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Changes in polyphenols content and biological activities on *Tetrapleura tetraptera* and *Aframomum citratum* fruits extracts using different extraction methods in an acid/ alkaline medium

Manga Eyenga [(1)], Patricia Ebai [(2)], Fedoung E. Fongnzossie [(2)], J. Laure Ngondi [(3)], M. Sindic [(4)]

[(1)] Interdisciplinary Laboratory of Territory, Society, and Sustainable Development, Higher Institute of Agriculture, Wood, Water Ressources and Environment of Belabo, Bertoua University (Cameroon). E-mail: emanga2013@yahoo.fr
[(2)] Process Engineering Laboratory, Higher Normal School of Technical Education, Douala University (Cameroon).
[(3)] Laboratory of Nutrition and Nutritional Biochemistry, Biochemistry department, Faculty of Sciences, Yaoundé 1 University, Yaoundé (Cameroon).
[(4)] Laboratory of Quality and Safety of Agro-food Products, TERRA research center, Gembloux Agro Bio-Tech, University of Liege, Gembloux (Belgium).

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Description. *Tetrapleura tetraptera* and *Aframomum citratum* fruits are ingredients in traditional medicines in some sub-Saharan countries. For medicinal purposes, the fruit extracts are obtained using different extraction methods and by adding lime juice or baking soda powder.
Objectives. Evaluate the influence of the solid-liquid ratio, the pH and the extraction procedures on the total polyphenols content and the biological activities (antioxidant, anti-inflammatory) of their extracts.
Method. Fresh fruits were harvested, crushed and various extracts were obtained using three extraction methods (infusion, 100 °C/30 min; decoction, 100 °C/30 min; ethanol maceration, 30 min at room temperature); four solid-liquid ratios (1/4, 1/6, 1/8, 1/10: mass of the sample in gram/volume of the solvent in ml) and two extraction mediums (alkaline and acidic). Polyphenol compounds of the aqueous extracts were identified and quantified using HPLC-DAD. The total polyphenols content, the total antioxidant capacity and the anti-inflammatory activities of both extracts were evaluated by spectrophotometric methods. Principal Component Analysis providing a transparent view of information was performed.
Results. *Aframomum citratum* extracts revealed the presence of major compounds like: gallic, protocatechuic, caffeic and syringic acids, epicatechin, catechin while *T. tetraptera* extracts revealed the presence of major compounds like protocatechuic, chlorogenic, kojik and sinapic acids, and catechin. The solid-liquid ratio, the pH and the extraction method significantly ($p < 0.05$) affect the total polyphenols content and the biological activities. The best results were obtained with *T. tetraptera* extracts using decoction in alkaline medium at a solid-liquid ratio of 1/4. *Aframomum citratum* best results were obtained by alcohol maceration in an alkaline medium at the same solid-liquid ratio.
Conclusions. All these extraction factors are important to optimize the use of the plant extracts. They should be controlled carefully during the various processes due to their significant effects on the biological activities.
Keywords. Bioactive properties, Zinziberaceae, Leguminosae, extraction, solid-liquid ratio, pH, maceration, infusion, decoction.

Variations des teneurs en polyphénols et des activités biologiques des extraits de fruits de *Tetrapleura tetraptera* et *Aframomum citratum* obtenus suivant plusieurs méthodes d'extraction en milieux acide et alcalin
Description du sujet. Les fruits de *Tetrapleura tetraptera* et *Aframomum citratum* sont utilisés en pharmacopée traditionnelle en Afrique subsaharienne. L'extraction des principes actifs utilisent plusieurs techniques après ajout du jus de citron ou du bicarbonate de soude alimentaire.

Objectifs. Évaluer l'influence du rapport solide-liquide, du pH et des procédés d'extraction sur la teneur en polyphénols et les activités biologiques antioxydante et anti-inflammatoire de leurs extraits.
Méthode. Les fruits frais récoltés sont broyés et des extraits sont obtenus suivant trois méthodes d'extraction (infusion, 100 °C/30 min ; décoction, 100 °C/30 min ; macération éthanolique, 30 min/26 °C), quatre ratios d'extraction solide/liquide (1/4, 1/6, 1/8 et 1/10 : masse de l'échantillon en g/volume du solvant en ml) et deux milieux d'extraction alcalin (pH : 7,5-10,5) et acide (4,0-6,7). Les composés phénoliques des extraits aqueux ont été identifiés et quantifiés (HPLC-DAD). La teneur en polyphénols, les activités biologiques (antioxydante et anti-inflammatoire) des extraits ont été évaluées par des méthodes spectrophotométriques. Une analyse en composantes principales fournissant une meilleure appréciation de l'information récoltée a été effectuée.
Résultats. Les extraits d'*A. citratum* révèlent la présence de composés tels que les acides gallique, protocatéchique, caféique, syringique et l'épicatéchine, la catéchine. Les extraits de *T. tetraptera* contiennent les composés majoritaires suivants : les acides protocatéchique, chlorogénique, kojique, sinapique et catéchine. Le rapport solide-liquide, le pH et la méthode d'extraction affectent significativement ($p < 0,05$) la teneur en polyphénols et les activités biologiques. Les meilleurs résultats sont obtenus avec *T. tetraptera* en utilisant la décoction en milieu alcalin à un ratio de 1/4. Pour *A. citratum*, la macération alcoolique en milieu alcalin au même ratio a été plus efficace.
Conclusions. Tous ces facteurs d'extraction sont importants pour optimiser l'utilisation de ces extraits de plantes et doivent être contrôlés au cours des différents process en raison de leurs effets significatifs sur les activités biologiques étudiées.
Mots-clés. Propriété bioactive, Zinziberaceae, Leguminosae, extraction, rapport solide-liquide, pH, macération, infusion, décoction.

1. INTRODUCTION

Spices are incorporated in diets around the world because of their aromatic and medicinal properties. In Cameroon markets, spices produced by harvesting non-timber forest products (Voukeng et al., 2012) are mixed with spices of conventional agriculture (thyme, cinnamon, garlic, onion, clove, ginger, turmeric, etc.). Among these spices, *Tetrapleura tetraptera* (Schumach. & Thonn.) Taub. and *Aframomum citratum* (J.Pereira) K.Schum. fruits represent a significant nutritional and therapeutic amount. Indeed, adding to their nutritional properties, they possess many traditional uses in the treatment of some diseases (cancer, cough, diabetes, arthritis, and ulcer) in Cameroon, Nigeria, Ghana and Uganda (Voukeng et al., 2012; Amadi et al., 2016; Atchan et al., 2023). The spices contain bioactive compounds that confer to them many interesting pharmacological properties (anticonvulsive, hypotensive, antioxidant, hepatoprotective, anti-malaria) according to Alade et al. (2016) and Amadi et al. (2016). It has been reported that the major components revealed on the different parts of the plant species (barks, fruits, leaves, seeds) are phenolic acids, flavonoids and alkaloids. They are also used for food preservation due to their antibacterial and insecticidal capacities (Fankam et al., 2011; Voukeng et al., 2012; Zakaria, 2012; Alade et al., 2016; Amadi et al., 2016).

Although polyphenols exist in several plants, their quantity and type after extraction are associated to the extraction methods used, the chemical structure, the interfering compounds, the particle size of samples, and the storage conditions (Suwal & Marciniak, 2019). Also the solubility and separation properties of polyphenols are affected by their structural differences. The structure of any compound has a significant influence on its polarity level, conjugation, and interaction with the sample medium (Karishma et al., 2015). High molecular weight phenolic compounds are frequently insoluble because of their complex chemical structure. Polyphenols recovery from different sources is a challenge because of the high level of enzyme activity in plant cells. Hence, choosing the extraction process must be done carefully to avoid the chemical modification of the targeted compounds (Alara et al., 2021).

Crude extracts are prepared using new alternative methods like solvent extraction and assisted extraction methods (use of ultrasounds, microwaves, and pressurized/supercritical fluids). Those methods are also used for phenolic compounds extraction (Alara et al., 2021). Besides, the phenolic compounds could exist in complex forms with macromolecules (proteins, carbohydrates, insoluble high molecular weight phenolic compounds) according to Alara et al. (2021). To obtain the crude form of phenolic compounds in this case, a specific extraction method should be applied to remove the unwanted compounds. To achieve this task, "Solid-phase extraction methods, fractionation and purification based on acidity are usually the methods of choice for removing these interferences" (Zwir-Ferene & Biziuk, 2006 cited by Alara et al., 2021). To optimize the extraction process, some critical factors (solvent, nature of the material, light, duration of extraction period, pH, temperature, material size, solvent/substrate ratio, liquid-liquid or solid-liquid ratio) should also be considered, according to the previous authors.

In a home food consumption context, the conventional extraction techniques of plant extracts are maceration, decoction, percolation and infusion. Water and ethanol are used as solvents because of their safety and non-harmful effects for human consumption. Sometimes alkali or lime is added during the extraction process to improve the taste or for other purposes like deodorizing. Extraction by maceration consists of soaking a crushed sample in the appropriate solvent, followed by constant agitation (Fadjare et al., 2021). According to the same authors, decoction involves boiling the samples for a short period of time and it is mostly suitable for heat-stable and water-soluble drugs phytochemicals (Rachmat & Wulandari, 2021). After the maceration time for both extraction methods, a separation process is applied to collect the supernatant. These are easy and low-cost extraction techniques. Indeed, skilled operation is not needed compared to other modern techniques, and those techniques are economically profitable (Fadjare et al., 2021).

Concerning *A. citratum* and *T. tetraptera* fruits, all these extraction methods have been used by many authors to obtain the plant extracts (Fankam et al., 2011; Voukeng et al., 2012; Zakaria, 2012; Moukette et al., 2015; Adesina et al., 2016; Amadi et al., 2016). However, information is not yet available about the comparative study on the effects of these traditional extraction techniques, the solid-liquid ratio and the pH medium on the recovery of polyphenols contents. Knowledge on such studies can help to optimize the extraction efficiency, especially when extracted traditionally for therapeutic purposes.

2. MATERIALS AND METHODS

2.1. Plant material

Tetrapleura tetraptera and *A. citratum* fresh fruits were harvested respectively from Bonepoupa II in Nkam Division, Littoral region (Lat: 4.074193; Long: 10.03148), and from Boanda in Mbam and Kim Division, Centre Region (Lat: 5.4431417; Long: 11.8891720) in Cameroon. Each specimen was identified and voucher specimens (N°31611/NHC, N°37796/NHC respectively) were deposited in the National Herbarium.

2.2. Fruits processing and various extraction conditions

The fresh fruits were selected, sliced before finely crushed using IKA Universal Mill M 20. The obtained paste was used for aqueous and alcohol extraction. De-ionized water and ethanol (98%) were used as solvents. Different solid-liquid ratios were investigated for extraction during preliminary studies.

2.3. Extraction protocols description

Preliminary studies were performed to investigate the influence of the solid-liquid ratio on the extract total polyphenols content and their total antioxidant capacity while the other extraction parameters were fixed. Five solid-liquid ratios (1/2, 1/4, 1/6, 1/8, 1/10 m/v) were randomly selected. By using ½ m/v for both plant species, the supernatant collected was not enough for the planned tests. Then the solid-liquid ratios used for the rest of the trials were 1/4, 1/6, 1/8, and 1/10 m/v.

Three extraction procedures were used to obtain plant extracts: infusion, decoction and ethanol maceration. Concerning infusion, 1 g of plant material is introduced into a conical tube (Corning© centrifuge tube: 15 ml) and the quantity of distilled water heated to 100 °C is added. After homogenization, the extract is left to soak and shake for 30 min at room temperature. Using decoction, the same amount of plant material is introduced into a screw tube and the quantity of distilled water which depends on the solid-liquid ratio is added. The screwed tube is placed in a water bath preheated to 100 °C and boiled for 30 min. Ethanol maceration was done in a conical tube with the same amount of plant material. The quantity of ethanol (98%) which depends on the solid-liquid ratio was added and the mixture was left to macerate during 30 min after homogenization at room temperature. All the tubes were then centrifuged (4,500 rpm/10 min) and the collected supernatants were lyophilized, vacuum-packed, refrigerated and used for subsequent analyses.

2.4. Extraction using different pH

To adjust the pH, acetic acid ($C_2H_4O_2$) and baking soda ($NaHCO_3$) were used. Stock solutions of acetic acid and baking soda of 1 g·100 ml^{-1} were prepared. Concerning the incorporation rates of acetic acid and baking soda, they varied from 0.05 to 1% of the total extraction volumes for both plant species. The solid-liquid ratio with the best total polyphenols content (1/4 m/v) was chosen for this analysis.

The pH ranges of the solutions obtained after adding acetic acid and baking soda for both plant species extracts were measured (**Table 1**). The measurements were realized using a digital pH meter (Hildebrant, 2016) in triplicate and this assay was done for the three extraction procedures. For each extraction procedure (infusion, decoction, ethanol maceration), the influence of the pH was investigated.

2.5. Characterization of polyphenols: HPLC-DAD analysis

Plant extracts preparation. The solid-liquid ratio with the best total polyphenols content (1/4 m/v) was chosen

Table 1. The pH ranges of the variable solutions obtained for both plant extracts after adding acetic acid or baking soda — *Les gammes de pH des solutions variables obtenues pour les deux extraits végétaux après ajout d'acide acétique ou de bicarbonate*.

Incorporation rate (%)	PH			
	Tetrapleura tetraptera		*Aframomum citratum*	
	Acetic acid ($C_2H_4O_2$)	Baking soda ($NaHCO_3$)	Acetic acid ($C_2H_4O_2$)	Baking soda ($NaHCO_3$)
0.05	6.5 ± 0.3	7.5 ± 0.3	6.7 ± 0.2	8.0 ± 0.2
0.1	6.0 ± 0.1	8.0 ± 0.2	6.3 ± 0.3	8.5 ± 0.0
0.2	5.7 ± 0.3	8.1 ± 0.2	6.1 ± 0.2	8.8 ± 0.1
0.4	5.1 ± 0.2	8.4 ± 0.4	5.3 ± 0.2	9.1 ± 0.1
0.6	4.8 ± 0.0	9.0 ± 0.0	5.1 ± 0.0	9.5 ± 0.3
0.8	4.4 ± 0.1	9.5 ± 0.3	4.7 ± 0.1	10.1 ± 0.0
1	4.0 ± 0.2	10.1 ± 0.1	4.2 ± 0.0	10.5 ± 0.3

for the HPLC-DAD analysis and the extracts biological activities tests. Ten grams of each spice paste were mixed with 40 ml of boiling de-ionized water (100 °C) and the mixture was let to brew for 30 min at room temperature. The mixture was then filtered and centrifuged. The filtrate was treated twice with equal volume of n-hexane (to remove non-polar compounds and reduce interference during polyphenols identification) in a separating funnel. The aqueous phase was centrifuged, collected, filtered (whatman paper N°1) freeze dried and kept into a freezer for further analysis.

For phenolic compounds identification, 1 g of the dried aqueous extract was diluted into 4 ml of de-ionized water. The mixture was degassed by ultrasonic bath (Fisher scientific ultrasonic bath, Elma Germany) prior to use and filtered through 0.45 μm membrane filter (Millipore) directly on 2 ml HPLC vial.

Identification, quantification of polyphenols compounds. Stock solutions of standards were prepared in the HPLC mobile phase (0.025-0.2 mg·ml^{-1}) and were handled as previously described. The identification and the quantification of polyphenol compounds were determined according to the methods described in previous studies (Moukette et al., 2015; Eyenga et al., 2020a; Hafiz et al., 2020).

Total polyphenols (TPP) contents determination. The extract total polyphenol content was estimated using the method described by Eyenga et al. (2020b) and the amounts were calculated from the gallic acid standard curve (0.05–1 mg·ml^{-1}), and expressed as mg GAE·g^{-1} dw.

Antioxidant assay: Total Antioxidant Capacity (TAC). The extracts total antioxidant capacity was determined by the Phosphomolybdenum method (Prieto et al., 1999 cited by Kumari et al., 2024) and Trolox was used as a positive control. The reducing activity was expressed as mg TE·g^{-1} dw based on the Trolox calibration curve (0.02–0.1 μg·ml^{-1}).

Anti-inflammatory assay: Lipoxygenase (5-LOX) inhibition (INH.5-LOX). The soybean 5-LOX inhibition (INH.5-LOX) activity by the extracts at various concentrations (0.05, 0.5, 5.0, 50 mg·ml^{-1}) was estimated using the method described by Gunathilake et al. (2018) with few modifications at 234 nm, the negative and the positive controls were respectively Phosphate buffer and Diclofenac. The solid-liquid ratio with the best total polyphenols content (1/4 m/v) was chosen for this analysis.

2.6. Statistical analysis

Results are reported as the mean of three replicates ± SD. Statistical significance between groups was analyzed by one-way ANOVA. The significance level was set at $p < 0.05$. The main multivariate statistical analysis approach was based on principal component analysis (PCA) to explain significant associations among quality parameters, total phenolic compounds contents, and biological activities data. Pearson's correlations ($p < 0.05$) were used to identify correlations between all the variables included in the dataset. All the statistical analyses were carried out using JMP PRO 16.0.

3. RESULTS

3.1. Phenolic compounds characterization of aqueous extracts of *A. citratum* and *T. tetraptera* fruits using HPLC-DAD

At the various wavelengths and using the solid-liquid ration 1/4 (m/v), many compounds were identified and quantified on both aqueous extracts (**Figures 1** and **2**). However, at 280 nm, concerning *A. citratum*, the total

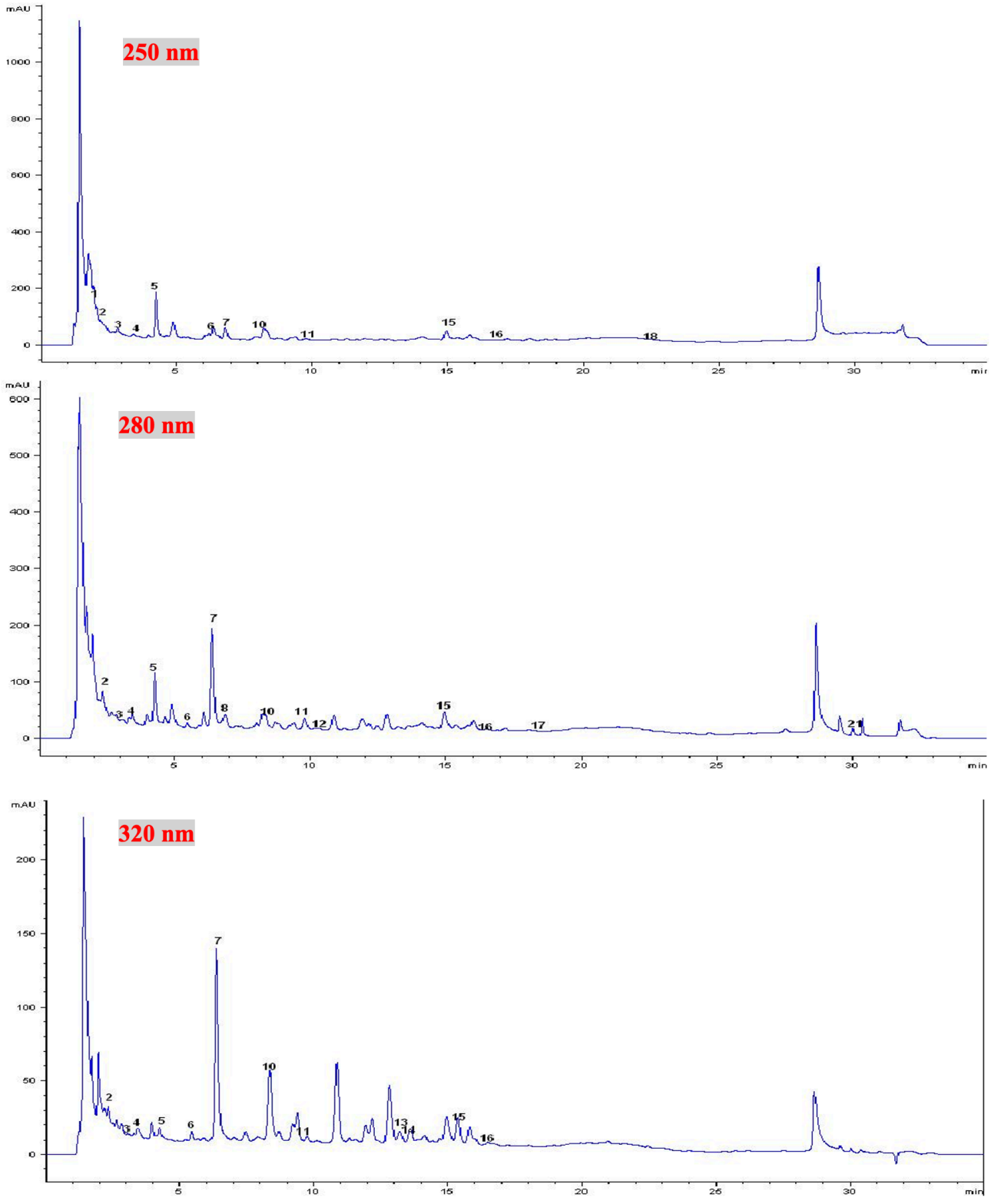

Figure 1. Chromatograms of aqueous extract of *Aframomum citratum* at different wavelengths (250, 280 and 320 nm) — *Chromatogrammes des extraits aqueux d'*Aframomum citratum *à trois longueurs d'onde (250, 280 et 320 nm)*.

1: kojic acid — *acide kojique*; 2: gallic acid — *acide gallique*; 3: theobromine — *théobromine*; 4: (-) gallocatechin — *(-) gallocatéchine*; 5: protocatechuic acid — *acide protocatéchique*; 6: epigallocatechin — *épigallocatéchine*; 7: chlorogenic acid — *acide chlorogénique*; 8: (+-) catechin — *(+-) catéchine*;10: caffeic acid — *acide caféique*; 11: epicatechin — *épicatéchine*; 12: epigallocatechin gallate — *épigallocatéchine gallate*; 13: p-coumaric acid — *acide p-coumarique*; 14: cyanidin chloride — *cyanidine chloride*; 15: syringic acid — *acide syringique*; 16: trans-OH-cinnamic acid — *acide trans-OH-cinnamique*; 17: benzoic acid — *acide benzoïque*; 18: oleuropein — *oleuropéine*; 21: *trans*-chalcone — trans-*chalcone*.

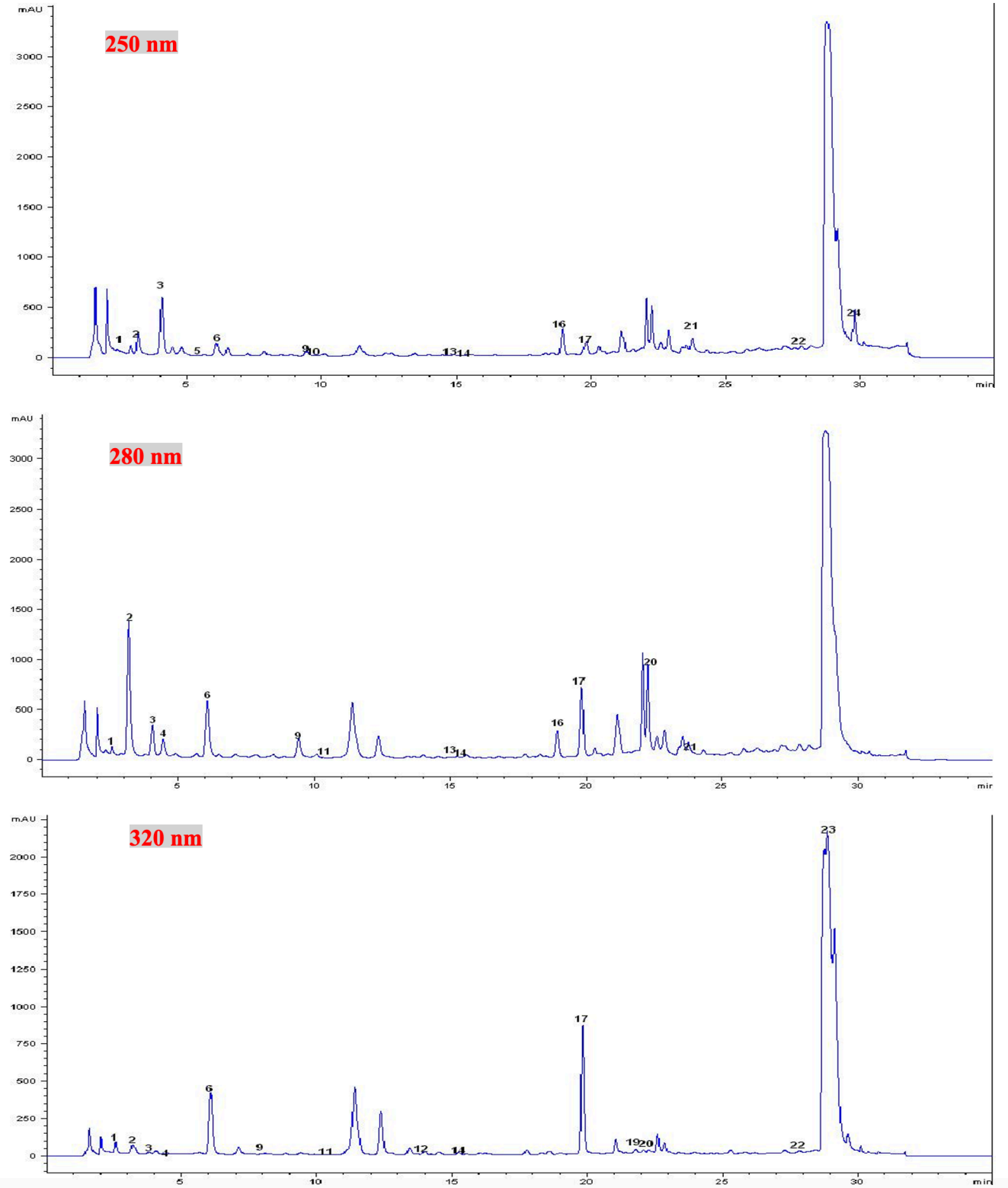

Figure 2. Chromatograms of aqueous extract of *Tetrapleura tetraptera* aqueous extracts at different wavelengths (250, 280 and 320 nm) — *Chromatogrammes des extraits aqueux de* Tetrapleura tetraptera *à trois longueurs d'onde (250, 280 et 320 nm)*.

1: gallic acid — *acide gallique*; 2: theobromine — *théobromine*; 3: (-) gallocatechin — *(-) gallocatéchine*; 4: protocatechuic acid — *acide protocatéchique*; 5: epigallocatechin — *epigallocatéchine*; 6: chlorogenic acid — *acide chlorogénique*; 9: 3-oh-benzoic acid — *acide 3-oh-benzoïque*; 10: epicatechin — *epicatéchine*; 11: epigallocatechin gallate — *epigallocatéchine gallate*; 12: cyanidin chloride — *cyanidine chloride*; 13: syringic acid — *acide syringique*; 14: ferulic acid — *acide ferulique*; 16 : benzoic acid — *acide benzoïque*; 17: 2-oh cinnamic acid — *acide 2-oh cinnamique*; 19: 3-oh-tyrosol — *3-oh-tyrosol*; 20: phlorhidzin — *phlorhidzine*; 21: resveratrol — *resvératrol*; 22: quercetin — *quercétine*; 24: flavone — *flavone*.

amount of phenolic acids identified was higher than the amounts of the other phenolic compounds (**Table 2**). Gallic acid, protocatechuic acid, chlorogenic acid, benzoic acid, caffeic acid, p-coumaric acid and syringic acid were identified (**Figures 1**). Flavonoids were also identified and quantified (**Table 2**), among them catechin, epicatechin, cyanidin chloride, oleuropein and *trans*-chalcone.

In *T. tetraptera* extracts (**Figure 2**), phenolic acids (gallic acid, protocatechuic acid, chlorogenic acid, benzoic acid, syringic acid, ferulic acid, cinnamic acid), flavonoids (catechin, gallocatechin, epigallocatechin gallate, cyanidin chloride, myricetin, phloridzin, resveratrol, quercetin, naringenin, flavone), tyrosol, theobromine were identified and valued (**Table 2**).

3.2. Solid-liquid ratio influence on total phenolic (TPP) content and antioxidant activity (TAC) of plant extracts

The solid-liquid ratio showed a significant ($p < 0.05$) effect on the TPP contents and the TAC of the extracts (**Figure 3** for *T. tetraptera* extracts and **Figure 4** for *A. citratum* extracts). Solid-liquid ratio 1/4 (m/v) exhibited the highest TAC, as well as extracted the highest TPP content in both plant extracts. In general, there was an increase of the TAC of extracts and the TPP while increasing the solid-liquid ratios in the two plant species. Trend of the TPP yields were similar with the trend obtained in TAC assays.

3.3. Extraction methods effects on total phenolic content (TPP) and antioxidant activities (TAC) of plant extracts

The various results are presented in **Tables 3** and **4** and in **Figures 3** and **4**. The decoction extraction mode significantly ($p < 0.05$) presents the highest extraction yields as well as the highest biological activities (antioxidant and anti-inflammatory) in *T. tetraptera* aqueous extracts, while alcohol maceration was the most effective regarding the parameters studied concerning *A. citratum* extracts.

3.4. Effect of the pH medium on the total phenolic content (TPP) and biological activities (TAC) of plant extracts

The results indicate that TPP contents and the studied *in vitro* biological activities (**Tables 3** and **4**) were significantly ($p < 0.05$) affected by the pH. Indeed, alkaline medium seems to be more effective for TPP recovery than acidic medium in plants extracts obtained by infusion, decoction and alcohol maceration.

Table 2. Phenolic compounds contents ($\mu g \cdot 100\ g^{-1}$ of dw) of aqueous extracts of *Tetrapleura tetraptera* and *Aframomum citratum* fruits at 280 nm — *Teneur en polyphenols ($\mu g \cdot 100\ g^{-1}$ de ms) des extraits aqueux des fruits de* Tetrapleura tetraptera *et d'*Aframomum citratum *à 280 nm*.

Type of compound	***Aframomum citratum***		***Tetrapleura tetraptera***	
	Identified compounds	**Amount** ($\mu g \cdot 100\ g^{-1}$ of dry weight)	**Identified compounds**	**Amount** ($\mu g \cdot 100\ g^{-1}$ of dry weight)
Phenolic acid	Gallic acid	6.31 ± 1.10	Protocatechuic acid	83.85 ± 3.27
	Protocatechuic acid	33.43 ± 3.51	Gallic acid	4.55 ± 1.01
	Chlorogenic acid	19.53 ± 2.34	Chlorogenic acid	31.42 ± 1.96
	Caffeic acid	13.47 ± 1.12	Syringic acid	Traces
	Syringic acid	22.77 ± 2.34	Ferulic acid	Traces
	Trans-OH-cinnamic acid	2.62 ± 0.27	Benzoic acid	56.23 ± 4.95
	Benzoic acid	0.33 ± 0.05	3-OH Benzoic acid	43.09 ± 3.12
			2-OH Cinnamic acid	71.88 ± 0.51
Flavonoid	(-)Gallocatechin	3.33 ± 0.64	(-)Gallocatechin	67.05 ± 5.62
	Epigallocatechin	1.95 ± 0.34	Resveratrol	Traces
	Epicatechin	10.93 ± 0.74	Phloridzin	137.45 ± 10.01
	Epigallocatechin gallate	Traces	Epigallocatechin gallate	Traces
	(+-)Catechin	8.15 ± 0.46		
	Trans-chalcone	0.38 ± 0.11		
Other	Theobromine	Traces	Theobromine	125.01 ± 8.21

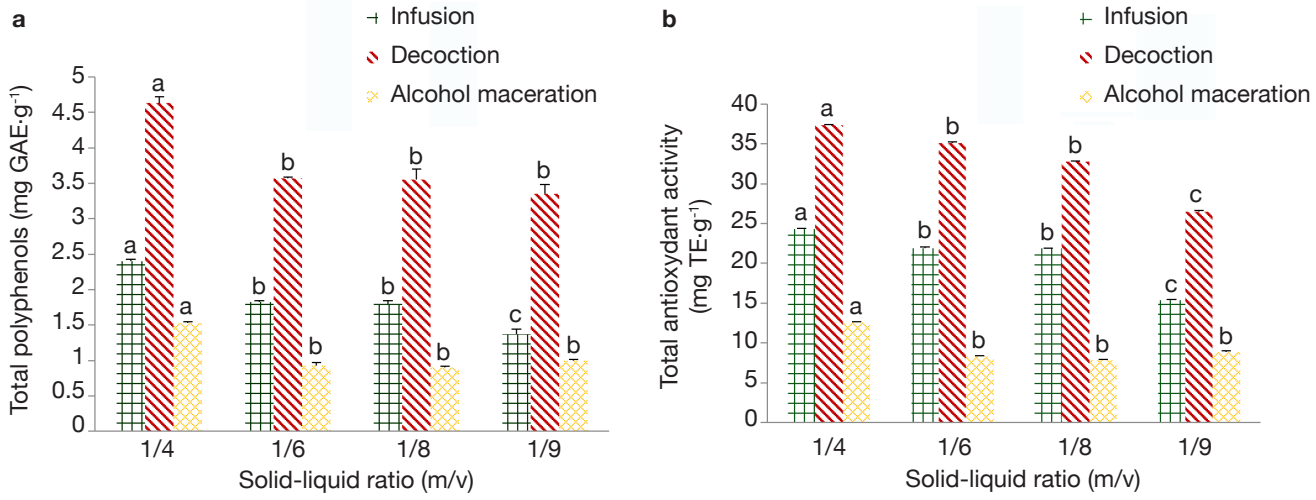

Figure 3. Total polyphenols content (**a**) and total antioxidant capacity (**b**) of *Tetrapleura tetraptera* extracts using different extraction ratios (1/4; 1/6; 1/8. 1/10: m/v) and extraction methods (infusion, decoction, alcohol maceration) — *Teneur en polyphénols totaux (**a**) et capacité antioxydante totale (**b**) des extraits de* Tetrapleura tetraptera *suivant différents modes d'extraction (infusion, décoction, macération alcoolique) et ratios solide-liquide (1/4; 1/6; 1/8. 1/10 m/v).*

The amount of total polyphenols and the total antioxidant capacity of the extracts followed by different letters (a, b, c) in the same colored column are significantly different at 5% level — *Les moyennes suivies de lettres différentes (a, b, c) sur les cylindres de même couleur sont statistiquement différentes au seuil de 5 %*; GAE: Gallic Acid Equivalent — *Équivalent Acide Gallique*; TE: Trolox Equivalent — *Équivalent Trolox*.

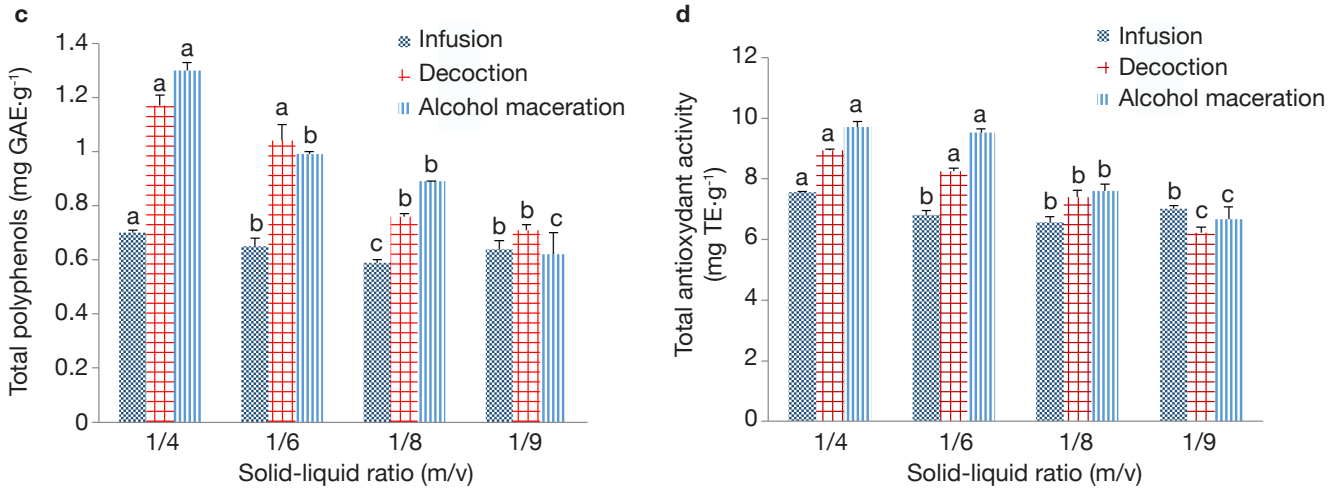

Figure 4. Total polyphenols content (**c**) and antioxidant activity of *Aframomum citratum* (**d**) extracts using different extraction ratios (1/4; 1/6; 1/8. 1/10: m/v) and extraction methods (infusion, decoction, alcohol maceration) — *Teneur en polyphénols totaux (**c**) et capacité antioxydante totale (**d**) des extraits d'*Aframomum citratum *suivant différents modes d'extraction (infusion, décoction, macération alcoolique) et ratios solide-liquide (1/4; 1/6; 1/8. 1/10 m/v).*

a, b, c; GAE; Te: see **figure 3** — *voir **figure 3***.

3.5. Comparative analysis of phytochemical content, biological activities of plant extracts obtained by different extraction procedures in different medium, extraction methods using PCA

Principal component analysis was performed (**Figure 5**) using three quantitative variables (TPP, INH.5-LOX, and TAC) and two qualitative variables (extraction procedures, pH) distributed into the two plant species (*A. citratum* and *T. tetraptera*).

The applied multivariate analysis helped assess the impact of the qualitative variables on the aqueous extracts, total polyphenols contents and the *in vitro* biological activities. The simple biplot presents the

Table 3. Total polyphenols (mg GAE·g^{-1}), total antioxidant capacity (mg TE·g^{-1}) and anti-inflammatory (INH-5-LOX: IC_{50} mg·ml^{-1}) activities of *Tetrapleura tetraptera* extracts using few extraction procedures in acid/alkaline medium — *Teneurs en polyphénols totaux (mg EAG·g^{-1}), capacité antioxydante totale (mg ET·g^{-1}) et activité anti-inflammatoire (INH-5-LOX: IC_{50} mg·ml^{-1}) des extraits de* Tetrapleura tetraptera *suivant plusieurs méthodes d'extraction en milieux acide et alcalin*.

Plant species	Extraction methods	$C_2H_4O_2$/$NaHCO_3$ incorporated rates (%)	Antioxydant activity (TAC : mg TE·g^{-1})		Total polyphenols (TPP : mg GAE·g^{-1})		Anti-inflammatory activity (INH-5-LOX: IC_{50} mg·ml^{-1})	
			Acid extract	Alkaline extract	Acid extract	Alkaline extract	Acid extract	Alkaline extract
Tetrapleura tetraptera	Infusion	0	30.21 ± 0.05^{c}	30.25 ± 0.06^{c}	3.04 ± 0.02^{c}	2.92 ± 0.01^{d}	1.28 ± 0.03	1.25 ± 0.11^{c}
		0.05	30.21 ± 0.08^{c}	33.18 ± 0.06^{d}	3.67 ± 0.02^{a}	3.37 ± 0.01^{c}	1.18 ± 0.14	1.05 ± 0.08^{bc}
		0.1	32.79 ± 0.1^{a}	30.57 ± 0.17^{c}	3.40 ± 0.10^{b}	3.15 ± 0.05^{c}	1.21 ± 0.04	0.91 ± 0.03^{b}
		0.2	32.70 ± 0.31^{b}	33.07 ± 0.24^{b}	3.51 ± 0.02^{a}	3.47 ± 0.05^{b}	1.17 ± 0.06	0.95 ± 0.14^{b}
		0.4	32.97 ± 0.24^{a}	33.20 ± 0.14^{b}	3.59 ± 0.05^{a}	3.49 ± 0.01^{b}	1.12 ± 0.07	0.82 ± 0.11^{a}
		0.6	32.9 ± 0.14^{a}	33.48 ± 0.09^{b}	3.57 ± 0.01^{a}	3.57 ± 0.01^{b}	1.10 ± 0.02	0.85 ± 0.06^{a}
		0.8	32.88 ± 0.19^{a}	38.36 ± 0.14^{a}	3.56 ± 0.06^{a}	3.71 ± 0.01^{a}	1.02 ± 0.09	0.83 ± 0.07^{a}
		1	32.82 ± 0.16^{a}	38.05 ± 0.14^{a}	3.57 ± 0.05^{a}	3.76 ± 0.04^{a}	1.11 ± 0.05	0.85 ± 0.02^{a}
	Decoction	0	40.08 ± 0.04	40.36 ± 0.06^{f}	3.59 ± 0.00^{a}	3.52 ± 0.02^{e}	1.07 ± 0.05^{c}	1.10 ± 0.11^{d}
		0.05	40.01 ± 0.36	44.39 ± 0.12^{e}	3.64 ± 0.00^{a}	3.57 ± 0.02^{e}	0.89 ± 0.03^{b}	0.89 ± 0.01c
		0.1	39.97 ± 0.1	47.53 ± 0.1^{d}	3.54 ± 0.01^{ab}	3.71 ± 0.08^{e}	0.88 ± 0.11^{b}	0.84 ± 0.23^{c}
		0.2	39.95 ± 0.24	49.87 ± 0.14^{c}	3.46 ± 0.01^{b}	4.09 ± 0.15^{d}	0.80 ± 0.05^{a}	0.79 ± 0.04^{b}
		0.4	40.03 ± 0.23	50.98 ± 0.19^{b}	3.29 ± 0.04^{b}	4.45 ± 0.08^{c}	0.77 ± 0.34^{a}	0.62 ± 0.15^{a}
		0.6	40.12 ± 0.10	50.76 ± 0.07^{b}	3.29 ± 0.01^{b}	4.95 ± 0.00^{ab}	0.81 ± 0.07^{a}	0.65 ± 0.04^{ab}
		0.8	39.62 ± 0.21	52.63 ± 0.1^{a}	3.14 ± 0.00^{c}	5.13 ± 0.05^{a}	0.85 ± 0.18^{a}	0.60 ± 0.03^{a}
		1	39.97 ± 0.2	52.43 ± 0.26^{a}	3.14 ± 0.04^{c}	4.88 ± 0.04^{b}	0.82 ± 0.11^{a}	0.57 ± 0.03^{a}
	Alcohol maceration	0	12.55 ± 0.11	12.53 ± 0.06^{b}	1.53 ± 0.02^{c}	1.53 ± 0.02^{d}	2.09 ± 0.00^{b}	2.11 ± 0.10^{d}
		0.05	12.60 ± 0.09	12.82 ± 0.01^{b}	1.60 ± 0.10^{b}	1.66 ± 0.05^{c}	1.97 ± 0.05^{b}	2.00 ± 0.05^{d}
		0.1	12.75 ± 0.01	12.80 ± 0.05^{b}	1.63 ± 0.03^{b}	1.73 ± 0.10^{c}	1.87 ± 0.10^{b}	1.69 ± 0.08^{c}
		0.2	12.66 ± 0.04	13.25 ± 0.04^{ab}	1.67 ± 0.05^{b}	1.84 ± 0.02^{b}	1.80 ± 0.02^{a}	1.63 ± 0.00^{c}
		0.4	12.90 ± 0.08	13.55 ± 0.00^{a}	1.71 ± 0.00^{ab}	1.83 ± 0.06^{b}	1.85 ± 0.14^{ab}	1.60 ± 0.13^{bc}
		0.6	13.01 ± 0.00	13.55 ± 0.04^{a}	1.94 ± 0.02^{a}	2.03 ± 0.11^{b}	1.77 ± 0.10^{a}	1.47 ± 0.12^{b}
		0.8	12.85 ± 0.09	13.83 ± 0.06^{a}	1.90 ± 0.10^{a}	2.11 ± 0.00^{a}	1.77 ± 0.07^{a}	1.49 ± 0.03^{b}
		1	13.00 ± 0.06	14.05 ± 0.03^{a}	1.93 ± 0.00^{a}	2.33 ± 0.05^{a}	1.76 ± 0.00^{a}	1.28 ± 0.05^{a}

Means followed by different letters in the same column are significantly different at 5% and those without letter are significanly the same — *les moyennes suivies de lettres différentes sur la même colonne sont statistiquement différentes au seuil de 5 % et celles sans lettres sont significativement identiques*; TE : Trolox Equivalent — *Équivalent Trolox*; GAE : Gallic Acid Equivalent — *Équivalent Acide Gallique*.

Table 4. Total polyphenols (mg GAE·g^{-1}), total antioxidant capacity (mg TE·g^{-1}) and anti-inflammatory (INH-5-LOX: IC_{50} mg·ml^{-1}) activities of *Aframomum citratum* extracts using few extraction procedures in acid/alkaline medium — *Teneurs en polyphénols Totaux (mg EAG·g^{-1}), capacité antioxydante totale (mg ET·g^{-1}) et activité anti-inflammatoire (INH-5-LOX: IC_{50} mg·ml^{-1}) des extraits d'*Aframomum citratum *suivant plusieurs méthodes d'extraction en milieux acide et alcalin.*

Plant species	Extraction methods	$C_2H_4O_2$/$NaHCO_3$ incorporated rates (%)	Antioxydant activity (TAC : mg TE·g^{-1})		Total polyphenols (TPP : mg GAE·g^{-1})		Anti-inflammatory activity (INH-5-LOX : IC_{50} mg·ml^{-1})	
			Acid extract	Alkaline extract	Acid extract	Alkaline extract	Acid extract	Alkaline extract
Aframomum citratum	Infusion	0	2.79 ± 0.03	2.76 ± 0.02^{d}	0.60 ± 0.00	0.58 ± 0.00	3.05 ± 0.11^{a}	2.99 ± 0.22
		0.05	2.69 ± 0.05	2.64 ± 0.03^{d}	0.58 ± 0.01	0.53 ± 0.01	2.85 ± 0.08^{ab}	2.98 ± 0.11
		0.1	2.70 ± 0.04	3.09 ± 0.02^{b}	0.59 ± 0.00	0.56 ± 0.00	3.01 ± 0.12^{a}	3.01 ± 0.08
		0.2	2.57 ± 0.02	3.05 ± 0.04^{bc}	0.58 ± 0.00	0.56 ± 0.01	2.85 ± 0.00^{ab}	2.83 ± 0.07
		0.4	2.42 ± 0.02	2.95 ± 0.01^{c}	0.54 ± 0.01	0.53 ± 0.01	2.82 ± 0.11^{b}	2.92 ± 0.33
		0.6	2.59 ± 0.04	2.92 ± 0.03^{c}	0.59 ± 0.01	0.55 ± 0.01	2.75 ± 0.06^{c}	3.05 ± 0.21
		0.8	2.58 ± 0.01	3.02 ± 0.01^{bc}	0.57 ± 0.00	0.56 ± 0.01	1.65 ± 0.11^{d}	2.97 ± 0.33
		1	2.44 ± 0.02	3.23 ± 0.02^{a}	0.57 ± 0.01	0.59 ± 0.00	2.73 ± 0.02^{c}	2.94 ± 0.12
	Decoction	0	4.55 ± 0.05^{c}	4.69 ± 0.08^{e}	0.83 ± 0.01^{c}	0.85 ± 0.01^{e}	2.39 ± 0.11	2.39 ± 0.31^{d}
		0.05	4.53 ± 0.03^{c}	5.46 ± 0.02^{d}	0.84 ± 0.03^{bc}	1.19 ± 0.00^{d}	2.41 ± 0.01	2.09 ± 0.22^{c}
		0.1	4.56 ± 0.06^{c}	5.47 ± 0.04^{d}	0.82 ± 0.04^{c}	1.31 ± 0.00^{c}	2.40 ± 0.23	2.05 ± 0.00^{b}
		0.2	4.58 ± 0.04^{bc}	6.02 ± 0.04^{c}	0.91 ± 0.04^{ab}	1.27 ± 0.01^{c}	2.41 ± 0.11	2.01 ± 0.31^{b}
		0.4	4.63 ± 0.01^{b}	6.42 ± 0.01^{b}	0.89 ± 0.09^{b}	1.3 ± 0.03^{c}	2.32 ± 0.05	2.02 ± 0.02^{b}
		0.6	4.74 ± 0.1^{ab}	6.74 ± 0.02^{a}	0.88 ± 0.00^{b}	1.71 ± 0.03^{a}	2.35 ± 0.04	1.85 ± 0.04^{a}
		0.8	4.83 ± 0.05^{a}	6.83 ± 0.05^{a}	0.88 ± 0.04^{b}	1.51 ± 0.02^{b}	2.49 ± 0.13	1.79 ± 0.10^{a}
		1	4.95 ± 0.04^{a}	6.25 ± 0.03^{b}	0.96 ± 0.05^{a}	1.61 ± 0.02^{a}	2.27 ± 0.31	1.87 ± 0.01^{a}
	Alcohol maceration	0	9.71 ± 0.18^{b}	9.71 ± 0.18^{b}	1.30 ± 0.05	1.30 ± 0.03^{b}	2.19 ± 0.01^{c}	2.17 ± 0.04^{d}
		0.05	9.70 ± 0.20^{b}	9.95 ± 0.02^{b}	1.30 ± 0.10	1.35 ± 0.00^{b}	2.09 ± 0.11^{b}	1.94 ± 0.14^{c}
		0.1	9.71 ± 0.03^{b}	9.81 ± 0.22^{b}	1.29 ± 0.11	1.34 ± 0.11^{b}	2.02 ± 0.03^{b}	1.92 ± 0.03^{c}
		0.2	9.88 ± 0.05^{b}	10.21 ± 0.04^{b}	1.31 ± 0.02	1.42 ± 0.11^{b}	2.01 ± 0.05^{b}	1.85 ± 0.11^{bc}
		0.4	10.04 ± 0.04^{b}	10.52 ± 0.15^{ab}	1.34 ± 0.00	1.62 ± 0.04^{ab}	2.02 ± 0.12^{b}	1.72 ± 0.02^{b}
		0.6	10.11 ± 0.12^{b}	10.47 ± 0.09^{b}	1.39 ± 0.01	1.61 ± 0.00^{a}	1.91 ± 0.08^{a}	1.75 ± 0.08^{b}
		0.8	10.31 ± 0.08^{a}	10.81 ± 0.06^{a}	1.43 ± 0.05	1.79 ± 0.04^{a}	1.85 ± 0.07^{a}	1.67 ± 0.05^{a}
		1	10.21 ± 0.à3^{a}	11.19 ± 0.03^{a}	1.42 ± 0.07	1.73 ± 0.10^{a}	1.90 ± 0.01^{a}	1.65 ± 0.01^{a}

Small letters, TE, GAE: see **table 3** — *voir **tableau 3***.

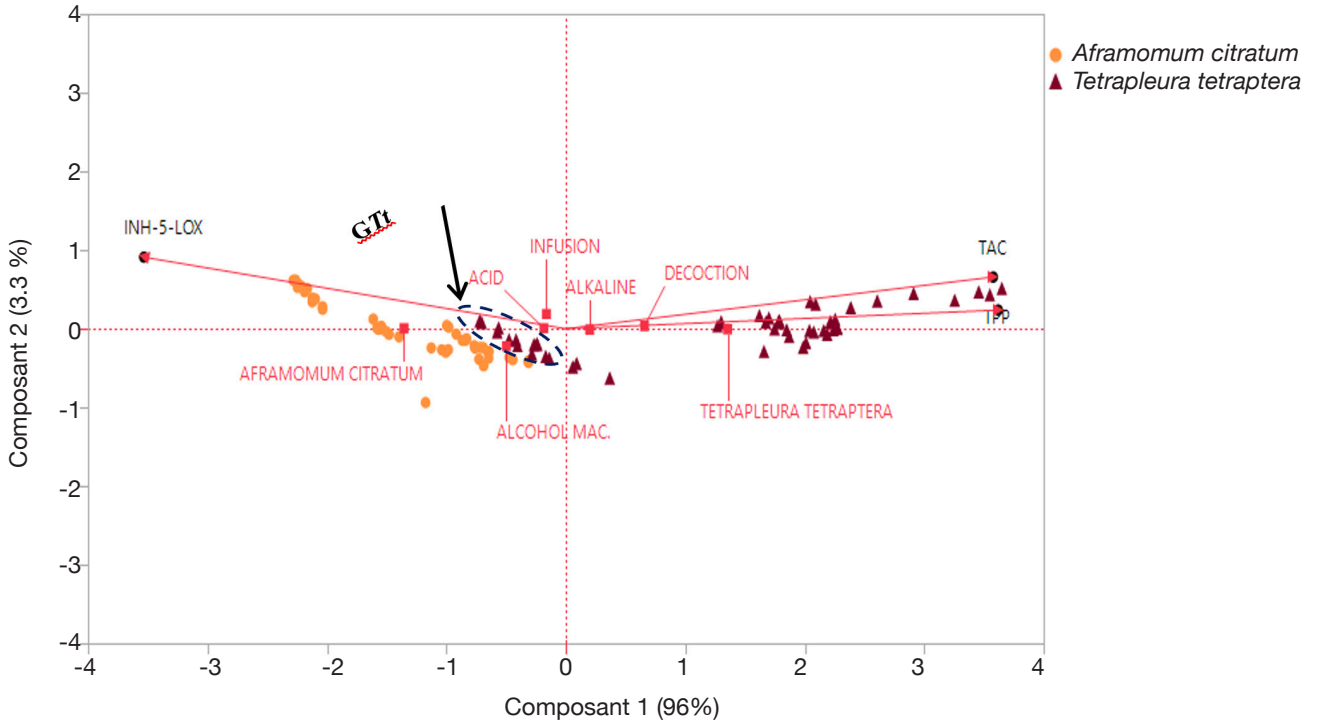

Figure 5. Principal Component Analysis (PCA) applied to polyphenol content and biological activities (quantitative variables) of *Tetrapleura tetraptera* and *Aframomum citratum* obtained using three extraction procedures in different pH mediums (qualitative variables) — *Analyse en Composantes Principales appliquée à la teneur en polyphenols totaux et aux activités biologiques des extraits de* Tetrapleura tetraptera *et d'*Aframomum citratum *obtenus suivants trois modes d'extraction en milieux alcalin et acide*.

TPP: total polyphenols — *teneur en polyphenols totaux*; TAC: total antioxidant capacity — *capacité antioxydante totale*; INH-5-LOX: lipoxygenase (5-LOX) inhibition — *inhibition de la lipoxygénase (5-LOX)*.

geographical representation of the plant species according to the studied parameters. The two axes explain a variance of 99.3%. The factor which explains better the component two is INH.5-LOX test while the one which contributes better to the component one is TPP. For both plant species extracts, the total polyphenols content is strongly and positively correlated with the antioxidant activity ($r = 0.97$; $p < 0.05$) and negatively correlated to the anti-inflammatory activity ($r = -0.94$; $p < 0.05$) of the plant extract.

Tetrapleura tetraptera extracts present the best TPP, TAC and anti-inflammatory (INH.5-LOX) activities while the highest scores are obtained by decoction using the alkaline medium. Extracts obtained by alcohol maceration and infusion (GTt: **figure 5**) possess less polyphenols content and are less effective concerning the biological activities studied. Those samples are not different to *A. citratum* samples treated on the same ways.

Concerning *A. citratum* fruit extracts, all the parameters studied are less than the one measured on *T. tetraptera* extracts. However, the best results (highest TPP content and *in vitro* biological activities) in general are clearly obtained in the alkaline medium using ethanol maceration.

4. DISCUSSION

By using 1/4 (m/v) solid liquid ratios during extraction, polyphenol characterization showed the presence of various compounds. Amongst them, resveratrol, oleuropein, luteolin, *trans*-chalcone, flavone, cyanidin chloride, phloridzin, kojic acid were noticed on recent trials (Eyenga et al., 2020a; Dzah, 2022) on *T. tetraptera* aqueous extracts. Gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, rutin, quercetin, catechin, ellagic acid, epicatechin, rutin, luteolin, apigenin and theobromine have already been identified and quantified (Moukette et al., 2015; Irondi et al., 2016; Odubanjo et al., 2018) on *T. tetraptera* methanolic extracts. Sokamte et al. (2019) report the presence of ferulic acid, t-cinnamic acid, epicatechin, quercetin on *A. citratum* extracts. However, for both phenolic compounds, the concentrations registered during this assay are higher than the ones stated on previous reports. The differences within the results noticed can be explained by the various analytical procedures used to quantify and identify the phenolic compounds or by the plant geographical origins. The detected polyphenols are probably responsible for the biological activities (TAC, INH-5-LOX) of

T. tetraptera and *A. citratum* extracts noticed on this trial. Indeed, both plant species extracts have already been reported to be antioxidant and anti-inflammatory by former reports (Onoja et al., 2014; Famobuwa et al., 2016; Eyenga et al., 2020a). The identified compounds on both plant species are also described as hypotensive, antitumor, antiviral, neuroprotective, antimicrobial and anti-ageing (Li et al., 2014; Pei et al., 2016; Jucà et al., 2018; Sie et al., 2018; Teplova et al., 2018; Espindola et al., 2019; Lakshmi et al., 2019).

According to Wong et al. (2013) and Herman et al. (2021), the solid-liquid ratio factor has a critical role during extraction and is correlated to the contact area between the sample and the solvent used for extraction. In fact, that area "can reach optimum conditions when the liquid phase is saturated to solid". In this study, the influence of the solid-liquid ratio on TPP contents and the extracts antioxidant activity is similar to the results previously mentioned (Wong et al., 2013; Predescu et al., 2016; Herman et al., 2021). Indeed, the phenolic compounds yields and the extract antioxidant activity increased gradually with the increase of the solid-to-solvent ratio.

Concerning *T. tetraptera* extracts and by comparing the solvent used (water and ethanol), the results obtained are the same as those previously published by Josiah et al. (2017) when studying the comparative antioxidant capacity of aqueous and ethanol fruit extracts. In fact, the aqueous extract was more efficient than the ethanol extract for both biological activities and phytochemical analysis. The suitable explanations are the strongest extractability of phenolic content by water and the positive correlation between the phenolic content and the biological activities studied.

Concerning *A. citratum* extracts and among the extraction methods and the solvent tested, ethanol maceration seems to be more efficient for extracting polyphenols. The observation made could be due by neo-formation of phenolic compounds with more phenol groups or with higher molecular weights comparing to the phenolic in water. These compounds seem to be soluble in hydro alcoholic extract (Moldovan et al., 2017; Rachmat & Wulandari, 2021). Another explanation can be the increase in the medium of non-phenolic compounds (terpene, organic acids, sugars, soluble proteins) during infusion which could interfere with the TPP recovery (Nguikwie et al., 2013).

A comparison between infusion and decoction shows that decoction is the most efficient for both plant species. The highest quantity of phenolic compounds obtained by decoction could have few justifications according to Moldovan et al. (2017): the release of cellular constituents by hydrolysis (as well as tannins); "the degradation of complex phenolic tannins as well as the enzymatic or non-enzymatic oxidation process leads to supplementary content of phenolic compounds"; the production of new phenolic compounds during Maillard reactions.

The pH has also an influence during the extraction of polyphenols. The results obtained in this trial are in close agreement to those obtained by Bouras et al. (2015). Similarly to our results, these authors found that alkaline medium extraction was more efficient for phenolic compounds recovery than acidic medium. Adding hydroxide sodium (NaOH) in the medium is increasing the extraction rate by the removal of tannins; the breakage of ester bonds and lignin physical barrier; the reduction of plant material viscosity; the increase of the extract reactivity. But Wiyono et al. (2020) reported that the optimum pH varies from one extraction to another. It was pH 6 for polyphenols extraction from *Phoenix dactylifera* leaves and pH 4 from *Litchi chinensis* fruit peel. On the contrary, another report mentioned that the pH did not affect the polyphenol extraction from *Cyperus esculentus* by-product (Ruenroengklin et al., 2008 cited by Wiyono et al., 2020). This shows that the polyphenol extraction efficiency is also closely related to the polyphenol class of the samples studied.

5. CONCLUSIONS

Tetrapleura tetraptera and *A. citratum* fruits aqueous extracts are good sources of bioactive compounds (phenolic acids, flavonoids, tannins, etc.) as it has been revealed after the chromatographic analysis. The solid-liquid ratio, the pH and the extraction methods used affect the effectiveness of polyphenol extraction and the *in vitro* biological activities studied of both fruits' extracts. Indeed, solid-liquid ratio 1/4 (m/v), alkaline medium and decoction method were the best features concerning *T. tetraptera* extracts. Concerning *A. citratum* fruits, the solid-liquid ratio 1/4 (m/v), the alkaline medium and the alcohol maceration extraction method recorded the highest results (total polyphenols content and biological activities). Because the total polyphenols content is positively correlated to the biological activities tested, the various factors (solid-liquid ratio, pH, extraction mode) should be carefully handled by herbalists, traditional healers and common users during the extraction process. Complementary studies on the same topic can also be investigated to compare the phenolic compounds profiles of the plant extracts after the three extraction procedures tested.

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