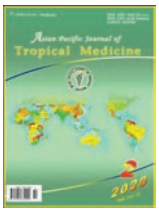

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In vitro biological activities of aqueous extracts of *Tetrapleura tetraptera* (Schumach & Thonn.) taub. and *Aframomum citratum* (C. Pereira) K.Schum from three Agro-ecologic Zones in Cameroon

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ABSTRACT

Objective: To investigate some phytochemical constituents and biological activities of twelve samples of *Tetrapleura tetraptera* (Schumach & Thonn.) taub. and nine samples of *Aframomum citratum* (C. Pereira) K. Schum fruits collected in the bimodal forest zone (ZONE V), the unimodal forest zone (ZONE IV) and the highlands zone (ZONE III) in Cameroon.

Methods: Fresh fruits extracts were obtained by aqueous infusion (100 ℃ during 15 min) and evaluated by spectrophotometric methods for total polyphenol (TPP), total flavonoids (TFLV) contents and antioxidant (DPPH, total antioxidant capacity by the phosphomolybdenum method, iron reducing power or ferric reducing antioxidant power and inhibition of beta carotene discoloration assays) and anti-inflammatory (inhibitions of protein denaturation and 5-LOX represented by INH.PROT and INH.5-LOX respectively) properties. Principal component analysis was performed.

Results: For both species, fruits from ZONE V have the highest TPP, TFLV levels and biological activities. TPP and TFLV content of *Aframomum citratum* and *Tetrapleura tetraptera* fruits are positively and significantly (P<0.05) correlated. The biological activities of all extracts (0.25, 2.5, 25, 250 mg/mL) were dose-dependent and the extracts have shown strong antioxidant and anti-inflammatory activities, but less than references (ascorbic acid, diclofenac, quercetin, and butylated hydroxytoluene). There was a positive correlation between TPP, TFLV and total antioxidant capacity, ferric reducing antioxidant power, and inhibition of beta carotene discoloration assays, and inverse correlations were observed with the IC_{50} (g/mL) of DPPH, INH.5-LOX and INH. PROT assays for both species.

Conclusions: The fruits exhibit variabilities and those from ZONE V for both species are economically and healthcare challenging for herbalists, pharmaceutical firms, scientists and consumers. Indeed, most important extraction yield of bioactive compounds correlated with significant biological activities and the use of less material compared with an implementation in other Agro-ecologic Zones with the same results are noted.

KEYWORDS: Biological activities; Non-Timber forest products; Antioxidant; Anti-inflammatory; Agro-ecologic Zone; Protein denaturation

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1. Introduction

Aframomum (A.) citratum and *Tetrapleura (T.) tetraptera* fruits were identified as being among the best available non-timber forest products in the Cameroonian markets[1]. *T. tetraptera* is a Fabaceae[2] native to the tropical rainforest of West Africa while *A. citratum*, a Zingiberaceae is endemic to the tropical flora of South Saharan Africa, Madagascar and the Guinea Gulf Islands[3]. In Cameroon, these fruits are mainly collected in the bimodal forest zone (ZONE V), unimodal forest zone (ZONE IV), the highlands zone (ZONE III)[4] and are sold throughout the national territory.

Used as spices, they demonstrate their socio-cultural importance in many Cameroonians regions[5]. Indeed, *T. tetraptera* and *A. citratum* show their importance in many food habits, where they are commonly used together for many traditional recipes ("pepe soup", "yellow soup", "Mbongo Tchobi", "nkwui"). Adding to their nutritional properties, they possess many traditional uses such as the treatment of inflammatory diseases (cancer, cough, diabetes, arthritis) in Cameroon, Nigeria, Ghana and Uganda[4,6-8]. While inflammatory states are an important source of oxygenated radicals that cause many diseases[8-10]. Indeed, inflammation involves high levels of arachidonic acid from damaged cell membrane phospholipids. The arachidonic acid metabolized by cyclooxygenase and lipoxygenase will produce highly inflammatory mediators. In addition, denaturation of proteins tissue and the red blood cell membrane lysis are also well-documented causes of inflammatory diseases[11]. *In vitro* anti-inflammatory mechanisms through which the extracts of both plant species act are still unknown.

Despite of their geographical diversity and origins, there is no report on the characterization of those fruits as regards to the respective Agro-ecologic Zones where they are harvested in Cameroon. Indeed, previous studies[5,12-14] were used to assess their phytochemical composition and the biological activities to which they are related mostly regardless of the place of picking as they are acquired on the markets. Therefore, for further investigation, we initiated a study of some phytochemical characteristics (total polyphenols and flavonoids) and *in vitro* biological activities (antioxidant and anti-inflammatory) of fresh fruits harvested in different Agro-ecologic Zones to serve as a scale for a comparative study and to determine if the locality where fruits are collected is a determinant of their chemical content and biological activities.

2. Materials and methods

2.1. Research areas and sample processing

The origin and geographical features of localities where spices were collected are recorded in Figure 1. The territory is divided into five Agro-ecologic Zones[15]: Sudano-Sahelian (ZONE I) in the North and Extreme North regions; Sudano-guinea (ZONE II) in the Adamawa Region; Western Highlands (ZONE III) in West and North-west regions with a relief consisting of steep land and depressions rich in alluvial and volcanic materials; Humid Forest with unimodal rainfalls (ZONE IV) in the Littoral, Southwest and the coastal edge of the South regions. The last Agro-ecologic Zone named ZONE V is located in the Centre, East and South regions with bimodal rainfalls.

Fresh fruits of *T. tetraptera* were harvested in twelve villages (Figure 1) spread over three regions: Centre (Bibey, Koumou, Boanda, Efok), East (Diang, Ngato, Kwo, Mbama) and Littoral (Nyanon, Mantem II, Bare-Bakem, Nkwangsi II) and three Agro-ecologic Zones (ZONES V, IV, III) while *A. citratum* fresh fruits were harvested in nine villages spread over two Agro-ecologic Zones (ZONES V, IV) and four regions: Centre (Makak, Messondo), East (Ngato, Ngola, Angossas), South (Lolodorf, Djoum) and Littoral (Nyanon, Bonépoupa). The localities were chosen based on a survey of wholesalers in major distribution markets. A specimen of each sample was authenticated by a taxonomist in comparison to the voucher specimens (31310/HNC and 37736/NHC respectively for *T. tetraptera* and *A. citratum*) in the National Herbarium, Cameroon. Cleaned, sorted and dried (45 ℃ during 72 h), fruits were grounded and the powders were vacuum bagged and refrigerated for further analysis.

2.2. Plant extracts preparation

Plant extracts were obtained by infusion in hot water (100 ℃ /15 min) from an extraction ratio of ¼ (g/mL). The mixture was centrifuged (4 500 rpm during 10 min) and the supernatant was lyophilized. The dried extracts were vacuum-packed and refrigerated.

2.3. Total polyphenols (TPP) and Total flavonoids (TFLV) determination

TPP was evaluated using Folin-Ciocalteu colorimetric method[16] with minor modifications. The TPP are calculated from the Gallic Acid standard curve (0.05-1.00 mg/mL) and are expressed as mg GAE/g dry plant weight. Total flavonoids (TFLV) content was also quantified[17] from Catechin standard curve (0.05-1.00 mg/mL) and expressed as mg CE/g dry plant weight.

2.4. Free radical scavenging activities

These activities are studied by 1,1-diphényl-2-picrylhydrazyl (DPPH) radical trapping assay[16] and by the inhibition of beta carotene discoloration method (beta-CAROT)[18] with slight modifications. DPPH discoloration by extracts (0.25, 2.5, 25, 250 mg/mL) is monitored at 517 nm after 30 min of incubation in the dark at room temperature. The IC_{50} of each extract were obtained by linear

regression. The method has been standardized by Ascorbic acid. The inhibition of the beta-CAROT by the extracts (250 mg/mL) was followed at 490 nm at the beginning and after 24 h against Butylated hydroxytoluene (BHT) prepared under the same conditions.

2.5. Reducing activities

The reducing power of the different extracts (0.25, 2.5, 25, 250 mg/mL) is measured by the "Ferric reducing antioxidant power" (FRAP)[19] and by the Phosphomolybdenum method of total antioxidant capacity (TAC)[20]. For the first test, ascorbic acid was used as positive control and the reducing activity was expressed as mg AAE/g of extract. The TAC is expressed as mg TE/g (milligram Trolox Equivalent).

2.6. Protein denaturation inhibition

The inhibition of bovine serum albumin denaturation (INH.PROT) by the extracts (0.25, 2.5, 25, 250 mg/mL) was performed with few modifications[21]. Absorbance measurements were made at 660 nm and diclofenac was used as positive control to evaluate the inhibition percentage. The IC_{50} of the extracts were obtained by linear regressions.

2.7. Lipoxygenase (5-LOX) inhibition

The inhibition of soybean 5-LOX (INH.5-LOX) activity by extracts (0.25, 2.5, 25, 250 mg/mL) was also tested[21]. The absorbance is measured at 234 nm, phosphate buffer solution is used as negative control and quercetin as reference or positive control. The inhibition percentage is calculated and the IC_{50}'s of extracts were obtained by linear regressions.

2.8. Statistical analysis

MINITAB 18, R (3.6.1), Microsoft Excel (2013) softwares were used for the statistical and graphical analysis and $P<0.05$ was considered statistically significant. Obtained results were recorded from triplicate observations and articulated as mean ± SD. The difference between two averages is determined by ANOVA from the Student-Newman-Keuls test. Tukey's test was used to separate two by two significantly different averages. Principal component analysis providing a transparent view of the accumulated information was performed and Pearson correlations were tested using Student's *t* test.

Table 1. Total polyphenols and flavonoids contents of *Aframomum citratum* and *Tetrapleura tetraptera* fruits aqueous extracts from various Agro-ecologic Zones.

Plant specie	Agro-ecologic zones	Regions	TPP (mg GAE /g)	TFLV (mg CE /g)
Aframomum citratum	Bimodal Forest zone (ZONE V)	Centre	2.50±0.03[b]	0.17±0.03[b]
		East	3.71±0.12[a]	0.22±0.02[a]
		South	2.81±0.05[b]	0.19±0.04[b]
	Unimodal Forest zone (ZONE IV)	Littoral	1.71±0.03[c]	0.11±0.05[c]
Tetrapleura tetraptera	Bimodal Forest area (ZONE V)	East	20.00±0.40[b]	0.51±0.01[b]
		Centre	27.31±0.70[a]	0.78±0.06[a]
	Unimodal Forest zone (ZONE IV)	Littoral	15.66±0.62[c]	0.28±0.01[c]
	Highlands zone (ZONE III)		14.13±0.72[c]	0.33±0.02[c]

Averages ($n = 3$) followed by letters (a-c) in the same column are significantly different ($P<0.001$); TPP: total polyphenols: TFLV: total flavonoids; GAE: gallic acid equivalent; CE: catechin equivalent

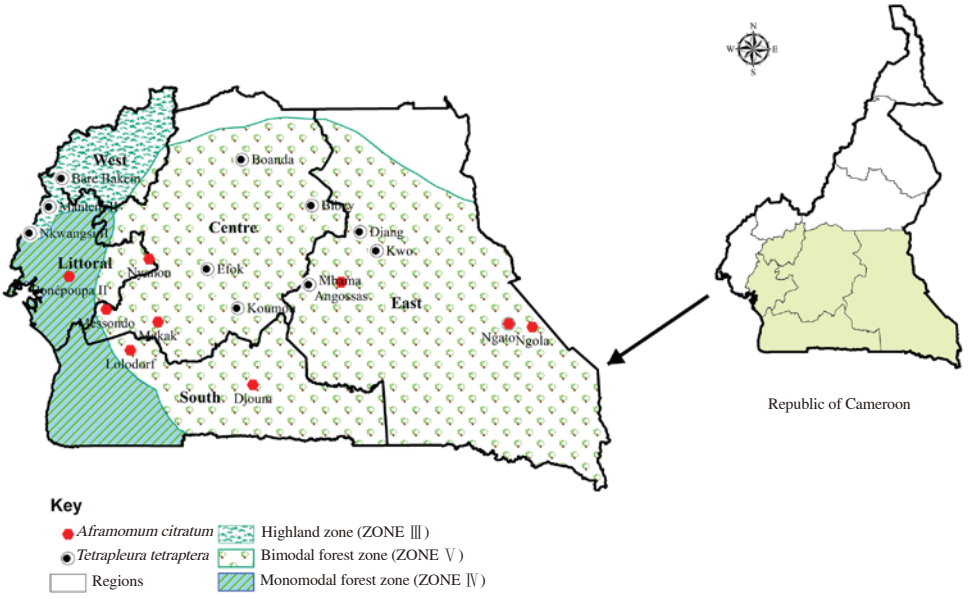

Figure 1. Agro-ecologic Zones and collection sites of the plant species.

Table 2. Correlations (P<0.05) among the various biological tests and the total polyphenols and total flavonoids content of *Aframomum citratum* aqueous extracts.

	FRAP (mg AAE/g)	DPPH (IC_{50}: g/mL)	beta-CAROT (IP: %)	TAC (mg TE/g)	INH. PROT (IC_{50}: g/mL)	INH. 5-LOX (IC_{50}: g/mL)	TPP (mg GAE/g)	TFLV (mg CE/g)
FRAP (mg AAE/g)	1							
DPPH (IC_{50}: g/mL)	-0.58836285	1						
P-value	0.0441805							
beta-CAROT (IP: %)	0.55516867	-0.7775522	1					
P-value	0.0609644	0.0029121						
TAC (mg TE/g)	0.5901457	-0.73767799	0.74234045	1				
P-value	0.0433838	0.0061729	0.0056922					
INH. PROT (IC_{50}: g/mL)	-0.70362208	0.69051614	-0.49083061	-0.6296132	1			
P-value	0.0408821	0.0129203	0.10517	0.0282404				
INH. 5-LOX (IC_{50}: g/mL)	-0.59589935	0.62736672	-0.7951954	-0.78662535	0.57657554	1		
P-value	0.0408821	0.0289810	0.0019891	0.002404	0.0497098			
TPP (mg GAE/g)	0.62356366	-0.64350545	0.58457349	0.83423173	-0.46702092	-0.74335949	1	
P-value	0.0302667	0.02396	0.045908	0.000741	0.1258392	0.0055911		
TFLV (mg CE/g)	0.67249821	-0.75669627	0.83100209	0.7808361	-0.56104026	-0.89964534	0.7604291	1
P-value	0.0165706	0.004388	0.0008114	0.0027196	0.057717	0.0000675	0.0040894	

AAE: ascorbic acid equivalent; CE: catechin equivalent; TE: trolox equivalent; IP: inhibition percentage; TAC; total antioxidant capacity; TPP: total polyphenols; TFLV: total flavonoids; IC: inhibitor concentration; beta-CAROT: beta carotene discoloration inhibition assay; INH.PROT: protein denaturation inhibition assay; INH.5-LOX: lipoxygenase inhibition assay

Table 3. Correlations (P<0.05) among the various biological tests and the total polyphenols and total flavonoids of *Tetrapleura tetraptera* aqueous extracts.

	FRAP (mg AAE/g)	DPPH (IC_{50}: g/mL)	beta-CAROT (IP: %)	TAC (mg TE/g)	INH. PROT (IC_{50}: g/mL)	INH. 5-LOX (IC_{50}: g/mL)	TPP (mg GAE/g)	TFLV (mg CE/g)
FRAP (mg AAE/g)	1							
DPPH (IC_{50}: g/mL)	-0.3423171	1						
P-value	0.3671951							
beta-CAROT (IP: %)	0.46402597	-0.66047182	1					
P-value	0.208309	0.0528246						
TAC (mg TE/g)	0.80786123	-0.7726559	0.805590645	1				
P-value	0.0084362	0.0146496	0.0087695					
INH. PROT (IC_{50}: g/mL)	-0.7020889	0.415077833	-0.495367276	-0.736212072	1			
P-value	0.0349848	0.2665995	0.1751036	0.023712002				
INH. 5-LOX (IC_{50}: g/mL)	-0.18995025	0.236982074	-0.194303218	-0.328093784	0.706954686	1		
P-value	0.6244835	0.539251	0.6164115	0.388703495	0.033200303			
TPP (mg GAE/g)	0.73470162	-0.36505048	0.573805364	0.77205369	-0.680655817	-0.298772159	1	
P-value	0.0241515	0.3340386	0.1061925	0.014776463	0.043583604	0.434823273		
TFLV (mg CE/g)	0.71169794	-0.56990087	0.575679422	0.848013045	-0.832242526	-0.609109628	0.905615581	1
P-value	0.0315188	0.1091478	0.1047914	0.0387149	0.00538123	0.047671123	0.000774725	

AAE: ascorbic acid equivalent; CE: catechin equivalent; TE: trolox equivalent; IP: inhibition percentage; TAC; total antioxidant capacity; TPP: total polyphenols; TFLV: total flavonoids; IC: inhibitor concentration; beta-CAROT: beta carotene discoloration inhibition assay; INH.PROT: protein denaturation inhibition assay; INH.5-LOX: lipoxygenase inhibition assay

3. Results

3.1. Total polyphenols (TPP) and Total flavonoids (TFLV) contents

TPP and TFLV were estimated from Gallic Acid (Y = 0.773 X +0.002) and Catechin (Y = 0.015 X +0.093) standard curves. TPP and TFLV contents were greater in *T. tetrapleura* extracts (Table 1) and there was a strong positive correlation between TPP and TFLV for both plant species (Table 2 and 3) meaning that the spices with the highest TPP contain the highest TFLV. *T. tetraptera* fruits from ZONE IV and III showed the lower TPP and TFLV contents compared with those from ZONE V. *A. citratum* fruits from ZONE V have registered higher phytonutrients concentration which is around twice greater than the content of ZONE IV fruits. However inside the different Agro-ecologic Zones, *T. tetraptera* fruits from the Centre region and *A. citratum* fruits from the East region recorded higher amounts.

3.2. DPPH radical trapping test trend

The extracts contained electron donors that inhibit the radical and the inhibition percentage is dose-dependent. The inhibition percentages of extracts from ZONE V are statistically greater for both plant species (Table 4). The concentration of extract needed to decrease the initial DPPH concentration by 50% is a parameter widely used to measure the antioxidant activity and a lower IC_{50} value corresponds to a higher antioxidant power. Regarding *A.*

Table 4. Biological activities of *Tetrapleura tetraptera* and *Aframomum citratum* fruits aqueous extracts from different Agro-ecologic Zones.

Plant specie and reference	Agroecologic zones	Regions	Antioxidant activities			Anti-inflammatory activities	
			DPPH IC_{50} (g/mL)	beta-CAROT IP (%)	TAC (mg TE/g)	INH.PROT IC_{50} (g/mL)	INH.5LOX IC_{50} (g/mL)
Aframomum citratum	Bimodal forest zone (ZONE V)	Centre	0.33 ± 0.15^{b}	72.11 ± 1.43^{b}	2.56 ± 0.28^{a}	0.21 ± 0.03^{b}	0.18 ± 0.07^{b}
		East	0.52 ± 0.18^{c}	62.13 ± 9.03^{bc}	2.82 ± 0.65^{a}	0.20 ± 0.01^{b}	0.17 ± 0.02^{b}
		South	0.57 ± 0.05^{c}	59.05 ± 3.74^{c}	2.22 ± 0.21^{a}	0.18 ± 0.01^{b}	0.15 ± 0.04^{b}
	Unimodal forest zone (ZONE IV)	Littoral	0.76 ± 0.06^{d}	48.72 ± 1.43^{d}	1.01 ± 0.10^{b}	0.26 ± 0.01^{c}	0.23 ± 0.01^{c}
Tetrapleura tetraptera	Bimodal forest zone (ZONE V)	East	0.18 ± 0.02^{b}	70.12 ± 5.48^{b}	24.33 ± 3.80^{a}	0.14 ± 0.02^{b}	0.11 ± 0.01^{b}
		Centre	0.15 ± 0.02^{b}	72.74 ± 2.46^{b}	27.20 ± 1.25^{a}	0.15 ± 0.01^{b}	0.10 ± 0.00^{b}
	Unimodal Forest zone (ZONE IV)	Littoral	0.34 ± 0.04^{c}	50.57 ± 4.17^{c}	17.38 ± 0.82^{b}	0.25 ± 0.04^{c}	0.12 ± 0.00^{b}
	Highlands zone (ZONE III)		0.35 ± 0.07^{c}	47.74 ± 6.90^{c}	17.67 ± 0.72^{b}	0.17 ± 0.04^{b}	0.12 ± 0.02^{b}
References			AA: 0.07 ± 0.00^{a}	BHT: 94.25 ± 0.19^{a}	-	Diclof: 0.05 ± 0.00^{a}	Quer: 0.05 ± 0.01^{a}

Averages (n = 3) followed by letters (a,b,c,d) in the same column are significantly different (P<0.001); TAC: total antioxidant capacity; TE: trolox equivalent; IP: inhibition percentage; AA: ascorbic acid; BHT: butylated hydroxytoluene; Diclof: diclofenac; Quer.: querctine

Table 5. Average iron reducing activity (mg AAE /g) of *Tetrapleura tetraptera* and *Aframomum citratum* fruits aqueous extracts from various Agro-ecologic Zones.

Plant specie	Agro-ecologic zones	Regions	Concentration (mg/mL)			
			0.25	2.5	25	250
Aframomum citratum	Bimodal forest zone (ZONE V)	Centre	0.27 ± 0.14^{a}	0.94 ± 0.13^{a}	8.50 ± 0.03^{a}	15.15 ± 0.35^{bc}
		East	0.11 ± 0.08^{a}	0.79 ± 0.28^{a}	11.37 ± 0.95^{a}	18.58 ± 1.39^{a}
		South	0.48 ± 0.09^{a}	2.04 ± 1.65^{a}	12.50 ± 0.57^{a}	16.54 ± 1.19^{ab}
	Unimodal forest zone (ZONE IV)	Littoral	-	0.86 ± 0.27^{a}	10.29 ± 1.68^{a}	13.86 ± 0.26^{c}
Tetrapleura tetraptera	Bimodal forest zone (ZONE V)	East	0.92 ± 0.42^{a}	5.12 ± 1.25^{a}	18.50 ± 2.83^{a}	20.48 ± 1.56^{b}
		Centre	0.58 ± 0.21^{a}	3.81 ± 0.28^{b}	17.06 ± 2.20^{a}	22.69 ± 2.63^{a}
	Unimodal forest zone (ZONE IV)	Littoral	0.85 ± 0.13^{a}	3.96 ± 0.64^{b}	14.45 ± 1.54^{b}	18.41 ± 0.80^{b}
	Highlands zone (ZONE III)		0.88 ± 0.43^{a}	3.49 ± 0.85^{b}	16.92 ± 1.68^{ab}	20.75 ± 0.77^{b}

Averages (n = 3) with different letters (a-c) are significantly different (P<0.05)

citratum, the average IC_{50} (g/mL) from ZONE IV extract was ten times higher than the reference IC_{50}'s and about 1/3 higher than the average of the IC_{50} of extracts from ZONE V. As for *T. tetraptera* extracts, after comparing extracts according to the localities, there was no significant difference between Diang (In the East region, ZONE V), Efok, Bibey, Koumou (In the Centre region, ZONE V) IC_{50}'s and the reference IC_{50}'s. But while relating the various Agro-ecological Zones as a whole, IC_{50} extracts were significantly different and ZONES III and IV extracts possess low trapping activity. Globally, IC_{50} extracts were following this pattern; Ascorbic acid < ZONE V < ZONE III = ZONE IV. Correlations between the IC_{50} of both plant species extracts and TPP, TFLV are significantly negative (Table 2 and 3).

3.3. Inhibition of beta carotene discoloration (beta-CAROT)

The extracts inhibited beta-CAROT and *T. tetraptera* extracts were more effective after 24 h (Table 4). The average inhibition capacity of *A. citratum* extracts from ZONE IV was less than the one noted in ZONE V and the reference (BHT). Regarding *T. tetraptera*, the average inhibition percentage of extracts from ZONE IV was about half lower than those of BHT and about a third smaller than those of ZONE V extracts. There was no difference between among the inhibition percentages of extracts coming from ZONE V. Significant positive correlations between the beta-CAROT activity and TPP, TFLV content of *A. citratum* extracts were also registered. It reveals that the spices with the highest TPP and TFLV content possessed the greatest inhibition power of beta-CAROT.

3.4. Iron reducing activity

Iron reducing activity by the extracts is dose-dependent. *A. citratum* extracts from ZONE IV recorded the lowest reducing power at all concentrations 0.25 and 250 mg/mL (Table 5) and for concentrations lower than 25 mg/mL there was no difference within the Agro-ecologic Zones. On the other hand, at 250 mg/mL, the reducing powers of ZONE V extracts are significantly higher than those of ZONE IV. For *T. tetraptera* at 25 mg/mL, extracts in ZONE

V recorded higher reducing activity. Correlations between the FRAP activity and TPP, TFLV of both plants species extracts are significantly positive (Tables 2 and 3).

3.5. Total antioxidant capacity by the Phosphomolybdenum method (TAC)

The total antioxidant capacity of spices extracts varied significantly from one site to another. Concerning *A. citratum*, there was no significant difference among the TAC of spices from ZONE V. *T. tetraptera* extracts TAC's from ZONE IV and ZONE III were less effective but there was no significant difference among extracts from ZONE V. Tables 2 and 3 shows significant positive correlations between TAC of both spices extracts and the TPP and TFLV contents.

3.6. Protein denaturation inhibition

The plant extracts recorded significant anti-inflammatory dose-dependent activity but lower than that of Diclofenac (Table 4). For *A. citratum* extracts, the highest Bovine Serum Albumin inhibition denaturation is noted on extracts from ZONE V showed higher Bovine Serum Albumin inhibition denaturation. *T. tetraptera* extracts inhibition percentage from ZONE V were higher than those of the other Agro-ecologic Zones and there was also significant negative correlations (Table 3) between TPP, TFLV and the extracts IC_{50}'s.

3.7. Lipoxygenase inhibition activity

The extracts have inhibited the soybean 5-LOX and the inhibition percentage is increasing with the rise of the extracts concentration (Table 4). Whatever the concentration of both plants species, Quercetin had the largest inhibition percentage. There is no difference between the inhibition percentages of extracts from ZONE V at the highest concentration for *A. citratum* and IC_{50} (g/mL) of the extracts were almost the same within that area. We noticed also significant negative correlations between the IC_{50}'s extracts and TPP, TFLV (Table 2). Concerning *T. tetraptera*, there is no significant difference between the inhibitions percentages registered in all the Agro-ecologic Zones.

3.8. Multivariate analysis of biological activities and TPP and TFLV of the two plant species

Principal component analysis was performed (Figures 2 and 3) using eight variables (TPP, TFLV, DPPH, INH.PROT, INH.5-LOX, beta-CAROT, TAC, FRAP) of nine observations distributed in three Agro-ecologic Zones (Centre, South, East and Littoral regions) for *A. citratum* and twelve observations distributed in two Agro-ecologic Zones (Centre, East and Littoral regions) for *T. tetraptera* extracts.

About *A. citratum*, the two main axes explain a variance of 79.7%. The factors which explain better component two (Dim2) are the TPP and FRAP assay while those which contribute better to the component one (Dim1) are DDPH and INH.5-LOX tests. The sites that are similar are Nyanon-Bonepoupa II or Angossas-Ngola-Djoum-Messondo groups. But spices from the locality of Ngato present the highest content of TPP and TFLV while samples from Bonepoupa II the smallest. The positively correlated variables are TPP, TFLV, TAC, FRAP, beta-CAROT (Figure 2 and Table 2). The negatively correlated variables are DPPH, INH.PROT, and INH.5-LOX assays. Indeed, expressed in IC_{50}, those biological activities are more effective when this concentration is small. The Biplot interpretation indicates that spices from Nyanon and Bonepoupa II (ZONE IV) have the weakest TPP, TFLV and biological activities as it was just confirmed by the *in vitro* tests results.

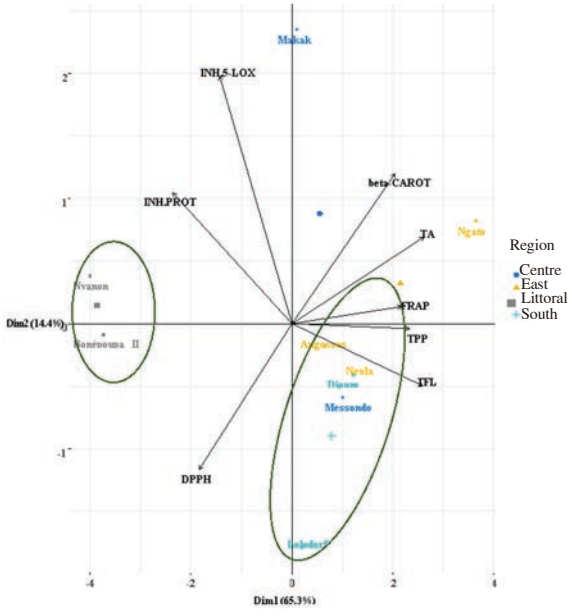

Figure 2. Simple biplot of different sites and variables (biological activity assays, total polyphenols and flavonoids contents) of *Aframomum citratum* fruits aqueous extracts.

As for *T. tetraptera* the two main axes explain a variance of 81.5%. The best important factors explaining component two are FRAP and INH.PROT assays. Measures of TAC and DPPH assays contribute the most to component 1. Similar sites are those from the East and Centre regions (ZONE V). But, samples from Bibey, Boanda, Efok, Koumou localities contains the biggest amount of TPP and TFLV while those from the unimodal Forest zone (ZONES IV) and the highlands zone (ZONE III) have the smallest amounts. Positively correlated variables are TPP, TFLV, TAC, FRAP, beta-CAROT (Figure 3 and Table 3). Negatively correlated variables are DPPH, INH.PROT and INH.5-LOX assays.

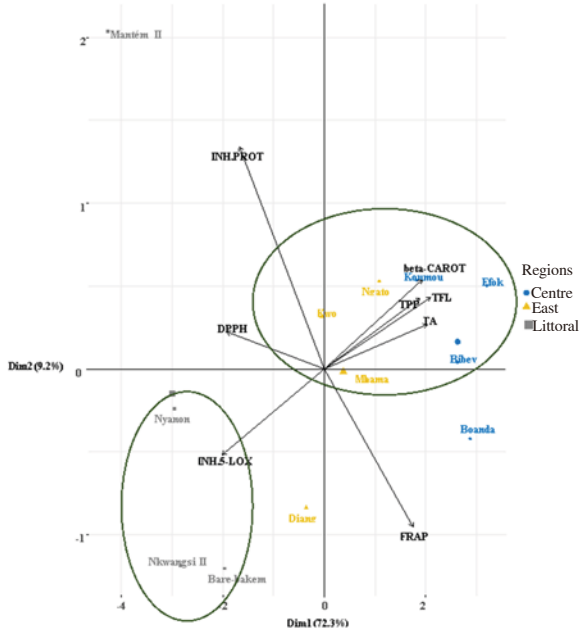

Figure 3. Simple biplot of different sites and variables (biological activity assays, total polyphenols and flavonoids contents) of *Tetrapleura tetraptera* fruits aqueous extracts.

4. Discussion

This study highlighted the levels of total polyphenols and total flavonoids and some biological activities of infused aqueous extracts of *T. tetraptera* and *A. citratum* fresh fruits taken from three different Agro-ecologic Zones in Cameroon. For both plants species ZONE V possesses the most important levels of TPP and TFLV. *T. tetraptera* have the highest studied characteristics certainly lower than those already published[12] in the aqueous extracts but above those reported in the ethanolic extracts of the two plant species[22]. The correlation between TPP and TFVL is similar to previous results published by Bouba *et al*[13].

When assessing the antioxidant activities, the change in absorbance of DPPH and beta-carotene and the reducing activity evaluated through TAC and FRAP assays after addition of the extracts suggest their antioxidant property. For all the antioxidant activities, *T. tetraptera* and *A. citratum* extracts from ZONE V are the most effective. However, the average intensity of these activities remained below those of the references used (Ascorbic acid, Butylated hydroxytoluène, Diclofenac and Quercetin). The results are similar to those already published[12,22-24]. Additionally, the highest activities are noticed in spices (for both plants species) with the most important TPP and TFLV contents as reported by Rice *et al*[25]. Without a doubt, polyphenols have an ideal chemistry structure for free radical elimination activities and therefore have a significant antioxidant activity. This activity is particularly important when the concentration in phenolic compounds is high[7,25]. Indeed, plant phenols have drawn increasing attention due to their significant effects in the prevention of multiple oxidative stress and flavonoids (hydroxylated phenolic substances) have shown great potential in management of coronary heart disease. The results obtained with the study of correlations are similar to former reports[22,24]. Some investigations show results different from those obtained. For example, Etoundi *et al*.[12] have reported at lower concentration (0.25 mg/mL), a scavenging activity of 83.5 % with aqueous extracts of *T. tetraptera* fruit. An activity ranging from 10.56% to 66.01% at concentrations between 25 mg/mL and 100 mg/mL was also recorded in the same extract by Famobuwa *et al*[23]. Concerning *A. citratum*, Josiah Sunday *et al*.[24] have recorded in the methanolic extracts a IC_{50} of (196.13 ± 1.44) μg/mL, while tests with *A. danielli* seed oil (100 μg/mL) exhibited the highest inhibition percentage of the DPPH radical (77.96 % / IC_{50}: 45.50 μg/mL) compared to rhizomes and leaves extracts[26]. beta-CAROT by the plant extracts gives them a good potential in the food industry and in the medicinal field because they can control oxidation in food systems and protect them (food safety implications) due to the importance of carotenoids or pro-vitamin A[27] in the diets. According to extracts reducing activities, at 250 mg/mL for both plant species, the results have the same tendency as those published earlier[14,22,24] on the aqueous and organic extracts even at lower concentrations with respect to the TAC. The iron reducing power presented results contrary to ours in Sokamte *et al*.[22] report. They recorded a higher reducing ability of *A. citratum* methanolic extract than that of *T. tetraptera* extract [(154.43 ± 3.02) AAE mg/g against (56.53 ± 0.66) AAE mg/g)]. In this study, results obtained with *A. citratum* extracts approached those noted by Ene-Obong *et al*[28]. The reducing ability of these extracts could be explained by the presence of reductones[29] causing the formation of hydrogen peroxide. These compounds therefore reflect the capacity of different extracts to give electrons that stabilize and block radicals to reduce their production.

Inflammation is implicated in the pathogenesis of many diseases (arthritis, stroke, cancer, diabetes *etc.*) and protein denaturation, lipoxygenase activity are well correlated with the appearance of the inflammatory response[30]. The extracts from ZONE V are the most active and *T. tetraptera* extracts have at equal concentration the most effective activities. Therefore, its uses in traditional medicine are justified for handling inflammatory diseases[2,8]. The results obtained have the same outline as those of Odukoya *et al*.[31] with regard to the ethanolic extracts and petroleum ether extract of *A. danielli* for the 5-LOX inhibition. They are related to the physicochemical content of extracts[14,22], especially phenolic

acids, flavonoids, metals, vitamins, *etc*. As an example, flavonoids are known to act on the inflammatory response *via* many routes and block molecules like COX, iNOS, cytokines, nuclear factor-κB and matrix metalloproteinases and they are good free radical scavengers that donate hydrogen, inhibit Lipid Peroxidation and are metal ion chelators[7]. The characterization of *T. tetraptera* fruits reveals the presence of phenolic acids (ellagic, caffeic, coumaric, chlorogenic, syringic, gallic, vanilic, benzoic, ferulic, cinnamic) flavonoids (rutin, quercetin, catechin, epicatechin, apigenin, luteolin, *etc.*) while *A. citratum* citratum fruits contain Ferrulic and T-cinnamic acids, epicatechin, quercetin and eugenol[22]. Meffo *et al*.[32] have also reported that the main component of the essential oils of *A. citratum* is geraniol. The majority of these compounds[7,32,33] has antioxidant and anti-inflammatory activities in animal models. As examples, rutin is only effective in the chronic inflammatory process (arthritis); flavanones are also effective in neurogenic inflammation induced by xylene and quercetin reduces paw oedema induced by carrageenan. Geraniol was found to significantly decrease lipid peroxidation, inhibit nitric oxide release (64.61%) and reactive oxygenated species generation in the pre-treated cells as compared to stresses cells[34]. Similarly, Albanoa and Miguel[35] stated that phenols, terpenoids have the ability to inhibit 5-LOX and reports[7,36] have mentioned the presence of diterpenoids, sesquiterpenoids in Zingiberaceae in general.

The differences noticed between the Agro-ecologic Zones according to characteristics studied on the plants species can be internal or external to the various ecosystems. Within the ecosystem, ZONE V is characterized by abundant rainfall (nine months/year and two distinct wet seasons) and rich (high agricultural value) and very fertile lateritic soils as long as the plant cover protects the soil against leaching[37]. Indeed, these soils are formed under forest cover and organic matter is very developed at the surface layers[38]. Organic matter is the main indicator of soil fertility because of its impact on the physicochemical and biological soil properties. It helps if all the conditions are encountered for soil quality and plant yields by increasing aeration, water retention capacity, stabilizing aggregates, providing a shelter for soil organisms that regulate the nutrients cycle and therefore promoting the retention and delivery of essential elements to increase soil productivity[39]. The particularities of ZONE IV and ZONE III are the rainfall and the soil[40,41]. In ZONE IV, the waterfalls are more important (2 500-4 000 mm/year) and the waterlogged and/or alluvial consist of fine clay, silt and sediment brought in the deltas and floodplains by many rivers (Bonepoupa and Nyanon localities). Nkwangsy II, Mantem II, Bare-Bakem (ZONE III) soils are instead volcanic (composed of sandstone with clay matrix certainly rich in minerals) and immediately more fertile. However, because of their crumbly consistency and the intensive rainfall, steep slopes and altitude, they are exposed to the risk of increased erosion[42]. Climatic factors and soils are added to biotic and human factors because those localities are intensive agricultural production zones for several cash crops (Robusta and Arabica coffee, bananas, plantains, pepper) and food crops are under significant pressure due to the intensive and sometimes uncontrolled use of pesticides[42,43]. Without a sustainable land management policy, intensive exploitation and pollution of soil by chemical fertilizers or pesticides decrease soil fertility and sustainability[43]. External to the ecosystem, the differences observed may be due to the genetic variability[44].

In summary, the area where to collect the two plant species is determinant and the biological activities expressed by the extracts reflect their different *in vitro* mechanisms. This preliminary study gives a graphic representation of some phytochemical characteristics and biological activities of the two spices in Cameroon. This decision-making graphic tool can therefore help for sampling and guide the various stakeholders involved in the valuation and exploitation of *A. citratum* and *T. tetraptera* fruits. But there is a need of additional *in vivo* tests in order to confirm the different biological activities observed because some of the tests used are not occurring in the human body. Furthermore, the phytochemical composition and the biological activities found on these fruits can constitute raw or base materials for pharmaceutical firms, scientists, food processing companies, herbalists, consumers who can save valuable time and money when looking for the raw material in a context where the resource's origins varied.

Conflict of interest statement

The authors declare they have no conflict of interest

Authors' contributions

ME, MS and MLF conceived of the presented idea, developed the theory, verified the analytical methods, conceived and planned the experiments, supervised the project, worked out almost all of the technical details, and performed the numerical calculations for the suggested experiment. ME, JLN, NAM planned and carried out the simulations, carried out the implementation, performed the analytic calculations and performed the numerical simulations, contributed to sample preparation, verified the numerical results. ME wrote the manuscript with input from all authors, carried out the experiments, , derived

the models and analyzed the data. ME, NAM aided in sampling, interpreting the results and worked on the manuscript by designing the Cameroon map and by drawing the various geographical areas parameters. All authors discussed the results and contributed to the final manuscript.

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