic and linoleic acids, which were found to be major fatty acid components in BAO, play an important role in the inflammation process by inhibiting protein denaturation [3]. Our findings demonstrated that BAO has an excellent ability to maintain the three-dimensional structure of proteins; this ability could be attributed to the presence of some fatty acids, particularly oleic acid.

In vivo anti-inflammatory activity from BAO

The topical anti-inflammatory effects of BAO are also evaluated in vivo using a Xylene-induced ear edema model. The results are presented in Table 7 and Fig. 2.Topical application of Xylene (20µL) induces ear erythema, which causes local acute exudative inflammation and edema after application to the auricles of mice, which forms the basis of a reliable inflammation model [74]. When compared to the control group, pretreatment with BAO at (100%) (20 µL) showed the most promising anti-inflammatory effect by attenuating xylene-induced ear edema to (88.56%) ($p < 0.001$). Diclofenac sodium, a non-steroid anti-inflammatory drug, served as a positive control,and showed relatively similar effects as BAO at a higher concentration ($p < 0.001$) with the percentage of inhibition of ear edema (69.76%). BAO at a lower concentration (50%) exhibited a strong effect (62.31%) comparably closer to the reference drug ($p < 0.01$). Thus, it could be considered that BAO has anti-inflammatory capacity in a dose-dependent way.

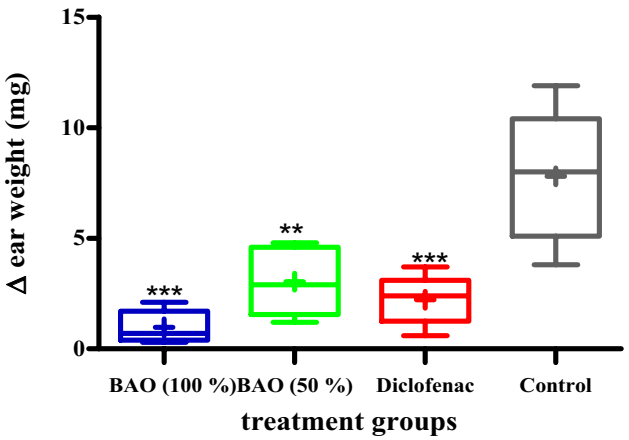


Fig. 2 In vivo anti-inflammatory potential from *P.amygdalus var. Amara.* **values are significantly different (Tukey test, $p < 0.01$). ***values are significantly different (Tukey test, $p < 0.001$)

Moreover, our findings confirmed previous studies that investigated the topical anti-inflammatory potential of vegetable oils with oleic acid as a major fatty acid compound, to treat skin diseases as well as for skin care, such as babassu oil, grape seed oil, and olive oil. In the present study, oleic acid was found as a major component in BAO. It has been confirmed previously that this compound has a topical anti-inflammatory activity via glucocorticoid receptors without causing any side effects, such as increasing blood glucose levels, which is a potential adverse effect related to glucocorticoids like dexamethasone [74].

Histopathological examination

The protective effects of BAO on ear edema were further supported by histopathological examinations. As shown in the H & E staining images presented in Fig. 3, treatments

Table 7 In vivo anti-inflammatory potential of BAO

Groups	Right ear (mg)	Left ear (mg)	Δ Ear weight (mg)	%Edema inhibition
Control (vehicule)	14.36 ± 3.17	7.52 ± 1.02	7.8 ± 2.16	–
Diclofenac (reference molecule	9.46 ± 1.52	7.24 ± 1.4	2.26 ± 0.72***	69.76 ± 6.94*
BAO (100%)	8.24 ± 1.23	7.52 ± 1.05	0.72 ± 0.26***	88.56 ± 7.39*
BAO (50%)	10.44 ± 1.60	7.4 ± 0.44	3.04 ± 1.55**	62.31 ± 6.49*

Data are shown as mean ± standard deviation. Statistical differences from the control were determined by ANOVA followed the Tukey test different from the control group. *P < 0 .05, **P < 0 .01 and ***P < 0.001 as right ear (pretreated with vehicle solvent and then with Xylene) vs. right ear (pretreated with BAO or Diclofenac, and then with Xylene) of mice in each group

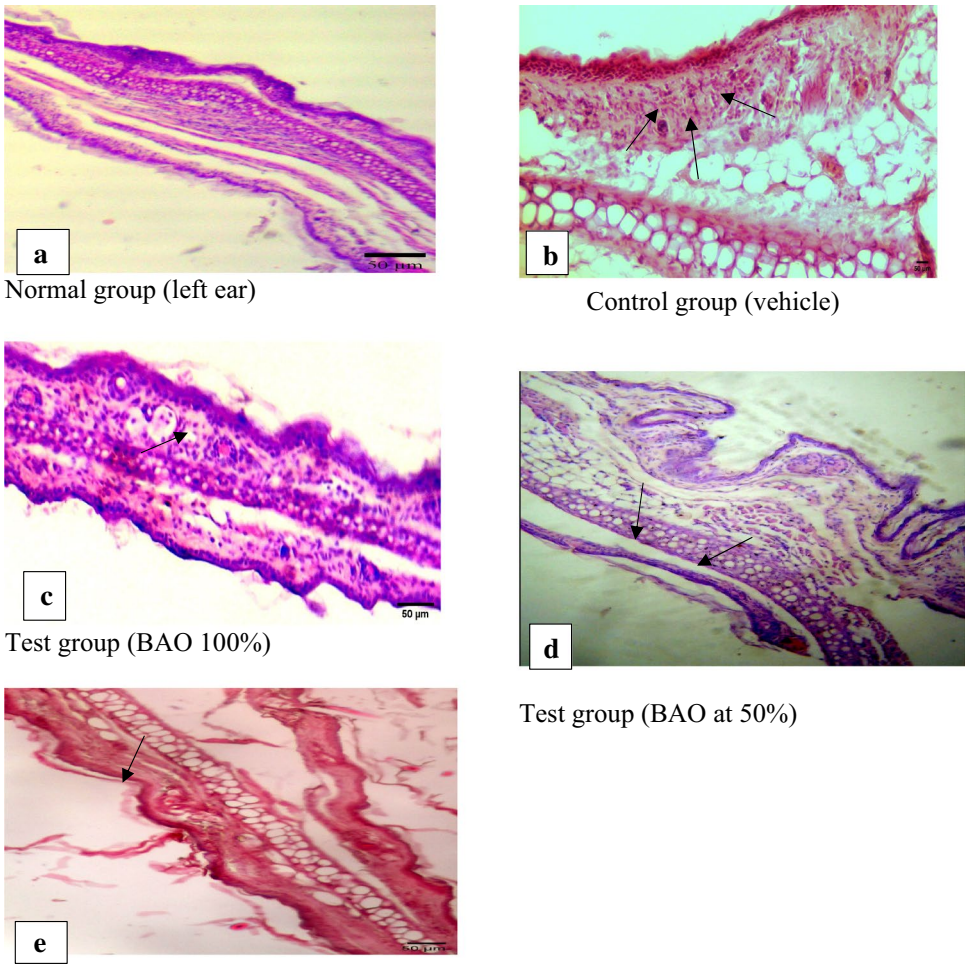


Fig. 3 Effect of the BAO at 100, 50%, and diclofenac sodium (0.5%) (20 µl/ear) on xylene induced ear edema in mice. Histological changes (A-H; hematoxylin–eosin 10 × and 20 × objectives) of the ear tissue of mice at 5 h after xylene application treatments. **a**normal group; **b** control group (no treatment); **c** test group BAO at 100%; **d** test group BAO at 50%; **e** reference group (diclofenac sodium);The black arrows indicate the presence of cellular infiltration in the ear tissue. Scale bar of 50 µm

with BAO reduced xylene-induced changes in mouse skin thickness and accumulation of granulation tissue.

Untreated control group

The inflammation induced by application of xylene on ear skin is characterized by cellular infiltration, mainly polymorphonuclear leukocytes (PMNL), eosinophils, lymphocytes at the borderline between the dermis and subcutis. Blood congestion, edema, loosening of connective tissue, and disorganization of fibers from the extracellular matrix were also observed (Fig. 3b).

BAO at 100%

Pure BAO was capable of reducing inflammation, where there was a significant decrease in inflammatory cell infiltration with the absence of blood congestion and a low intensity of edema compared with the untreated group. (Fig. 3c).

The test group at 50%

when compared to the control group showed mild inflammatory cell infiltration, mild capillary congestion, and mild edema intensity. (Fig. 3d).

The standard group

When compared to the control group, the diclofenac sodium-treated group showed a significant decrease in the inflammatory cell infiltration, as well as the absence of blood congestion edema in the dermal layer. (Fig. 3e).

To conclude, BAO provides a strong topical anti-inflammatory potential in a dose-dependent manner, by reducing cellular infiltration and blood congestion owing to its high content in unsaturated fatty acid, especially the oleic acid

one. Consequently, it could be recommended their use as an excellent ingredient for skin care in cosmetic fields.

Conclusion

The current study reports for the first time the chemical profile (fatty acid composition, tocopherol content, and polyphenols and flavonoids compounds) of *P. amygdalus var. amara* oil seeds cultivated in Algeria. The oil is rich in tocopherols and unsaturated fatty acids, particularly the oleic and linoleic ones. Therefore, it exhibited significant anti-diabetic activity with both α-glucosidase and α-amylase, which was higher than the reference molecule used, and demonstrated in vivo and in vitro anti-inflammatory efficacy similar to diclofenac sodium, a chemical drug widely used in inflammatory diseases, supported by histological investigations. This could provide an alternative solution to the use of synthetic anti-inflammatories without causing side effects on the organism. Furthermore, it has moderate antioxidant and anticholinesterase activities, which could be attributed to its low content in phenolic and flavonoid compounds. Above all, the results here reported suggest that BAO could find applications as cosmetic, pharmaceutical, and food ingredient agents. Future in vitro and in vivo toxicological studies are required to confirm the oral use of this oil. This work could pave the way for a larger number of Algerian almond varieties through the determination of their chemical composition and their biological activities.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11694-022-01629-2.

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