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ORIGINAL ARTICLE

Phenolic profile, antioxidant and enzyme inhibitory activities of underutilized spices from Central Africa

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Abstract The aim of the present study is to evaluate phytochemical profile, antioxidant and enzyme inhibitory activity of some underutilized Cameroon spice extracts, viz. The fruit of *Xylopia africana*, the fruit and the bulb of *Aframomum sulcatum*; and the bark of *Hypodaphnis zenkeri*. Four different solvent combinations were used for extraction of phytochemicals. Highest total polyphenol, flavonoid and tannin contents were observed in *X. africana* extract 10.32 ± 0.49 g gallic acid eq/100 g of dry matter, 146.66 ± 4.15 mg quercetin eq/100 g of dry matter, 452.44 ± 2.7 mg tannic acid eq/100 g spices dry matter. Methanol extracts revealed the presence of chlorogenic acid (0.790–4.312), vanillic acid (0.830–19.768), epicatechin (25.386–34.707), *p*-coumaric acid (1.127–12.652), protosynapic acid (0.221–186.562) and T-cinnamic acid (3.794–52.58) mg/100 g dry spice by HPLC analysis. Extracts of *X. africana* and *H. zenkeri* exhibited higher antioxidant activity: DPPH (182.24 ± 2.41 mg ascorbic acid eq/g dry spice), ABTS (9.247 ± 0.004 g trolox eq/100 g spice), hydroxyl free radicals (729.27 ± 3.07 mg mannitol eq/100 g spice) and reducing power (2.351 ± 0.002 g ascorbic acid equivalent/100 g spice). Positive and high correlations existed between the antioxidant activity of extracts obtained with different methods and their corresponding phenolic content. Extract of *A. sulcatum* highly inhibited porcine pancreatic lipase whereas, *X. africana* and *H. zenkeri* extracts highly inhibited α-amylase (98.82 and 99.54% respectively). These spices could be utilized as natural antioxidant sources for the management oxidative stress, lipid and carbohydrate metabolism related diseases.

Keywords Cameroon spices · Solvent extraction · Phenolic compounds · Antioxidant activity · Enzyme inhibitory properties

Introduction

Search for solutions to manage the increasing number of people suffering from chronic diseases worldwide is highly necessary and spices are reported to be one of them (Peter 2001; Villupanoor et al. 2008). Spices are sources of bioactive compounds that act against oxidative stress, inhibit some digestive enzymes involved in the development of diabetes and cardiovascular diseases; they are consumed regularly in food and as food supplements (Oben et al. 2010; De Krishna and Minakshi 2019). West and Central Africa have many underutilized spices which are traditionally used in food preparation and medicinal purposes. A study conducted in the western regions of Cameroon to identify spices consumed in traditional dishes revealed that some of the spices have so far not been studied for their biological activities (Djiazet et al. 2016, 2019). They include *Xylopia africana* (Benth.) Oliv fruits, *Aframomum sulcatum* bulbs and fruits and

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Hypodaphnis zenkeri bark. Moreover, no study has been conducted on the solubility of bioactive compounds from these spices in aqueous medium, as to estimate the quantity that may be available for absorption at the level of the gastro-intestinal tract.

The fruit of *X. africana* is commonly known as 'African pepper'. There are very few data in the literature on this plant, meanwhile the fruit is part of the food habits of the populations of the North-West and South-West Regions of Cameroon where it is highly and frequently consumed in yellow *achu* soup (Djiazet et al. 2019). *X. africana* plant is found in Cameroon, Nigeria, Sao Tome and Bikoko (Johnson and Murray 2018). To the best of our knowledge, few works have been done on *X. africana*. They include: isolation of some chemical compounds from the roots (Anam 1997), anti-yeast activity of the extracts (Taffou et al. 2017), proximate composition (Tchiégang and Mbougueng 2005) and data about consumption of the fruit as spice in yellow *achu* soup in Cameroon (Djiazet et al. 2019). *A. sulcatum* is better known in some parts of the West regions of Cameroon, where the fruits as well as the bulbs are consumed in the form of spices in some traditional foods (Djiazet et al. 2016, 2019). *A. sulcatum* has low habitat specificity, and for this reason, unlike *X. africana*, it belongs to the list of threatened species (IUCN 2013). Authors have reported on the bark of *H. zenkeri*, but have seldom tackled the biological activity of the extract, especially aqueous extracts. The bark of *H. zenkeri* is however highly appreciated and used at high proportion in *nah poh* preparation in Cameroon (Tchiégang and Mbougueng 2005; Djiazet et al. 2019). Following the remarkable finding on these spices, estimating its biological activities by the way it is consumed in most traditional soups will be of great interest to understand its influence on the health of consumers. Determining some biological activities of these spices by the way they are consumed may stimulate their cultivation and valorisation in the food habits of the populations to prevent and manage noncommunicable diseases. The present work was carried out in view of contributing to the search of edible plant materials used as spices, with biological potentialities for the management of chronic diseases.

Materials and methods

Images of the underutilized spices used in this study are given as Fig. 1.

Samples collection and preparation of extracts

Plant materials used for this study were the dried fruits of *X. africana*, the fruits and bulbs of *A. sulcatum* and the bark of *H. zenkeri* (Fig. 1). The samples were purchased from Bamenda and Bafoussam markets in Cameroon. The spices were dried at 45 °C in a ventilated oven, pounded and transformed into powder using an electric blender (Butter fly grand turbo). Four different solvent systems were used for extracting the phenolic compounds viz., distilled water, 1% HCl-methanol (v/v), ethanol/water (80/20, v/v), and methanol/water/acetic acid (70/25/5, v/v/v). The use of four different solvent systems was to estimate the quantity of bioactive compounds that can solubilised in water during food preparation. Estimations were done based on the those obtained with solvent systems recommended for better yields of various bioactive compounds in the literature, which was considered to be 100% extraction. The extracts were prepared by mixing 0.5 g of sample with 15 mL of solvent. The contents were mixed for 30 min, using Tarsons test tube mixer (CAT 3090, 240 VAC, 50 Hz), centrifuged at $3000 \times g$ for 15 min and the supernatant was recuperated and filtered using Whatman filter paper No 4. The procedure was repeated three times with each sample, and the final volume was completed to 50 mL.

Determination of some bioactive compounds

Three different analyses were done for the estimation of bioactive compounds in the spice extracts.

Total phenolic compounds

The total phenolic compounds content was estimated for various extracts with the method described by Marigo (1973). Briefly, aliquot (0.02 mL) of each extract was mixed with 1.2 mL of distilled water and then 0.2 mL of Folin-Ciocalteu reagent was added. To this, 0.4 mL of sodium carbonate solution were added and the absorbance was read at 725 nm against the blank (UV-1800, Shimadzu) after keeping the tubes in water bath at 40 °C for 2 h. The analysis was performed in triplicate and results expressed as gallic acid equivalents per 100 g of sample.

Total flavonoids

The total flavonoids content was determined using the aluminium chloride colorimetric assay method as described by Mimica-Dukic (1992). The calibration curve was prepared with 100, 200, 400, 600, 800 and 1000 µg/mL of quercetin. To 0.2 mL of quercetin solution, 4 mL of distilled water, 0.3 mL of 5% sodium nitrate were added and incubated for 5 min. A volume of 0.3 mL of 10% aluminium chlorite was added, then 2 mL of 1 M sodium hydroxide and the final volume was brought to 10 mL with distilled water. Samples were prepared in the same way using 0.2 mL of extract and the absorbance was read at

Fig. 1 Pictures of underutilized Cameroon spices. **a** *A. sulcatum,* **b** *X. africana*, **c** *H. zenkeri*

510 nm against a blank. Results were expressed as mg of quercetin equivalents/100 g of spices.

Tannins content

Tannins contents were determined as follows: 0.02 mL of extract was added to 3 mL of 4% vanillin diluted in methanol and 1.5 mL of concentrated hydrochloric acid was added. The mixture was incubated at 30 °C for 20 min and absorbance was read at 500 nm. The standard curve was plotted with 100, 200, 400, 600, 800, 1000 µg/mL of tannic acid. The quantity of tannins in each sample was expressed as mg of tannic acid per 100 g of spice powder (Bainbridge et al. 1996).

Antioxidant activity

Four different methods were used to evaluate the antioxidant activity of the spice extracts: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2-deoxyribose and the reducing power.

The 2,2-diphenyl-1-picrylhydrazyl

The radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined following the method edited by Brand-William et al. (1995). Briefly, a solution of DPPH (0.2 mM) in ethanol and 0.05 mol/L acetate buffer (pH 5.5) were prepared. A volume of 0.5 mL of extract was mixed with 2 mL of acetate buffer, 1.9 mL of absolute ethanol and 1 mL DPPH• solution. The mixture was vortexed immediately after adding DPPH• and allowed to stand at room temperature (25 °C) in dark environment for 30 min. The absorbance was read at 517 nm. The radical scavenging activity was determined as a decrease in absorbance of DPPH• and was calculated using the following equation.

$$\text{Activity } (\%) = \frac{\text{Abs control} - (\text{Abs sample} - \text{Abs blank})}{\text{Abs control}} \times 100$$

The 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS·)

ABTS· scavenging activity of the spice extracts was determined using the protocol proposed by Re et al. (1999). Three microlitres of the diluted ABTS (Initial absorbance 0.700) was added with 30 µL of spice extract; the content was vortexed, and the absorbance was read at 734 nm. The antioxidant activity was obtained using a standard curve plotted with varying concentrations of trolox (1.25 mM, 0.833 mM, 0.625 mM, 0.500 mM). Results were expressed in g trolox equivalent/100 g of dry matter. The percentage of inhibition or antioxidant activity (AA) was calculated with the following equation.

$$\text{AA } (\%) = \frac{\text{A initial} - \text{Assay}}{\text{Ainitial}} \times 100$$

Hydroxyl radical

Hydroxyl radical scavenging activity was determined by the 2-deoxyribose method described by Halliwell et al. (1987). To 0.1 mL of spice extract, reagents were added sequentially as follows: 500 µL of 2-deoxyribose (5.6 mM) prepared in a phosphate buffer KH_2PO_4–NaOH 50 mM, pH 7.4), 200 µL of the mixture of 100 mM of $FeCl_3$ and 100 mM EDTA (1/1, v/v), 100µL of 1.0 mM H_2O_2 and 100 µL of aqueous solution of ascorbic acid (1.0 mM). The contents were vortexed and incubate at 50 °C for 30 min; then 1 mL of thiobarbituric acid (1.0%) and 1 mL of trichloroacetic acid (2.8%) were added and re-incubated at 50 °C for 30 min. The oxidation (degradation of deoxyribose) was determined by the measurement of absorbance at 532 nm. The value of the inhibition percentage was calculated from the absorbance of the control (A control) and that of the spice extracts. The control was made up of all the reagents apart of the extract. The standard curve was plotted using mannitol at different concentrations. The anti-oxidant activity was expressed in terms of mannitol equivalent (mg mannitol/g of powder) using the following formula:

$$\text{AA } (\%) = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Total reducing power

Determination of the antioxidant activity by reducing power was assessed with the modified method of Oyaizu (1986). Reagents were mixed as follows: 0.5 mL of spices extracts, 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$. The mixture was incubated at 50 °C for 30 min and 1.25 mL of 10% of trichloroacetic acid was added. The tube contents were centrifuged at 3000 x *g* for 10 min and 1.25 mL of the supernatant was diluted with 1.25 mL distilled water and 0.25 mL $FeCl_3$ (0.1%). The absorbance was recorded at 700 nm and the reducing power was indicated by the increased of absorbance in the reaction. Results were expressed as mg ascorbic acid equivalent/100 g spice powder, using a standard curve.

Phenolic compounds profile

The phenolic profile was done to determine the compounds that are responsible for the antioxidant activity observed in various analyses. The phenolic profile of each spice extract was determined using the method described by Burin et al. (2011). One gram of sample was extracted with methanol (HPLC grade) by sonication for 20 min and centrifuged at 8000×*g* for 15 min. The supernatant was concentrated and syringe filtered. HPLC system (LC 20-AS, Shimadzu) was equipped with dual pump, UV detector (SPD 20A) and C18 silica column (YMC pack C18—Wilmington, North Carolina, USA). The separation and elution were done by binary gradient mode using the mobile phase, which consisted of solvent A (acetic acid filtered in Mili Q water adjusted to pH 2.6) and solvent B (20% of solution A in 80% acetonitrile) with injection volume of 20 µL of the sample at a flow rate of 1.2 mL/min for 60 min. Separated phenolic compounds were compared to standards used and were identified based on their retention time and area

Porcine pancreatic lipase inhibitory activity

The anti-lipase activity was assessed with the method reported by Vattem et al. (2007) modified from that used by Smeltzer et al. (1992). A suspension containing 1% of triolein and 1% Tween 80 (v/v) in 0.1 M phosphate buffer (pH 8) was prepared and emulsified. The assay was then initiated by adding 800 µL of triolein emulsion to 200 µL of porcine pancreatic extract (Type II, 100-500 units/mg sigma product form Germany) prepared in 0.1 M Phosphate buffer at pH 8. The content was emulsified by sonification for 3 min and the absorbance read immediately at 450 nm and designated as T_0. The test tubes were incubated at 37 °C for 30 min, after which the absorbance was measured for each emulsion at 450 nm and designated as T_{30}.Variation in absorbance [A450 (T_0)–A450 (T_{30})] was calculated for both the control and the treatment (Δ450) and the % inhibition was determined using the following formula.

$$\mathrm{Ip} = \frac{\Delta 450\mathrm{contro} - \Delta\mathrm{extract}}{\Delta\mathrm{control}} \times 100$$

Inhibitory activity of α-amylase

The inhibitory activity of α-amylase was measured for various extracts, using the method described by Komaki et al. (2003). About 20 µL of α-amylase solution (0.03 mg/mL) (sigma (Aldrich, ≥ 100 units/mg protein, Germany) was mixed with 1.3 mL of Tris–HCl buffer solution (0.01 M containing 0.006 M NaCl, pH 6.8) and 80 µL of extract. After incubation at 37 °C for 20 min, 100 µL of starch solution (0.1%) was added and the mixture re-incubated for 20 min, after which 2 mL of 0.01% acidic iodine solution was added and the absorbance measured at 565 nm. The inhibition percentage was calculated taking into consideration the control in which no extract was added.

$$\mathrm{Ip} = \frac{\mathrm{A} - \mathrm{C}}{\mathrm{B} - \mathrm{C}} \times 100$$

where A is the absorbance of the sample, B is the absorbance of the blank (no extract) and C is the control with no starch.

Statistical analysis

Experiments were done in triplicate and data were subjected to analysis of variance. Significant differences between the means was determined using the Duncan multiple range test at ($p < 0.05$) in Statgraphics centurion XVI. Results were expressed as mean ± standard deviation and the graphs were plotted using Sigma plot software 11.0.

Results and discussion

Total phenolic compounds

The quantity of phenolic compounds significantly varied in the various spice extracts as presented in Table 1. The aim of this assessment using water and other solvent systems was to estimate the quantity of phenolic compounds that can be solubilized when these spices are used in food. The quantity of phenolic compounds solubilized in the different extracts varied with the type of spice. The solvent systems prepared with 80% ethanol/water and 1% HCl-methanol gave higher yields for all the spices. *X. africana* and *H. zenkeri* were the spices with higher phenolic contents (10.32 ± 0.49 and 8.66 ± 29 g gallic acid eq/100 g of dry spice respectively), in 80% ethanol/water extract. The higher yields for *A. sulcatum* fruits and bulbs were recorded with 1% HCl/methanol (0.52 ± 0.04 and 0.88 ± 0.05 g gallic acid eq/100 g of dry spice respectively). This shows that the extraction yield of phenolic compounds is highly depends on the nature of plant material and the solvent used. Ethanol/water is reported in the literature as one of the good solvent systems for phenolic compounds extraction (Díaz-de-Cerio et al. 2018). Taking into consideration the higher phenolic content obtained with ethanol/water and 1% HCl methanol for the different spices, it can be estimated that 69.76% of phenolic compounds from *X. africana*, 69.05% from *H. zenkeri*, 88.46% from *A. sulcatum* fruits and 42.31% from *A. sulcatum* bulbs are soluble in water. This result indicates that consuming food prepared using these spices may contribute to significant intake of phenolic compounds from these spices. Abdou-Bouba et al. (2010) reported higher phenolic compounds content from other spices used in traditional dishes in Cameroon like *Dichrostachys glomerata*, *Fagara leprieurii* and *Tetrapleura tetraptera* fruits (38.8, 34.59 and 22.75 g gallic acid eq/100 g of dry spice respectively). The difference may be due to the fact that the extracts used by Abdou-Bouba et al. (2010) were partially concentrated, or to the specificity of the plant.

Total flavonoids

The flavonoids contents of the spice extracts are presented in Table 2, which shows that there was a significant difference ($p < 0.05$) in the quantity of flavonoids of the different spice samples. Based on these results, the flavonoids content was found to be higher with methanol/water/acetic acid (70/25/5: v/v/v) for *X. africana* and *H. zenkeri* (146.66 ± 4.15 and 116 ± 5.03 mg quercetin equivalent/100 g spice respectively). However, the best flavonoids yield of *A. sulcatum* fruit and bulb was obtained with ethanol/water extract 47.98 ± 3.49 and 82.04 ± 3.88 mg quercetin eq/100 g respectively mean while it was not found in methanol/water/acetic acid which is the solvent often used for flavonoids extraction. This suggests that theextraction yield of flavonoids depends on the type of plant material used. Quy et al. (2014); Dirar et al. (2019) reported variable concentrations of polyphenols and flavonoids for the same plant material, using different solvents. For the same plant material, these phytochemicals were found to be totally absent in some extracts, while they were detected in other extracts (Dirar et al. 2019). This may be due to the affinity of flavonoid with certain solvents, which mostly depends on the polarity of the solvent and the structure of the molecule. Zhang et al. (2017) conducted a study on the solubility of flavonoids, using water and 1-octanol. They showed that the differential solubility of flavonoids in these two solutions depends on the presence

Table 1 Total phenolic compounds of spice extracts expressed in g gallic acid eq/100 g of dry spices

Spices	Aqueous extracts	Ethanol/water (80/20, v/v)	Methanol/water/acetic acid (70/25/5, v/v/v)	HCl/methanol (1%, v/v)
X. africana fruit	7.12 ± 0.150^{d}	10.32 ± 0.49^{c}	5.87 ± 0.47^{c}	6.94 ± 0.85^{c}
A. sulcatum fruit	0.46 ± 0.014^{b}	0.49 ± 0.06^{a}	NF	0.52 ± 0.04^{a}
A. sulcatum bulb	0.11 ± 0.01^{a}	0.26 ± 0.02^{a}	0.17 ± 0.083^{a}	0.88 ± 0.05^{a}
H. zenkeri bark	5.98 ± 0.08^{c}	8.66 ± 0.29^{b}	3.85 ± 0.23^{b}	5.31 ± 0.090^{b}

Results were expressed as mean ± standard deviation

NF Not found

Superscript letters (a–d) represents the significant differences ($p < 0.05$) between the rows and within columns

Table 2 Flavonoids content of spice extracts expressed as mg quercetin eq/100 g spices

Spices	Aqueous extracts	Ethanol/water (80/20, v/v)	Methanol/water/acetic acid (70/25/5, v/v/v)	HCl/methanol (1%, v/v)
X. africana fruit	3.22 ± 0.61^{b}	119.23 ± 4.11^{d}	146.66 ± 4.15^{b}	649.88 ± 2.46^{c}
A. sulcatum fruit	NF	47.98 ± 3.49^{a}	NF	NF
A. sulcatum bulb	NF	82.04 ± 3.88^{b}	0.001 ± 0.000^{a}	13.26 ± 0.1^{a}
H. zenkeri bark	1.79 ± 0.34^{a}	93.023 ± 3.58^{c}	116.56 ± 5.03^{c}	255.69 ± 2.46^{b}

Results were expressed as mean ± standard deviation

NF Not found

Superscript letters (a–d) represents the significant differences ($p < 0.05$) between the rows and within columns

or absence of double bonds in ring C, the number of OH substitution on ring B. The presence or not of OCH3 substitution on ring B, the position of the carbon with which a ring is connected to the other. The flavonoids contents obtained with ethanol/water extract were in the same range with those obtained in other spices (Abdou-Bouba et al. 2010; Sokamte et al. 2019).

Tannins content

The tannins content of various spices is given in Table 3. From these results, it was noted that tannins were not present in aqueous extract, 80% ethanol/water, and methanol/water/acetic acid of the fruit of *A. sulcatum*. They were also not found in methanol/water/acetic acid extracts of the bulb of *A. sulcatum*. Tannins were nevertheless found in all the extracts obtained with 1% (v/v) HCl/methanol. The highest tannin content was obtained with 80% ethanol extract of *X. africana*. All the four extracts obtained with the different solvents for the bark of *H. zenkeri* contained tannins. The aqueous extract of the bark of *H. zenkeri* had the highest tannin content, compared to ethanol/water, methanol/water/acetic acid and HCl/methanol extracts. Tannins were generally more soluble in water compared to flavonoids. The solubility depends on the affinity of the compound extracted with the solvent use, but also to the structure of the molecule (Zhang et al. 2017). The use of *X. africana* and *H. zenkeri* in soups and other food in which they are consumed may therefore favour tannins intake. Spices in the form of bark of tree are also reported to be part of food habits in other parts of the world. In South-Asia, the bark of *Cinnamomum burmannii* is one of them. It has been reported to be source of bioactive compounds unlike the bark of *H. zenkeri*. Studies have been done on the optimization of aqueous extract of *C. burmannii* in view of producing an aqueous extract for the management of diabetes mellitus (Ervina et al. 2019). Results of the present study shows that the fruits of *X. africana* and the bark of *H. zenkeri* can be used in the future for the same purposes.

Antioxidant activity

Phytochemicals act by preventing, blocking or slowing down oxidation and autoxidation processes. The bioactive compounds in plants have different mechanisms through which they interact with oxidative agents; thus, the need of evaluating their activity using different antioxidant methods (Amorati and Valgimigli 2018).

Radical scavenging activity using DPPH

Table 4 contains the result of antioxidant activity determined using DPPH, expressed as mg of ascorbic acid equivalent per gram of spices. The highest antioxidant activity was obtained with methanol/water/acetic acid

Table 3 Tannin content of spice extracts (expressed as mg tannic acid eq/100 g spices)

Spices	Aqueous extracts	Ethanol/water (80/20, v/v)	Methanol/water/acetic acid (70/25/5, v/v/v)	HCl/methanol (1%, v/v)
X. africana fruit	383.33 ± 5.77[b]	452.44 ± 2.7[c]	NF	112.28 ± 2.34[c]
A. sulcatum fruit	NF	NF	NF	36.39 ± 1.25[b]
A. sulcatum bulb	0.23 ± 0.01[a]	5.83 ± 0.14[a]	NF	0.38 ± 0.048[a]
H. zenkeri bark	245.00 ± 5.00[b]	200.38 ± 3.8[b]	128.46 ± 1.5	131.75 ± 3.4[d]

Results were expressed as mean ± standard deviation

NF Not found

Superscript letters (a–d) represents the significant differences ($p < 0.05$) between the rows and within columns

solvent system for *X. africana* and *H. zenkeri* (182.24 ± 2.4 and 180.86 ± 2.73 mg ascorbic acid equivalent per g of spice respectively). A contrary observation was noted with *A. sulcatum* fruit and bulb. The ethanol/water solvent system gave the highest scavenging activity against DPPH radicals (44.44 ± 1.28 and 83.09 ± 2.33 mg of ascorbic acid equivalent per gram of spice respectively). Compared to the three other spice samples, *X. africana* aqueous extract exhibited the highest (165.8 mg ascorbic acid equivalent per g of spice) and significant ($p < 0.05$) scavenging activity against DPPH radicals. Looking at the proportions of phenolic compounds and the antioxidant activity, it can be suggested that, the radical scavenging activity is related to both the quantity and efficiency of the type of phenolic compound present in the various samples. There was a significant difference ($p < 0.05$) between the radical scavenging activity of the different spice extracts. The high scavenging activity obtained with *X. africana* and *H. zenkeri* can be attributed to flavonoids, knowing that ethanol/water/acetic acid extract is recommended for best yield of flavonoids. The solvent may contribute to maintain the activity of flavonoids, compared to 1% HCl methanol solvent system (Table 2). The phenolic content and scavenging activity with DPPH had the same trend for the aqueous extract, but was different from other solvents (Table 1 and 4). Likewise, Abdou-Bouba et al. (2010); Sokamte et al. (2019) reported a discordance between the phenolic content and the radical scavenging activity of methanolic extract. Dragan et al. 2003 showed that highly active flavonoids possess a 3′,4′-dihydroxy occupied B ring. This shows that two solvent systems can have the same flavonoids content, but their biological activities are different, due to the type of flavonoid present. Apart from the positions of hydroxyl groups, tannins have a smaller number of hydroxyl groups, which can affect their solubility in different solvent systems (Dragan et al. 2003)

There was a positive and high correlation with the phenolic content of the different extracts and the radicals scavenging activity of the corresponding extracts, using DPPH. The correlation values were 0.778 for water extracts, 0.961 for ethanol/water and 0.969 for methanol/water/acetic acid (Table 6). This shows that the radical scavenging activity is enhanced by phenolic compounds in these spices. The correlation between the flavonoid content of ethanol/water extract and its corresponding antioxidant

Table 4 DPPH and ABTS activities of extracts

Spices	DPPH assay (mg ascorbic acid eq/g spice)			ABTS assay (g trolox eq/100 g spice)		
	Aqueous extracts	Ethanol/water (80/20: v/v)	Methanol/water/acetic acid (70/25/5: v/v/v)	Aqueous extracts	Ethanol/water (80/20: v/v)	Methanol/water/acetic acid (70/25/5: v/v/v)
X. africana fruit	165.8 ± 2.68[d]	179.29 ± 3.48[c]	182.24 ± 2.41[c]	7.190 ± 0.003[c]	1.294 ± 0.002[d]	0.466 ± 0.013[a]
A. sulcatum fruit	27.47 ± 1.60[b]	44.44 ± 1.28[a]	37.71 ± 0.36[a]	0.529 ± 0.004[b]	0.454 ± 0.004[a]	2.628 ± 0.026[d]
A. sulcatum bulb	16.43 ± 2.86[a]	83.09 ± 2.33[b]	72.56 ± 2.14[b]	0.047 ± 0.002[a]	0.381 ± 0.00[b]	2.141 ± 0.026[b]
H. zenkeri bark	159.18 ± 2.88[c]	179.37 ± 0.33[c]	180.86 ± 2.73[c]	9.247 ± 0.004[d]	0.939 ± 0.004[c]	2.331 ± 0.066[c]

Results were expressed as mean ± standard deviation

Superscript letters (a–d) represents the significant differences ($p < 0.05$) between the rows and within columns

activity was also determined (0.89). It indicates that flavonoids may be mainly responsible of the scavenging activity of ethanol/water extract.

Hydroxyl radical scavenging activity

The determination of hydroxyl radicals scavenging activity using 2-deoxyribose showed that there was a significant difference with respect to the spice samples and for various extracts. The higher values were recorded with ethanol/water extract. Ethanol/water extract of the bark of *H. zenkeri* had the best activity (729.27 mg mannitol equivalent/100 g of spice) (Table 5). The lowest values were obtained with methanol/water/acetic acid extracts of *A. sulcatum* fruit and bulb (129.32 and 87.33 mg mannitol eq/100 g spice correspondingly). Low scavenging activity of hydroxyl radical compared to DPPH have also been reported by Lee et al. (2019) for other plants materials. The difference in hydroxyl radical scavenging activity may be attributed to the flavonoid content of these spices. The spices with high flavonoid content presented high activity against hydroxyl radicals; this was the case with ethanol/water and methanol/water/acetic acid extracts of *X. africana* and *H. Zenkeri*. This result also indicates that the hydroxyl radicals scavenging activity was almost the same with the water extracts of the different spices.

There was a negative correlation (− 0.056) with the aqueous extract of total phenolic compounds and the corresponding hydroxyl radical scavenging activity. Nevertheless, there were high and positive correlations with the ethanol/water (0.81), methanol/water/acetic acid (0.946) extracts (Table 6) and between the flavonoid content of ethanol/water extract and the corresponding hydroxyl radical scavenging activity (0.59). The correlation obtained suggest that in the aqueous extracts, the hydroxyl radical scavenging activity is due to other compounds like ascorbic acid and related molecules which are highly water soluble. It may also be suggested that the type of phenolic compounds in *A. sulcatum* fruit and bulb is specific for scavenging hydroxyl radicals (Dragan et al. 2003).

2-2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

The 2-2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) is reported in many studies to be used along with DPPH method to study antioxidant activity of plant extracts (Pisoschi et al. 2016). The ABTS scavenging activity of the four spice extracts showed that, *H. zenkeri* aqueous extract had the highest activity (9.247 g trolox eq/100 g spice), followed by *X. africana* (7.190 and 1.294 g trolox eq/100 g spice) respectively for the aqueous and ethanol/water extracts (Table 4). It was also noted that the aqueous extract generally had high activity compared to other solvents. From one spice to the other, the antioxidant activity was significantly different ($p < 0.05$). The values obtained were higher than those reported by Abdou-Bouba et al. (2010). The difference may be due the solvent used. Nevertheless, these results are closed to those obtained by Nikolic et al. (2019) who reported high values like those obtained in this study, on spices and herbs used in Serbia cuisine. The scavenging activity of the spice extracts towards ABTS radical showed a positive and strong correlations with the aqueous and ethanol extracts (0.953 and 0.976 respectively) (Table 6). There was also a positive correlation between the flavonoids of ethanol extracts and the antioxidant activity (0.845). The ABTS scavenging activity in these extracts can be attributed to the total phenolic compounds as well as flavonoids.

Table 5 Hydroxyl radical and reducing power activities of extracts

Spices	Hydroxyl radical scavenging assay (mg mannitol eq/100 g spice)			Total reducing power assay (g ascorbic acid equivalent/100 g spice)		
	Aqueous extracts	Ethanol/water (80/20: v/v)	Methanol/water/acetic acid (70/25/5: v/v/v)	Aqueous extracts	Ethanol/water (80/20: v/v)	Methanol/water/acetic acid (70/25/5: v/v/v)
X. africana fruit	420.45 ± 6.65^{a}	609.57 ± 4.40^{c}	541.28 ± 2.52^{d}	1.974 ± 0.003^{c}	2.351 ± 0.002^{d}	2.300 ± 0.009^{d}
A. sulcatum fruit	450.31 ± 6.65^{c}	493.69 ± 2.43^{a}	129.32 ± 3.05^{b}	0.164 ± 0.002^{b}	0.149 ± 0.001^{a}	0.098 ± 0.001^{a}
A. sulcatum bulb	433.07 ± 1.67^{b}	524.48 ± 3.27^{b}	87.33 ± 1.24^{a}	0.103 ± 0.002^{a}	0.262 ± 0.001^{b}	0.165 ± 0.004^{b}
H. zenkeri bark	466.40 ± 4.03^{d}	729.27 ± 3.07^{d}	474.75 ± 3.63^{c}	2.101 ± 0.005^{d}	2.099 ± 0.003^{c}	1.669 ± 0.001^{c}

Results were expressed as mean ± standard deviation

Superscript letters (a–d) represents the significant differences ($p < 0.05$) between the different spices and extracts

Table 6 Correlation coefficients between the extracts and antioxidant activity tests

Spice extracts	Correlation of phenolic compounds and antioxidant activity (r)			
	Radical scavenging assay			Total reducing power assay
	DPPH	ABTS	Hydroxyl	
Aqueous extracts	0.778	0.953	− 0.056	0.74
Ethanol/water	0.961	0.976	0.809	0.997
Methanol/water/acetic acid	0.969	–	0.946	0.998

Total reducing power

A reducing antioxidant reaction is a reaction during which, one reactive species is reduced at the expense of the oxidation of another. Most plant bioactive compounds have this potentiality. The total reducing power activity of the four spices extracts indicate that the values obtained were significantly different for various spices ($p < 0.05$) (Table 5). The highest value was obtained with ethanol/water extract (2.35 g ascorbic acid eq/100 g spice) and the lowest value was that of methanol/water/acetic acid of *A. sulcatum* fruit (0.098 g ascorbic acid eq/100 g spice). The extract with the highest total reducing power activity was ethanol/water for three out of the four spices. The differences in the reducing power activity shows the influence of the type of the plant material on the extraction of bioactive compounds. There were high correlations between the total reducing power of the different extracts and the corresponding phenolic content (0.74, 0.99 and 0.99 for the aqueous, ethanol/water and methanol/water acetic acid respectively) (Table 6). The correlation obtained with the ethanol/water extract and the corresponding flavonoid extract was also positive (0.851). This indicates that the total reducing power of extracts is due to the bioactive compounds found in each of them, at varying proportions.

Phenolic profile of the spice samples

The quantitative analysis of various families of phenolic compounds in the four spice samples is presented in Table 7. The phenolic compounds were identified as protocatechuic acid, chlorogenic acid, vanillic acid, *p*-coumaric acid, ferulic acid, protosynapic acid, T-cinnamic acid and epicatechin. Epicatechin was determined in two of the spices (*X. africana* and *A. sulcatum* fruit). It is a monomer of flavan-3-ol, reported to have several health benefits. Protocatechuic acid was found in small amount in *A. sulcatum* fruit (0.78 mg/100 g) and bulb (4.41 mg/100 g). Unlike protocatechuic acid, ferulic acid was also identified in these two spices only. The presence of these two phenolic acids in the bulb and fruit of the plant may be specified to *A. sulcatum* plant. Protosynapic acid and T-cinnamic acid were determined in *A. sulcatum* fruit and bulb. The maximum number of various phenolic compounds, as well as the highest quantity of T-cinnamic acid (52.58 mg/100 g) and epicatechin (34.707 mg/100 g) were found in the fruit of *A. sulcatum*. Observations made on hydroxyl radical scavenging activity can be attributed to these groups of phenolic compounds. Five different families of phenolic compounds were determined in *X. africana*: chlorogenic acid (5.160 mg/100 g), vanillic acid (19.768 mg/100 g), epicatechin (25.386 mg/100 g), *p*-coumaric acid (12.652 mg/100 g), T-cinnamic acid (6.214 mg/100 g). Only two of the phenolic compounds were present in the methanolic extract of *H. zenkeri* (chlorogenic acid, 4.312 mg/100 g and protosynapic acid, 186.562 mg/100 g). The high antioxidant activities of *H. zenkeri* can therefore be attributed to these two groups of phenolic compounds.

Porcine pancreatic lipase inhibition activity

Inhibition activity of lipase at the level of the gastrointestinal tract (GIT) is one of the means by which fat related diseases can be managed. Plant secondary metabolite are often studied for their ability to inhibit pancreatic lipase as to reduce fat harvest at the level of the gastrointestinal tract (Zhu et al. 2018). Porcine pancreatic lipase was used in this study to determine the effect of the various spice extracts to inhibit triglycerides breakdown in the gastrointestinal tract. Figure 2 shows that, 80% ethanol/water solvent generally inhibited pancreatic lipase for all the different spice extracts, compare to the extracts obtained with other solvents. The aqueous extract having the highest activity was that of *H. zenkeri*. 1% HCl methanol extract of *A. sulcatum* fruit and bulb highly inhibited pancreatic lipase compared to 1% HCl methanol extract of *X. africana*. These results indicate that the inhibition of pancreatic lipase does not depend on the quantity of bioactive compounds, but on the presence of a particular molecules (Dragan et al. 2003). Etoundi, et al. (2013) showed hypolipidemic effect of extract from the bark of *H. zenkeri* on rats with hyperlipidaemia induced by triton WR-1339. Oben et al. (2010) reported that the heated extract at 80 °C for 30 min gave no activity on pancreatic lipase inhibition. In the present study, the extract was not heated and the aqueous extract of

Table 7 Quantitative analysis of various phenolic compounds in methanolic extract of the spices by HPLC

Phenolics (mg/100 g dry spice)	*A. sulcatum* bulb	*A. sulcatum* fruit	*X. africana* fruit	*H. zenkeri* bark
Protocatechuic acid	4.409	0.780	NF	NF
Chlorogenic acid	0.790	NF	5.160	4.312
Vanillic acid	NF	0.830	19.768	NF
Epicatechin	NF	34.707	25.386	NF
p-Coumaric acid	NF	1.127	12.652	NF
Ferulic acid	3.165	1.374	NF	NF
Protosynapic acid	0.221	1.955	NF	186.562
T-Cinnamic acid	3.794	52.58	6.214	NF

NF Not found

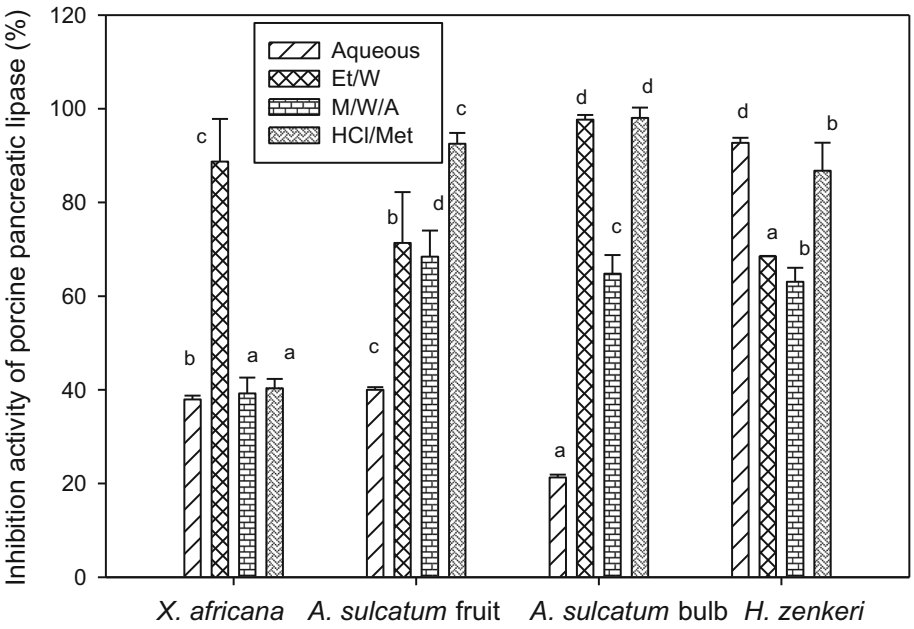

Fig. 2 Porcine pancreatic lipase inhibitory activity of spice extracts. *Et/W* Ethanol/water extract, *M/A/W* methanol/water/acetic acid extract, *HCl/M* hydrochloric acid/methanol extract. Values were expressed as mean ± standard deviation. Superscript letters (a–d) represents the significant differences ($p < 0.05$) between the different spices and extracts

H. zenkeri inhibited porcine pancreatic lipase by 92.71 ± 1.08%. The compound responsible of porcine pancreatic lipase could have been destroyed by heat in the study of Oben et al. (2010). Irushika et al. (2019) stated that non-boiled *Trigonellafoenum-graecum* seeds, *Myristica fragrans* seeds, and *Cuminum cyminum* seeds significantly inhibit porcine pancreatic lipase than the corresponding boiled ones. On the contrary, heat treatment of *Cinnamomum zeylanicum* stem bark, *Foeniculum officinale* seeds increase their activities. These results show the complexity related to the use of plant bio-active compounds on biological activities. The consumption of some spices at raw state could be advantageous to reduce lipids digestion and absorption at the level of GIT while some do not. The mechanism of used to lower lipids absorption proposed in the literature is that, secondary metabolites especially those of high molecular weight like condensed tannins bind to lipase by non-covalent bonding and electrostatic interactions. The binding of the secondary metabolite alters the molecular conformation of the enzyme, resulting to a decrease of its catalytic activity (Zhu et al. 2018).

Alpha amylase inhibitory activity

Alpha amylase is an enzyme which hydrolysis polysaccharides into oligosaccharides, maltose, dextrin and disaccharides (Kato et al. 2017). The hydrolysis results to an increase of absorption of sugars that further enhance the increase in blood glucose level or overnutrition in certain

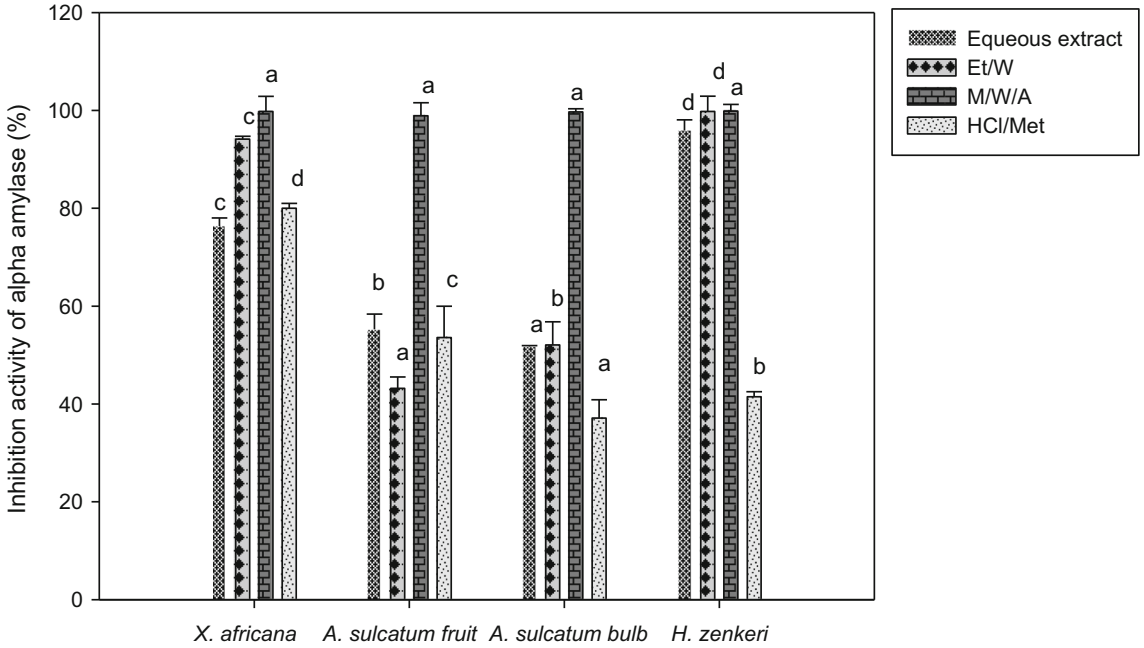

Fig. 3 Alpha amylase inhibitory activity of spice extracts. *Et/W* Ethanol/water extract, *M/A/W* methanol/water/acetic acid extract, *HCl/M* hydrochloric acid/methanol extract. Values were expressed as mean ± standard deviation. Superscript letters (a–d) represents the significant differences ($p < 0.05$) between the different spices and extracts

cases. Plant secondary metabolites are known to inhibit digestive enzymes, including -amylase. The inhibition of -amylase controls the elevation of blood glucose levels by delaying and blocking postprandial carbohydrate digestion and absorption (Jayaraj et al. 2013).

The four spices studied in this work were investigated for their activity to inhibit the α-amylase. Figure 3 shows that ethanol/water extracts of *H. zenkeri* and *X. africana* were the spice extracts with higher inhibitory activity towards α-amylase (99.54 ± 0.34 and 98.82 ± 0.42% respectively). The extracts of the two plants were generally highly active against α-amylase than those of *A. sulcatum* fruit and bulb. Oben et al. (2010) reported that the heated extracts (at 80 °C for 30 min) of other spice used in Cameroon cuisine inhibited α-amylase. It has been demonstrated in other studies that heat treatment can either activate or inhibit the activity of α-amylase (Irushika et al. 2019). The inhibition of this enzyme can be attributed to secondary metabolites common to *H. zenkeri* and *X. africana* (chlorogenic acid, vanillic acid, epicatechin, *p*-coumaric acid, protosynapic acid and T-cinnamic acid). The difference in the inhibition percentages of the various spice extracts can be attributed to the family of plant secondary metabolites present. Kato et al. (2017) studied the effect of hydrolysable and condensed tannins. They showed that the structure of tannins is an important factor on the inhibition of -Amylases. Hydrolysable tannins inhibition was concentration dependent, while condensed tannins were not.

Conclusion

In conclusion, *X. africana* and *H. zenkeri*, are sources of dietary bioactive compounds like flavonoids and tannins. The aqueous extracts of *X. africana* and *H. zenkeri* were shown to have high antioxidant activity. Ethanol/water extracts of *A. sulcatum* fruit and bulb inhibited porcine pancreatic lipase by 76.29 and 97.40% respectively. *X. africana* and *H. zenkeri* ethanol/water extracts inhibited α-amylase by 98.82 and 99.54%. These findings show that *X. africana* and *H. zenkeri* can be valued for the production of food supplements. While waiting for *invitro* analysis, these spices can be considered as a source of bioactive compounds through food, and may contribute to the management of some metabolic diseases.

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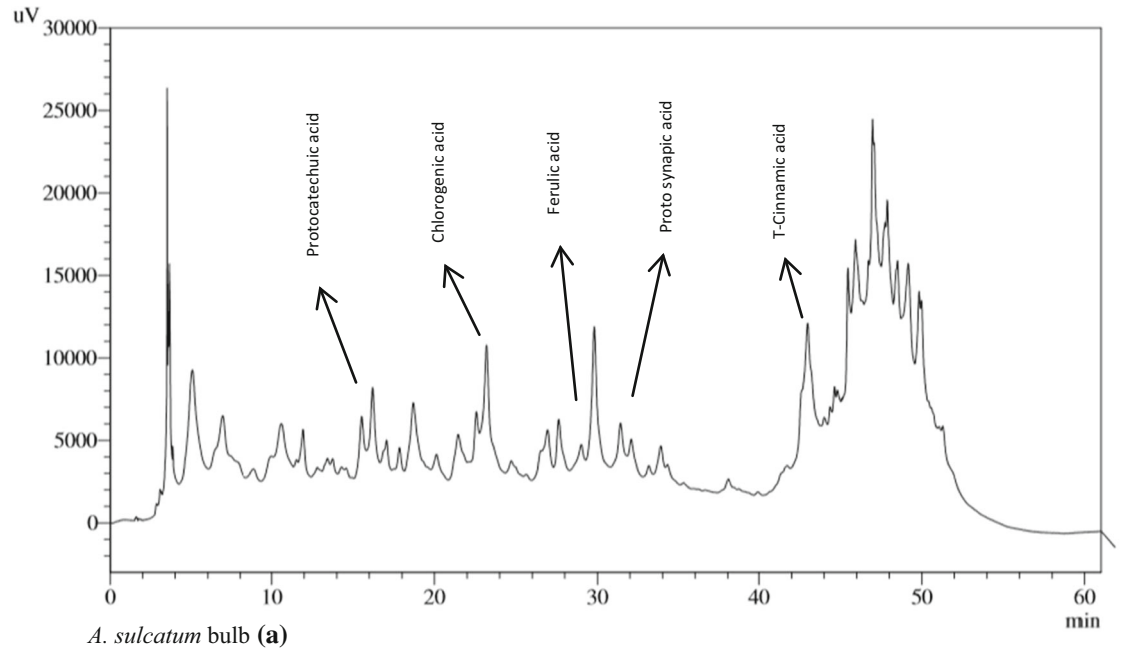
Compliance with ethical standards

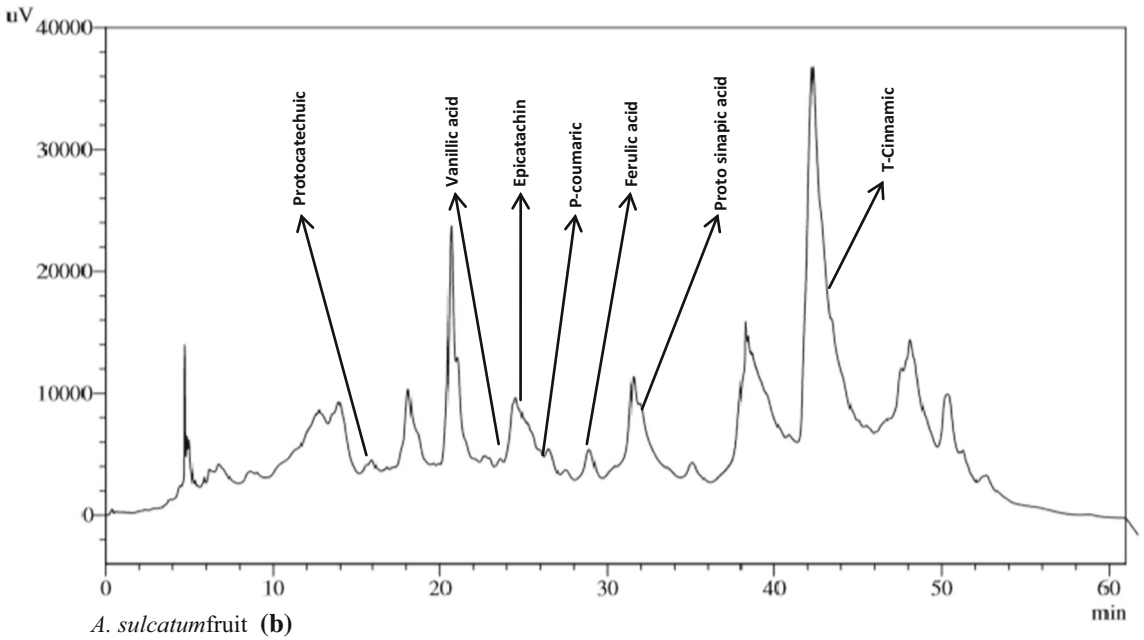
Conflict of interest The authors declare that they have no conflict of interest.

Appendix

See Fig. 4.

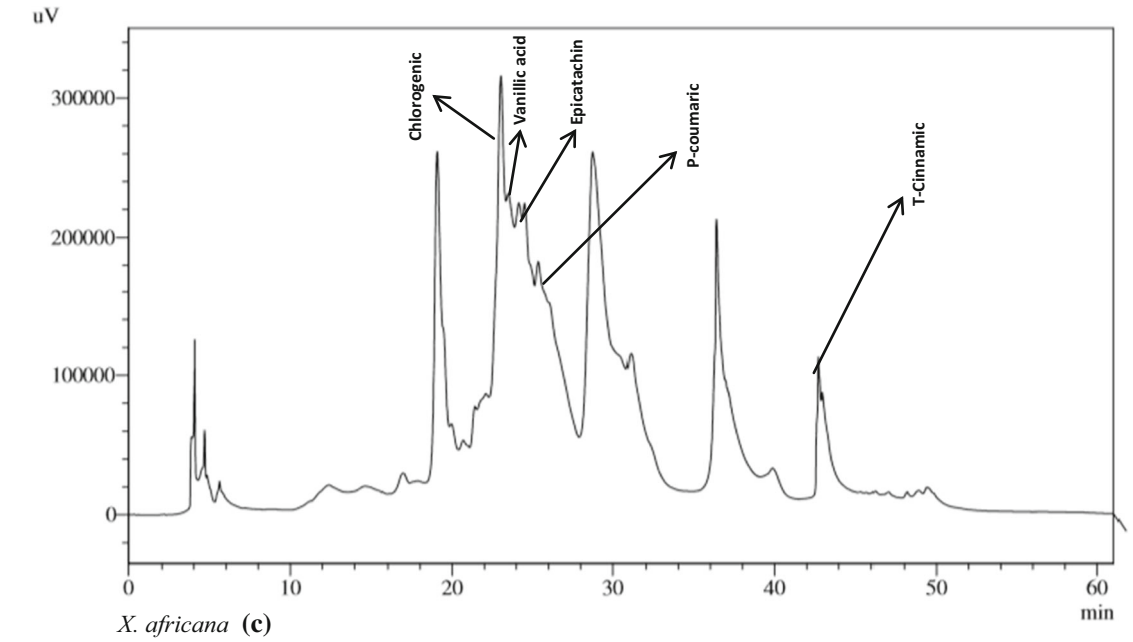


A. sulcatum bulb **(a)**

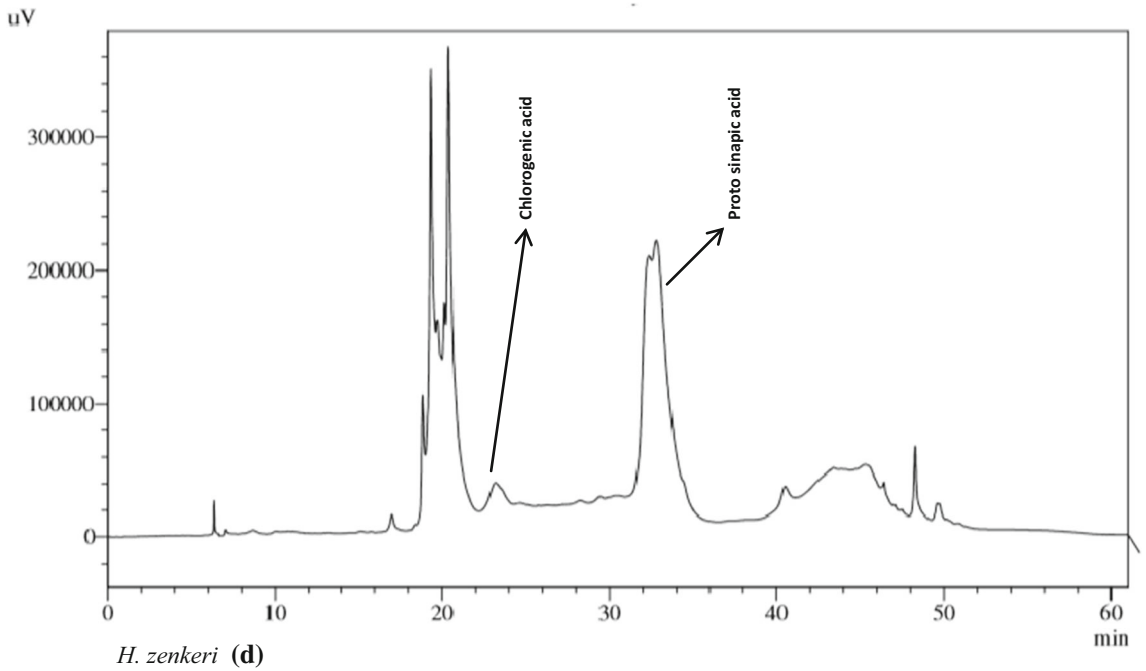


*A. sulcatum*fruit **(b)**

Fig. 4 Chromatograms of the phenolic profile of the *A. sulcatum* bulb (**a**), *A. sulcatum* fruit (**b**), *X. africana* (**c**), and *H. zenkeri* (**d**) C-18 silica column, solvent A: acetic acid/water, pH 2.6, solvent B: 20% acetic acid (solvent A)/80% acetonitrile, flow rate of 1.2 mL/min



X. africana **(c)**



H. zenkeri **(d)**

Fig. 4 continued

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