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Antioxidant and antimicrobial activities of two edibles spices from Cameroon and quantification of their major phenolic compounds

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Abstract

This study aimed to analyze the phytochemical composition, antioxidant capacity, antimicrobial activity and the phenolic profile of *Aframomum melegueta* and *Afrostyrax lepidophyllus* methanolic extracts. The mean phenolic compound contents were 160.77 ± 6.74 and 46.63 ± 0.93mg GAE/g extract, while that of the tannins was 136.40 ± 0.94 and 8.72 ± 1.60mg GAE/g, respectively for *A. melegueta* and *A. lepidophyllus*. Similarly, the flavonoids were higher in *A. melegueta* extract (70.62 ± 0.90mg CE/g) compared to *A. lepidophyllus* extract (3.95 ± 0.14 mg CE/g). *A. melegueta* extract showed strong antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP) and Phosphomolybdenum antioxidative power (PAP) tests compared to *A. lepidophyllus* extract. *A. lepidophyllus* extract showed strong antibacterial activity with a minimal inhibitory concentration (MIC) range of 312.5 to 5000µg/mL. *A. melegueta* extract has exerted a bactericidal effect against *Staphylococcus aureus* with minimal bactericidal concentration (MBC) = 5000 µg/mL. The qualitative and quantitative analysis of the phenolic compounds were carried out by HPLC coupled to a diode array detector. The most abundant flavonoid in *A. melegueta* was quercetin. Among the quantified phenolic acids, gallic acid was the most abundant in both spices extracts. The results of this study showed that *A. melegueta* extract has promising potential to the control of non-communicable diseases and could be used with *A. lepidophyllus* extract as natural sources of antimicrobial agents.

Introduction

Clinical and epidemiological studies in recent decades confirm that degenerative diseases remain a major public health problem and account for about 38 million (68%) of the 56 million deaths in developed and developing countries. Almost three quarters of all degenerative diseases deaths (28 million), and the majority of premature deaths (82%), occur in developing countries (WHO, 2014). The highest burden of diseases are cancer, heart diseases, chronic respiratory diseases, diabetes, ocular and nerve degeneration, etc. (WHO, 2014). Oxidative stress has been identified as the main initiator (Pham-Huy *et al*., 2008; Khansari *et al*., 2009). It occurs when there is a breakdown of the cell balance between antioxidants and prooxidants, in favor of the latter. To fight against these degenerative diseases, one of the practical strategies is to improve the antioxidant status of the body, which can be achieved by providing exogenous sources of antioxidants (Mark, 1998; Ohigashi *et al*., 2000). Indeed, although the organism possesses a complex system of endogenous antioxidants, it may happen that the ability of free radicals causing oxidative stress exceeds the body's defense capabilities, hence it is necessary to look for exogenous sources of antioxidants to restore the oxidative balance in the body.

It has been well established that the consumption of food derived from spices makes it possible to reduce the risk of certain degenerative diseases. Spices are considered to be one of the main sources of natural antioxidants because they possess beyond the basic nutrients (proteins, lipids, carbohydrates), certain bioactive substances derived from their secondary metabolism and which can confer beneficial effects on human health; Including: reducing the risk of

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cancer or altering their behavior in the body (Kaefer and Milner, 2008). The main secondary metabolites endowed with these virtues are phenolic compounds, which are increasingly taken intentionally through certain beverages (tea) considered to be natural medicinal preparations. The biological activity of phenolic compounds is expressed by their ability to chelate metals, scavenge free radicals and can also be expressed through their action to inhibit or activate a wide variety of enzymatic systems.

On the other hand, the pathways that regulate cell division and their proliferation can be modulated by phenolic compounds. Phenolic compounds can also interfere with pathways that regulate the immune response, detoxification and platelet aggregation (Hollman *et al*., 1996). Phenolic acids and flavonoids are phenolic compounds mostly found in plants (Silva *et al.*, 2004) and are of great interest for their important role in the control and prevention of certain noncommunicable diseases. Flavonoids are known for their antioxidant properties (Tamura and Yamagami, 1994) and their role in reducing the risk of certain cancers (Kamei *et al*., 1995) and coronary diseases (Bridle and Timberlake, 1996). Because of their richness in phenolic compounds, spices are considered natural food additives and have been used as seasoning, flavoring and coloring agents for decades to improve the quality and acceptability of certain foods.

In addition to their antioxidant properties, the phenolic compounds of the spices also have excellent antimicrobial properties, which give the spices some preservation properties. In Africa, many native spices used in culinary preparations and also exploited for their multiple medicinal virtues possess great antioxidant and antimicrobial properties. *Aframomum melegueta* and *Afrostyrax lepidophyllus* belong to this category and one of the most used parts is the seed (Onoja *et al*., 2014; Fogang *et al*., 2014). *A. lepidophyllus* belonging to the Huaceae family is a non-timber forest product found in the forests of Cameroon, Ghana and the Republic of Congo. The seeds of this plant have a strong smell of garlic or onion (bush onion) and are used as spices in African cuisine. Roots and leaves are used in traditional medicine in decoction form as an anthelmintic agent and to fight against urinary tract infections, hemorrhoids, and dental caries (Oliver-Bever, 1986; Kambu, 1990). Fogang *et al*. (2014) demonstrated antimicrobial, antioxidant and cytotoxic activity of seed essential oils. *A. melegueta* belonging to the family Zingiberaceae is found in Central and Western Africa. It is also used in traditional cooking to enhance the flavor and taste of food. The seeds and their essential oils possess antibacterial, antihelminthic, antifungal, anti-inflammatory, hepatoprotective, anti-cancer and antiparasitic properties (Musuyu *et al*., 2012; Fogang *et al.,* 2014; El-Halawany *et al*., 2015).

Although intensive studies have been carried out on the biological activity of many of these spices, data for the individual identification and quantification of bioactive phenolic compounds of increasing interest remain incomplete and insufficient. These additional data could provide a better understanding of the activity - structure relationship of phenolic compounds in some native spices.

The objectives of this study were to evaluate the antioxidant, antimicrobial activity and to determine the phenolic profile of the methanolic extracts of *A. melegueta* and *A. lepidophyllus*.

Materials and Methods

Plant material and bacterial strains

Collection of plant material and preparation of plant extracts

The seeds of *Aframomum melegueta* and *Afrostyrax lepidophyllus* were purchased in Bafoussam (West Cameroon) in June 2016 and were identified at National Herbarium of Yaounde (Cameroon). Voucher specimen of *Aframomum melegueta* (43129/HNC) and *Afrostyrax lepidophyllus* (44853/HNC) were deposited.

The seeds were dried at 45°C for 48 hrs, freed from their outer shell and reduced to a fine powder using a grinder machine (Preethi Spice MG203). 2.5 g of powder were macerated in methanol at a ratio of 1/10 (m/v) for 24 hrs at room temperature. The mixture was then centrifuged (5000 rpm, 10 mins, 4°C) and filtered through a Whatman No. 1 filter paper. The filtrate was introduced into a separatory funnel and partitioned with 25 mL of n-hexane. The methanolic fraction was subsequently concentrated using a rotavapor (HAHN VAPOR HS-2005V) set at 40°C and operating under reduced pressure. The residual methanol was removed under a stream of nitrogen followed by drying at 37°C for 60 mins using a ventilated oven. The samples were stored at 4°C and later diluted in the appropriate solvents just prior to analysis.

Bacterial strains and preparation of inoculum

The following bacterial strains: *Salmonella newport*; *Listeria monocytogenes, Salmonella* sp.; *Salmonella typhi*; *Yersinia* sp.; *Staphylococcus aureus* and *Listeria ivanovii* were used in this study. Activation of the bacterial strains was carried out by

inoculation of the Brain Heart Infusion (BHI) broth and then incubated at 37°C for 18 hrs, followed by subculturing on BHI agar. After incubation for 24 hrs, the colonies were transferred into sterile physiological water (0.9%) and then the turbidity was adjusted to 0.5 Mc Farland Standard. Finally, the bacterial suspension was diluted in Muller Hinton (MH) broth at a ratio of 1/99 (v/v) to get an approximate concentration of 1.5×10^6 colony forming units (CFU)/mL.

Phytochemical analysis

Determination of total phenolic content

The Folin-Ciocalteu method described by Sumczynski *et al.* (2015) was used with some changes. 25 µL of Folin-ciocalteu reagent (10% v/v) was added to a well of a microplate containing 20 µL of extract (0.1 mg/mL) and 200 µL of distilled water. Then, 25 µL of sodium carbonate (10% w/v) was added to the reaction mixture and incubated at room temperature for 40 mins under dark conditions. The absorbance was read at 760 nm. Gallic acid (0-0.5 mg/mL) was used to plot the calibration curve and the results were expressed in mg gallic acid equivalent / g dry extract (mg GAE/g dry extract).

Determination of total tannin content

The method described by Siddhuraju and Manian (2007) was used. In an Eppendorf tube containing 100 mg of polyvinyl polypyrrolidone, 500 µL of extract (0.1 mg/mL) and 200 µL of distilled water were introduced. The mixture was incubated at 4°C for 4 hrs and then centrifuged (5000 rpm, 5 mins, 4°C). Residual phenolic compounds were determined in 20 µL of the supernatant using the Folin-Ciocalteu method. The total tannin content was estimated in the extract using the formula below:

Total tannins (mg GAE/g)=Total phenol content (mg GAE/g)-Residual phenol (mg GAE/g)

Determination of total flavonoid content

The total flavonoid content was determined by the method described by Siddhuraju and Becker (2003). 50 µL of extract (1 mg/mL) was added to 75 µL of sodium nitrate (5% w/v). After 5 mins at room temperature, 75 µL of aluminium chloride (10% w/v) was added. After an additional 6 mins 1000 µL of 1M sodium hydroxide was added to the reaction mixture. The absorbance was read at 510 nm against the blank after 15 mins of incubation. Catechin (0-2.5 mg/mL) was used to plot the calibration curve and the results were expressed in mg catechin equivalent/g dry extract (mgCE/g dry extract).

Determination of antioxidant activity

DPPH assay

The method described by Brand-Williams *et al.* (1995) was used to assess the ability of the extracts to scavenge the free radical DPPH. A stock solution of DPPH was freshly prepared by dissolving 6mg of DPPH in 100 mL of methanol. 200 µL of this solution was added to 50 µL of variable concentration (2.44-909.09 µg/mL) of spice extract or standard Tert-Butylhydroquinone (TBHQ). 50 µL of methanol treated under similar conditions served as a control. The absorbance was read at 517 nm against the blank after 40 mins incubation at room temperature. The percentage inhibition of the DPPH radical was calculated using the following formula:

$$\%\text{DPPH inhibition} = (Ao - At)^{*}100/Ao$$

Where *Ao* is the absorbance of control, *At* represents the absorbance of the sample or standard. The results were expressed as IC_{50} determined by the exponential regression equation.

ABTS assay

The method described by Gülçin *et al.* (2010) was used to assess the ability of the extracts to scavenge the free radical ABTS. Some changes have been made. The ABTS stable stock solution was produced by adding 2640 µL of potassium persulfate solution (140 mM) in 150 mL of ABTS solution (7.47 mM). Then, the mixture was incubated at 25°C for 16 hrs in the dark. 405 mL of saline phosphate buffer (0.1M, pH 7.4, 150 mM NaCl) was added to 15mL of stock solution of stable ABTS radical to constitute the working solution whose absorbance was 0.9 ± 0.02 at 734 nm. 25 µL of a variable concentration of extract or TBHQ standard was mixed with 200 µL of ABTS radical generated. After 40 mins incubation at room temperature, the absorbance was read at 734 nm. 25 µl of methanol mixed with ABTS was used as a control. The percentage inhibition of the ABTS radical was calculated using the following formula:

$$\%\text{ABTS inhibition} = (Ao - At)^{*}100/Ao$$

Where *Ao* is the absorbance of control, *At* represents the absorbance of the sample or standard. The results were expressed as IC_{50} determined by the exponential regression equation.

FRAP assay

FRAP was determined using the method described by Benzie and Strain (1996). The working solution (FRAP reagent) was prepared by mixing 250 mL of acetate buffer (300 mM, pH 3.6), 25 ml of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) and 25 mL of anhydrous solution of $FeCl_3$ (20 mM). 240 µl of FRAP reagent was added to 10 µL of spice extract or ascorbic acid and incubated at 37°C for 30 mins. Then, the absorbance was measured at 593nm against the blank. The results were expressed in mg equivalent ascorbic acid / g dry extract (mg AAE/g dry extract).

PAP assay

The total antioxidant activity of the spice extracts was determined using the phosphomolybdenium method described by Prieto *et al.* (1999). 300 µL of extract or ascorbic acid was mixed with 3000 µL of molybdate reagent (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a centrifuge tube. The tubes were incubated at 95°C for 90 mins. After 20 mins, the absorbance was measured at 695 nm against blank. The results were expressed in mg AAE/g dry extract.

Antimicrobial activity

The method described by Mishra and Padhy (2013) was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), few changes were made. The dry extracts were diluted in 10% Dimethylsulfoxyde (DMSO) to have a concentration range of 312.5 - 5000 µg/mL. 200 µL of freshly constituted bacterial inoculum in the sterile MH broth was added to 50 µL of spice extract previously introduced into a well of a microplate. The negative control was consisted of 50 µL of 2.5% DMSO and of bacterial inoculum. The positive control was consisted of Gentamicin (0.49 - 250µg/ml). The phenol red salt (0.0018%) previously added to the MH broth supplemented with D-glucose (0.1%) was used to assess the quality of the result. After 18 hrs of incubation of the microplates at 37°C, the turning to yellowish of MH broth indicated bacterial growth, while no color change indicated the inhibition of bacterial growth, thereby allowing to define the minimum inhibitory concentration. Subcultures were made on BHI agar from bacterial inocula from wells where there was no growth and then incubated at 37°C for 24 hrs to determine the minimum bactericidal concentration.

Determination of the phenolic profile by HPLC

One gram of spice powder was macerated in 10 mL of HPLC grade methanol for 6 hrs and centrifuged (4°C., 5000rpm for 10 mins). The operation was repeated a second time on the supernatant which was then collected and diluted to ¼ in grade HPLC methanol, and filtered through a 0.45µm filter paper (PTFE). A Hitachi LaChrom Elite system (Hitachi High Technologies America, Inc.) was used for the identification and quantification of phenolic compounds. This system was equipped with the following components: a Shodex C18-120-5 4E column (250 mm; 4.6 mm; 5µ); an autosampler (L-2200), a Diode Array detector with variable wavelength (L-2455) and a pump (L-2130). The mobile phases consisted of the water / acetic acid mixture (phase A) and a methanol / acetic acid (phase B) mixture, each mixed separately in a ratio of 99/1, respectively. The elution program was as follows: 0-7 mins, 0-15% B; 7-30 mins, 15-50% B; 30-50 mins, 50-100% B; 50-60 mins, 100-0% B; 60-70 mins, 0% B. The HPLC parameters were as follows: elution rate 1.0 mL/min; Injection volume 20 µL; Column temperature 30°C; Wavelength 280 nm, 320nm and 360 nm. The retention time and spectral characteristics of the authentic phenolic standards were used for identification of phenolic compounds of the methanol extracts. Different concentrations of the mixture of phenolic standards were injected to plot the calibration curves necessary for the quantification of the phenolic compounds of the extracts studied.

Statistical analysis

All measurements were made in triplicate, which allowed to express the results as average ± standard deviation. One-way ANOVA (analysis of variance) was used for data analysis and significant differences between means were evaluated by Duncan Multiple Range test using STATGRAPHICS Centurion XVI Software version 16.1.18 (StatPoint Technologies, Inc.). SigmaPlot 12 version 12.5.0.38 (Systat Software, Inc.) was used for the analysis of exponential regression curves necessary for the determination of IC_{50}. The differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Total phenolic, flavonoid and tannin content

Table 1 shows the phytochemical composition of the methanolic extracts of *Aframomum melegueta* and *Afrostyrax lepidophyllus*. This table shows that the total phenolic compound content of *A. melegueta* extract is 3 times higher than that of *A. lepidophyllus*, i.e. 160.77mg GAE/g and 46.63 mg GAE/g, respectively. The total phenol content of *A.*

Table 1 Total phenolic content, total flavonoid content and total tannin content of methanolic plant extract.

Sample	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)	Total tannins (mg GAE/g)
A. melegueta	160.77±6.74[a]	70.62±0.90[a]	136.40±0.94[a]
A. lepidophyllus	46.63±0.93[b]	3.95±0.14[b]	8.72±1.60[b]

[a,b] Values in each column with different superscripts are significantly different ($p<0.05$).

Table 2 Scavenging and reducing capacities of methanolic plant extract expressed in mg AAE/g extract and IC_{50} (µg/ml).

	IC_{50} (µg/ml)		mg AAE/g extract	
Sample	DPPH	ABTS	PAP	FRAP
A. melegueta	391.43±3.72[b]	100.10±2.40[b]	133.24±1.38[a]	128.60±2.71[a]
A. lepidophyllus	831.70±7.43[a]	131±1.39[a]	93.84±0.84[b]	72.48 ± 0.27[b]
TBHQ	23.00±0.96[c]	26.67±0.68[c]	nd	nd

[a,b] Values in each column with different superscripts are significantly differents (p 0.05). nd: not determined

lepidophyllus extract found in this study is greater than that found by Moukette *et al.* (2015). They showed that the total phenolic compound content of the ethanolic extract of the roots and leaves of *A. lepidophyllus* was 35.33mg GAE/g and 34.11 mg GAE/g, respectively. This difference could be explained by the part of the plant subjected to extraction. In agreement with the literature, the difference of the solvents used for extraction could also be another reason. Indeed, it has been shown that the methanol allows extraction of a substantial amount of phenolic compounds compared to ethanol (Boulekbache-Makhlouf *et al.*, 2013; Medini *et al.*, 2014). Okugbo and Oriakhi (2015) showed that the total phenol content of the methanolic extract of *A. melegueta* leaves was 110 mg GAE/g. Similarly, the total flavonoid and tannin content was significantly ($p<0.05$) higher in the extract of *A. melegueta* (70.62 mg CE/g and 136.40 mg GAE/g, respectively) compared to the *A. lepidophyllus* extract (3.95 mg CE/g and 8.72 mg GAE/g, respectively). This high flavonoid content suggests the use of *A. melegueta* in the prevention of coronary diseases. However, although the *A. lepidophyllus* extract is low in flavonoid and tannin, it must contain other phenolic groups (probably phenolic acids), which are responsible for its relatively high total phenol content.

Antioxidant activity

Table 2 shows the scavenging activity of the DPPH and ABTS free radicals by the methanolic extract of *A. melegueta* and *A. lepidophyllus*. *A. melegueta* extract exerted a significant antiradical activity with IC_{50} values significantly ($p < 0.05$) lower than that of *A. lepidophyllus*. They are 306.56 µg/mL and 130.11 µg/mL respectively for the DPPH and ABTS tests. This is similar to the result previously reported by Adefegha and Oboh (2012) who showed that a concentration of 308.50 µg/mL of the methanolic extract of *A. melegueta* allowed to trap 50% of the free radical DPPH. For *A. lepidophyllus* extract, the IC_{50} values were 692.78 µg/mL and 211.24 µg/mL, respectively for the DPPH and ABTS tests. However, TBHQ used as a reference antioxidant showed better antiradical activity compared to plant extracts studied with low IC_{50} values (26.67 µg/mL and 23.00 µg/mL, respectively for ABTS and DPPH). This great antiradical power demonstrated by *A. melegueta* extract could be attributed to its high content of phenolic compounds. Phenolic compounds have a special structure enabling them to exercise certain functionalities. Indeed, they possess hydroxyl groups and some functional derivatives substituted on their aromatic ring, which allows them to neutralize free radicals which are promoters of the lipid peroxidation. Since DPPH and ABTS are very unstable free radicals, they are capable of reacting with a polyphenolic compound by accepting a proton or an electron of the latter, thus becoming a stable diamagnetic molecule (Mu *et al.*, 2004;

Table 3 Antimicrobial activity of plant extracts and gentamycin (expressed in µg/mL).

Sample		Bacteria strains						
		Salmonella sp.	*Listeria monocytogenes*	*Yersinia sp.*	*S. newport*	*S. typhi*	*L. ivanovii*	*S. aureus*
A. melegueta	MIC	-	-	-	2500	2500	-	5000
	MBC	-	-	-	-	-	-	5000
A. lepidophyllus	MIC	2500	312.5	2500	5000	5000	-	625
	MBC	-	-	-	-	-	-	-
Gentamycin	MIC	3.91	0.49	3.91	1.95	1.95	0.98	3.91
	MBC	125	1.95	125	125	250	250	250

The sign - indicates a value of CMI or CMB greater than 5000 µg/mL.

Moukette *et al.*, 2015). Flavonoids are considered one of the most important phenolic classes because they are abundantly found in the glycosides form in many plants and helps the human body to prevent or fight against diseases related to oxidative stress (Rajanandh and Kavitha, 2010).

The reducing capacity of the extracts was evaluated by the FRAP and PAP method, which make it possible to measure the reduction capacity of the Fe^{3+}-TPTZ complex in Fe^{2+}-TPTZ and Molybdenum (VI) in Molybdenum (V) respectively. It can also be observed in table 2 that the FRAP value of the methanolic extract of *A. lepidophyllus* (72.48 mg AAE/g) is significantly low ($p < 0.05$) compared to that of *A. melegueta* (128.60 mg AAE/g). Similar observations were made for the PAP test with PAP values of 93.84mg AAE/g and 133.24 mg AAE/g respectively for *A. lepidophyllus* and *A. melegueta* extracts. Like the anti-radical activity, the reduction power of plant extracts could be explained by the presence of phenolic compounds which may have as alternative antioxidant properties, to combine the metals of transitions involved in the formation of free radicals (Dai and Mumper, 2010), to act by electron donation to reduce the Fe^{3+}-TPTZ complex to its ferrous form Fe^{2+}-TPTZ (Agbor *et al.*, 2005) or by the formation of an inactive complex with Fe^{2+} (Bors and Michel, 2002). These results confirm the important role that *A. melegueta* can play in preventing the formation of free radicals by acting as a secondary antioxidant.

Antimicrobial activity

Table 3 presents the results of the antibacterial activity of methanolic extracts of *Aframomum melegueta* and *Afrostyrax lepidophyllus* evaluated *in vitro* against food borne pathogenic bacteria. *A. melegueta* and *A. lepidophyllus* extracts exhibits antibacterial activity against Gram positive bacteria and Gram negative bacteria. But this activity varies according to the bacteria strains. *Listeria ivanovii* showed no *in vitro* sensitivity under the effect of the various extracts. The antibacterial activity of *A. lepidophyllus* extract was comparatively higher than that of *A. melegueta*. Thus, *A. lepidophyllus* extract exerted strong activity against *Staphylococcus aureus, Listeria monocytogenes, Yersinia* sp. and *Salmonella* sp. with MIC values of 625 µg/mL, 312.5 µg/mL, 2500 µg/mL and 2500 µg/mL, respectively. The extract of *A. melegueta* showed a MIC value of 5000 µg/mL against *S. aureus* and was found to be more active against *Salmonella newport* and *Salmonella typhi* with a MIC value of 2500 µg/mL against both strains. However, unlike the *A. lepidophyllus* extract, no inhibitory activity was observed against *Salmonella* sp., *Listeria monocytogenes* and *yersinia* sp. No bactericidal activity was observed for *A. lepidophyllus* extract. These results are consistent with the work of Tekwu *et al*. (2012) who demonstrated that the methanolic extract of *A. lepidophyllus* seeds completely inhibited the growth of two strains of *Mycobacterium tuberculosis* (H37Rv and H37Ra) at 512 µg/mL but showed no bactericidal activity up to 2048 µg/mL. *A. melegueta* extract exerted bactericidal activity only against *S. aureus.* This activity could be attributed to its richness of bioactive phytochemical compound such as flavonoids, phenolic acids, tannins, saponins, terpernoids, cardiac glycosides and alkaloids (Odetunde *et al.,* 2015). The spectrum of antimicrobial activity of *A. lepidophyllus* extract compared to that of *A. melegueta* confirms that the antimicrobial efficacy of an extract is not always proportional to its phenolic compound content but may also depend on the nature of active phenolic compounds. Another possible cause could also be the synergistic interaction that may exist between

Table 4 Phenolic composition of methanolic extract (µg/g DW)

Groups	(Elution order) Phenolic standard	*A. melegueta*	*A. lepidophyllus*
Phenolics acids	(1) Gallic acid	61.85±5.03^{b}	340.38±7.07^{a}
	(2) Protocatechuic acid	nd	236.27±5.31
	(4) *p*- hydroxybenzoic acid	nd	nd
	(5) Gentisic acid	nd	nd
	(6) Chlorogenic acid	nd	nd
	(8) Vanillic acid	22.47±0.79^{b}	39.68±2.64^{a}
	(9) Caffeic acid	nd	nd
	(10) Syringic acid	4.34±0.61^{b}	23.93±1.94^{a}
	(11) *p*-coumaric acid	nd	28.85±4.51
	(12) Ferrulic acid	nd	68.39±3.51
	(13) Sinapic acid	nd	nd
	(16) Salicylic acid	28.72±2.52	nd
	(17) *t*-cinnamic acid	54.81±4.04^{a}	51.57±2.52^{a}
Flavonoids	(3) Catechin	nd	nd
	(7) Epicatechin	4.50±0.44^{a}	1.53±0.32^{b}
	(14) Rutin	6.34±0.94	nd
	(18) Quercetin	14.72±1.78	nd
Stilben	(15) Resveratrol	nd	nd

^{a,b} Values in each line with different superscripts are significantly different (p 0.05); nd: not detected.

the bioactive phytochemicals present in the extract, and which would give rise to a higher antimicrobial activity.

Identification of phenolic compounds by HPLC

Analysis of the phenolic profile of methanolic extracts was performed by HPLC coupled to a diode array detector reader operating at three different wavelengths (280, 320 and 360 nm). The spectral characteristics and the retention times of the peaks obtained were compared with those of the authentic phenolic standards. The HPLC chromatograms are shown in Figure 1 for phenolics standard and in Figure 2 (a, b; c; d) for samples. The figure 2 shows that a large number of phenolic compounds present in both extracts absorb at 280 nm, which corresponds to the flavanols, isoflavones, benzoic acids and their derivatives (Vidović *et al*., 2015). The low flavonoid content of *Afrostyrax lepidophyllus* extract (Table 1) suggests that it consists essentially of benzoic acid derivatives, which could, in addition to its low phenolic content, explain its low antioxidant potency compared to *Aframomum melegueta* extract. The cinnamic acid derivatives possess a high antioxidant activity associated with the presence of the propenoic chain grafted onto the benzene ring compared to the benzoic acid derivatives with their

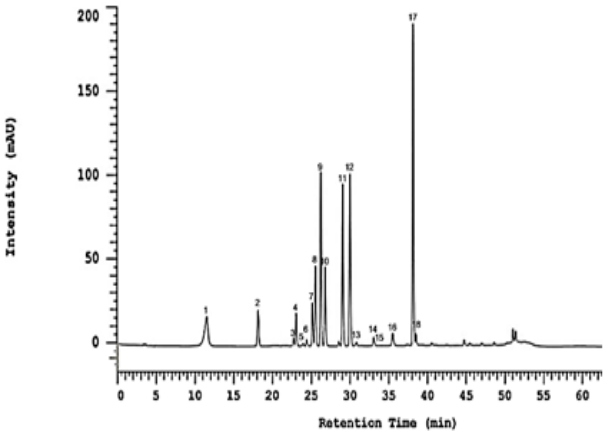

Figure 1. HPLC Chromatogram profile of phenolics standard at 280 nm: (1) gallic acid; (2) Protocatechuic acid; (3) Catechin; (4) p-hydroxybenzoic acid; (5) Gentisic acid; (6) Chlorogenic acid; (7) Epicatechin; (8) vanillic acid; (9) caffeic acid; (10) syringic acid; (11) p-coumaric acid; (12) ferrulic acid; (13) sinapic acid; (14) rutin; (15) resveratrol; (16) salicylic acid; (17) t-cinnamic acid; (18) quercetin.

carboxyl group. The phenoxide radical generated is in the case of cinnamic acid derivatives, stabilized by resonance due to the presence of the double bond of the propenoic chain conjugated to that of the benzene ring (Natella *et al.*, 1999). However, several derivatives of benzoic acid are known to have a strong antimicrobial activity and a broad spectrum of action, in particular against the Gram positive and the Gram negative bacteria (Alves *et al.,* 2013). The results of the quantitative analysis of the identified phenolic compounds are shown in Table 4. Among the desired compounds, the phenolic acids detected and quantified in *A. melegueta* extract were gallic acid, vanillic acid, syringic acid, salicylic acid and t-cinnamic acid. In *A. lepidophyllus* extract, gallic acid, protocatechuic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid and t-cinnamic acid were quantified. The dominant phenolic acid in the two extracts was gallic acid, which was significantly ($p < 0.05$) higher in *A. lepidophyllus* extract compared to *A. melegueta* extract (340.38 µg/g DW and 61.85 µg/g DW, respectively). Similarly, vanillic acid was significantly ($p < 0.05$) higher in *A. lepidophyllus* extract (39.68 µg/g DW) compared to the *A. melegueta* extract (22.47 µg/g DW). The protocatechuic acid identified only in the extract of *A. lepidophyllus* was also present at high concentration (236.27 µg/g DW), followed by ferulic acid (68.39 µg/g DW), t-cinnamic acid (51.57 µg/g DW), p-coumaric acid (28.85 µg/g DW) and finally syringic acid (23.93 µg/g DW). No significant difference ($p > 0.05$) was observed for the t-cinnamic acid content present in both methanolic extracts. Quercetin (14.72 µg/g DW) follow by rutin (6.34 µg/g DW)

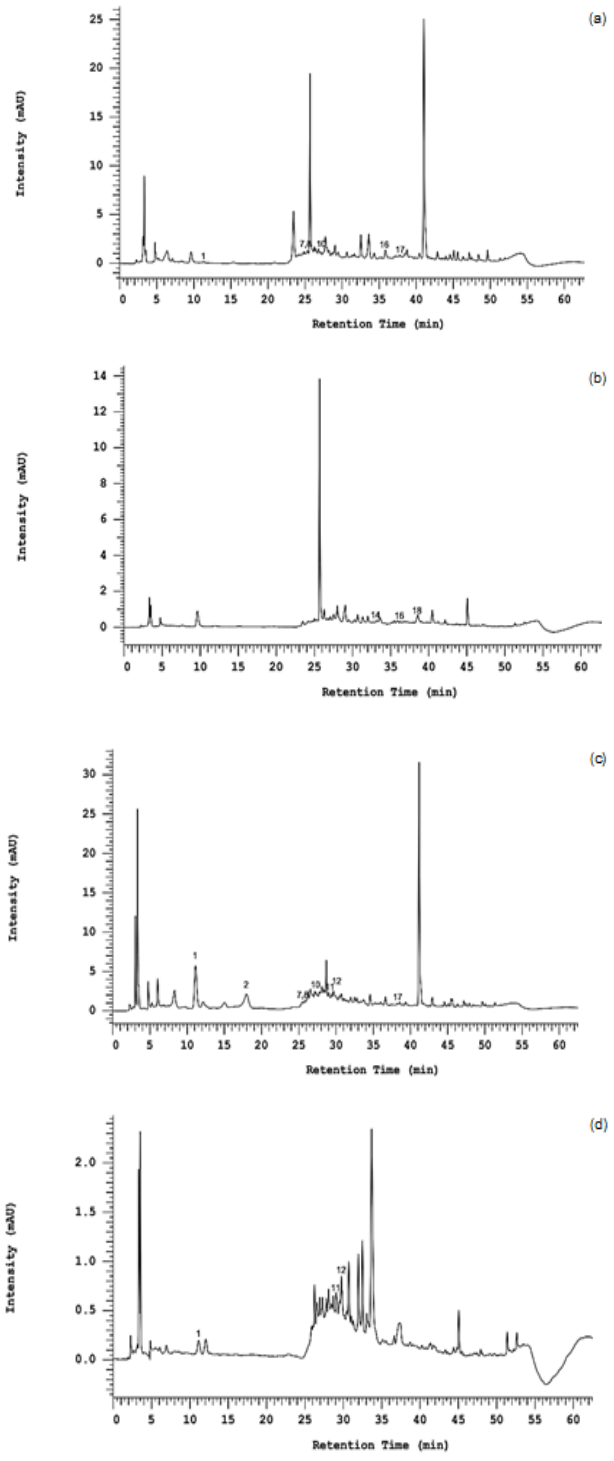

Figure 2. HPLC Chromatogram profile of methanolic extract of *A. melegueta* at (a) 280 nm, (b) 320 nm; and *A. lepidophyllus at* (c) 280 nm, (d) 320 nm.

were the most abundant flavonoids found only in *A. melegueta.* Epicatechin was detected in low amount in both extracts. Although the peaks representing a high percentage of the phenolic composition of the methanolic extract of *A. melegueta* (retention time: 23.45, 25.67 and 41.03) and *A. lepidophyllus* (retention time: 3.35 and 41.16) were not identified, they may also belong to the groups of flavanols, isoflavones, benzoic acids and derivatives because they exhibit a maximum absorption at 280 nm.

Adefegha and Oboh (2012) analyzed the phenolic composition of the methanolic extract of the seeds of two Aframomum species from Nigeria by RP-HPLC and found that in *A. danielli* extract chlorogenic acid was the most abundant phenolic acid, followed by p-hydroxybenzoic acid, caffeic acid, and p-coumaric acid, while quercetin and kaempferol were the most abundant in *A. melegueta* extract. Gallic acid, vanillin and hesperidin were also detected in the methanolic extract of *A. melegueta.* In accordance with the literature, extracts of the same plant species may have different phenolic profiles and the factors that may influence the chemical composition of an extract being plant age, harvest period, geographic origin, microbial contamination, climate, harvesting and storage conditions (Naghdi *et al.*, 2004). To the best of our knowledge, this is the first report on the phenolic profile of the methanolic extract of *A. lepidophyllus* seeds and *A. melegueta* from Cameroon.

Conclusion

In this study we showed that the methanolic extract of *Aframomum melegueta* had a high content of phenolic compounds, flavonoids and tannins whereas *Afrostyrax lepidophyllus* extract was mainly composed of phenolic acids with a low flavonoids and tannins content. The antioxidant activity evaluated in vitro by the DPPH, ABTS, FRAP and PAP tests was in all cases stronger with *A. melegueta* extract compared to that of *A. lepidophyllus* suggesting that *A. melegueta* may contain functional substances that can prevent or combat diseases associated with oxidative stress. *A. lepidophyllus* extract exhibited a broad spectrum of activity against Gram positive and Gram negative bacteria but showed no bactericidal activity. *A. melegueta* extract had a bactericidal effect against *Staphylococcus aureus.* The HPLC profiles obtained in this study revealed the presence of some phenolic compounds that might explain the biological activity of studied extracts. The results of this study suggest that *A. melegueta* could play an important role in the prevention of diseases such as cancer, Alzheimer's, cardiac and vascular accidents. In addition, it could be used in combination with *A. lepidophyllus* as an effective natural antimicrobial agent to control the growth of certain food borne pathogenic germs.

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Conflict of interest

The authors have no competing interest.

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