ORIGINAL RESEARCH PAPER

IN VITRO ANTIOXIDANT POTENTIAL AND ANTIMICROBIAL ACTIVITY OF SOME CAMEROONIAN PLANT EXTRACTS

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Abstract

A study on Cameroonian edible and medicinal plants was undertaken to assess their potential as food preservatives and natural food colorants. The ultrasound-assisted technique was used for the extraction of bioactive compounds. A total of 16 extracts prepared from 7 plants (*Aframomum melegueta* S., *Ageratum conyzoides* L., *Cympobogon citratus* Stapf, *Garcinia kola* Heckel, *Lantana camara* L., *Nauclea diderrichii*, *Vernonia amygdalina)* were assayed to detect phytochemicals and assess antioxidant potential using the scavenging DPPH radical method and antimicrobial activity (10 mg/mL) against ten microorganisms by the agar well diffusion method. The ethanolic extract from *L. camara* leaves revealed the highest polyphenols and flavonoids contents. In contrast, the hexane/acetone extract from *A. conyzoides* and *L. camara* leaves had the highest chlorophyll a (Chl a) and chlorophyll b (Chl b) contents, whereas the highest carotenoid content was found in *L. camara* leaves. The ethanol extracts exhibited more significant antioxidant activity than hexane/acetone extracts. All seven plants (100%) inhibited the growth of at least one tested foodborne and spoilage bacteria and fungi. The germination of both *Bacillus* spp. spores were also affected. The hexane/acetone extracts were by far the best antimicrobial agent, and extract from *L. camara* leaves was one of the most effective, with a large antimicrobial spectrum. The antimicrobial activity of extracts was correlated to the amount of carotenoids and chlorophylls. HPLC-DAD revealed bioactive compounds as chlorogenic and caffeic acids found as major phenols in ethanolic extract of *L. camara* leaves, whereas lutein, carotene, and zeaxanthin were the specific carotenoids present in hexane/acetone extract of the same plant.

Keywords: Cameroonian plants extracts, phytochemicals, antioxidants, antimicrobial activity

Introduction

Food poisoning and food safety resulting from microbial deterioration remain a concern for consumers and the food industry. According to the World Health Organization, foodborne hazards are annually responsible for 137,000 deaths and 91 million acute illnesses in Africa, more than 5000 deaths and 76 million foodborne illnesses in the USA, and 100,000 cases of food poisoning in England and Wales (Cock and van Vuuren, 2015). To counteract the perishable nature of food products due to bacterial and fungal growth and to improve food color and effectively prevent food poisoning, many banned synthetic chemicals are still used as food preservatives and food colorants in some countries (Amchova *et al.,* 2015). In recent years, foods preserved with natural additives have become very popular because of the excellent consumer awareness and concern regarding synthetic chemical additives (Lucera *et al.,* 2012). Consumers perceive natural colorants as safer than synthetic ones, which are harmful because of their possible links to hyperactivity in children and allergenicity in sensitive populations (Amchova *et al.,* 2015). Apart from their potent ability to color foods, natural colorants provide health benefits, organoleptic features and also act as antioxidants and even preservatives as food ingredients (Rodriguez-Amaya, 2016). Indeed, natural crude extracts from plant species used in traditional medicine may represent valuable sources for such new natural preservatives and colorants considered as safe, effective, and acceptable (Meot-Duros *et al.,* 2008). It is estimated that there are 250,000 plant species on the earth, and only one-tenth of these have been explored until date (Negi, 2012). There are approximately 60,000 plant species in Africa, and up to 90% of the population of many African countries still rely on plants as a source of medicines. However, Africa has only contributed to 83 of the world's 1100 leading commercial medicinal plants. Plant oils and extracts have been used for thousands of years; they serve many purposes, such as food preservatives and therapeutic agents (Hintz *et al.,* 2015). Given that the use of plant extracts with known biological properties can be of great significance in food preservation and dyeing, the search for natural substances from plants with antimicrobial activities, antioxidant potentials, and dyeing property constitutes a great interest of research and in industrial innovations. In this context, extracts from seven indigenous Cameroonian plants used as vegetables, spices, and medicines were investigated.

Aframomum melegueta K. Schum. is also known as African jujube or guinea pepper (Onoja *et al.,* 2014). In Cameroon, where its indigenous names include "ndôn" in the Bassa dialect, this plant is consumed as an edible spice and used in the folk headache treatment. Previous studies showed that different plant parts act as immune enhancers and have healing potential (Olajuyigbe *et al.,* 2020). *Ageratum conyzoides* L. is an annual herb with a long history of traditional medicinal uses in many tropical countries worldwide. The concentrate or infusion of the plant is used in folk medicine to treat various ailments such as malaria, dysentery, diarrhea (Ladoh-Yemeda *et al.,* 2016). *Cymbopogon citratus* Stapf, commonly known as lemongrass, is an aromatic plant used in herbal medicine for a wide range of applications (Prakash *et al.,* 2016). In folk medicine, this plant treats

cough, gastrointestinal disorders, malaria (Ladoh-Yemeda *et al.,* 2016). The decoction of *C. citratus* leaves is widely consumed alone as a relaxing herbal tea and flavoring agent. Studies on its phytochemical content revealed the presence of various essential oils in the plant (Ascension *et al.,* 2017). *Garcinia kola* Heckel also called bitter kola, is mainly found in the rainforest region of Central and West Africa (Esiegwu *et al.,* 2014). This plant is a medium-sized tree, which produces large fruits, containing 3-4 seeds coated brown. *G. kola* is used both as food and medicine (Ladoh-Yemeda *et al.,* 2016). Ethnobotanical studies showed that this plant is used to fight against gastroenteritis and some infectious diseases (Ladoh-Yemeda *et al.,* 2016). *Lantana camara* L. is a flowering ornamental plant, mainly found in the American and African continents (Ladoh-Yemeda *et al.,* 2016). The leaf extracts are widely used in folk medicine to treat malaria, stomach ache, fever, and cancers (Ladoh-Yemeda *et al.,* 2016). *Nauclea diderrichii* (De Wild.) Merr is an evergreen savannah tree widely used in traditional folklore medicine to treat indigestion, diabetes, and stomachache (Agnaniet *et al.,* 2016). *Vernonia amygdalina* known as the bitter leaf is one of the most studied plants in Africa. This plant is consumed as a vegetable and used in folk medicine in many African countries. Researchers have recorded that this plant is used to control gastrointestinal parasites, treat malaria, boast immunity of HIV patients and stomach pains (Ladoh-Yemeda *et al.,* 2016).

In Cameroon, many studies on the biological properties of indigenous plants deal essentially with the plant oils (Ascension *et al.,* 2017; Voundi *et al.,* 2015), but enough has to be done to explore the urge potential of the plant extracts. Furthermore, no information has been reported on crude extracts as a source of natural food additives. In this study, we have highlighted for the first time a collaborative study on the phytochemical, antioxidant, and antimicrobial properties of the extracts of seven plants to assess their biological activities and to confirm their potential use as food preservatives and natural colorants. Therefore, the purpose of this study was (i) to investigate the phytochemical profile and antioxidant activity of the plant extracts obtained from two solvent systems, (2i) to evaluate their antimicrobial property (3i) to characterize the phytochemical content of the most active extract.

Materials and methods

Collection of plant material and extraction

Native Cameroonian plants used in this work were either used as food or identified as plants with ethnobotanical interest to treat food poisoning associated with diarrhea and dysentery. The plants chosen were harvested or purchased in April 2018 in Maroua (Far North, Cameroon), Yaoundé (Centre, Cameroon), and Douala (Littoral, Cameroon). The plants' identity was authenticated by the botanical experts of the national herbarium, Cameroon (Table 1). Plants collected were washed and dried shade in an oven at 45°C for a day. The dried material was milled into powder using an electrical blender (MLynek Laboratory JNY Tip WZ/2, Poland) and kept in sealed containers for further use. The extracts were

prepared using ultrasound-assisted extraction protocol as described by Dadi *et al.* (2019). One gram of each plant powder was weighed in four Eppendorf tubes. Ten milliliters of ethanol 70% or hexane/acetone (3:1, v/v) were added. The samples were left at 35°C for 30 min in an ultrasonic bath (MRC AC-150H series, UK) designed with a fixed frequency of 35 kHz and power intensity of 150 W. The samples were centrifuged three times at 5000 rpm at 4°C for 30 minutes. The resulting supernatants were collected, centrifuged once at 9000 rpm for 10 min at 4°C, and concentrated in an air-dried vacuum (Rotavapor R-124, Buchi, Switzerland). Ethanolic and hexane/acetone dried extracts of each plant were stored at 4°C for further analysis.

Table 1. Common names and local usage of the plants used.

Plant names	Common/local names	Family	Part used
Aframomum melegueta S.	-/ndon	*Zingiberaceae*	Seeds
Ageratum conyzoides L.	-/ Kaïtoro	*Asteraceae*	Entire plant
Cympobogon citratus Stapf	Citronella	*Poaceae*	Leaves
Garcinia kola Heckel	Bitter kola	*Clusiaceae*	fruits
Lantana camara L.	Lantana	*Verbenaceae*	Leaves, rods
Nauclea diderrichii	Bilinga	*Rubiaceae*	Stem bark
Vernonia amygdalina	Bitter leaf/Ndole	*Asteraceae*	Leaves

Chemicals

Folin-Ciocalteu reagent, 2,2- diphenyl- 1- picrylhydrazyl, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, gallic, caffeic, chlorogenic and hydrochloric acids, catechin, lutein, zeaxanthin, ß-carotene, sodium carbonate, sodium hydroxide, sodium acetate, sodium nitrite, potassium chloride, aluminum chloride, ethanol, methanol, acetone, n-hexane, Tryptic Soy Broth, Nutrient Agar, Muller Hinton Agar, Sabouraud Agar were obtained from Sigma (Steinheim, Germany) and Merck (Darmstadt, Germany).

Determination of pigment content: chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids

The pigment content of hexane/acetone plant extracts was determined spectrophotometrically according to the modified protocol used by Elbatanony *et al.* (2019). Three milliliters of each plant extracts prepared at 1 mg/mL were put into a reading tube, and the absorbance values were recorded at λ = 450 nm, 645 nm, and 663 nm. The content of Chl a, Chl b, and carotenoids were calculated using the formula (1), (2), and (3), respectively:

Chlorophyll a (mg/g) = $(11.75 \times Abs_{663}) - (2.35 \times Abs_{645})$ (1)

Chlorophyll b (mg/g) = $(18.61 \times Abs_{645}) - (3.96 \times Abs_{663})$ (2)

Carotenoids (mg/g) = (Abs$_{450}$ × Vs x D$_f$) / (m × l × ξ)   (3)

where Abs$_{450}$, Abs$_{645}$, Abs$_{663}$ are O.D. values at 450 nm, 645 nm, 663 nm, Vs is volume of solution; D$_f$ is dilution factor; l= 1; m=9.35; ξ = 2590

Determination of total phenolic content

Total phenolic contents were analyzed using the Folin-Ciocâlteu method as described by Bolea and Vizireanu (2017). Briefly, 100 µL of extract (1mg/mL) was mixed with 7.9 mL of deionized water and 0.5 mL of Folin-Ciocalteu reagent (0.25 mol/L). After 10 minutes, 1.5 mL of 20% Na$_2$CO$_3$ solution was added, and the mixture was kept in the dark for one h. Finally, the absorbance was measured against a blank at λ = 765 nm. Gallic acid was used as a standard (50-250 mg/L), and the total phenolic content was expressed as gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Determination of flavonoids content

Flavonoids contents were assessed using a modified aluminum chloride colorimetric method as described by Pękal and Pyrzynska (2014). 250 µl of the plant extracts (1mg/mL) were diluted with 1.25 mL of deionized water, and 0.075 mL of 5 % NaNO$_2$ solution was added. After 5 minutes in the dark, 0.15 mL of 10% AlCl$_3$ solution was introduced. After 6 minutes, 0.5 mL of 1M NaOH and 0.775 mL of deionized water were added into the reaction mixture, and absorbance was read at λ = 410 nm using a UV-Vis spectrophotometer (Libra S22, Biochrom, UK). Catechin was used as standard (20-100 mg/L), and the flavonoids content was expressed as catechin equivalent per gram of dry weight (mg CE/g DW).

Determination of the antioxidant activity of the plant extracts

The DPPH assay was determined following the colorimetric method described by Shahinuzzaman *et al.* (2020) with slight modifications. A volume of 0.1 mL of extracts (1 mg/mL) was mixed with 3.9 mL of diluted (1:10) DPPH/methanol solution. Then, the preparation was kept for 90 min at room temperature in the dark. The decrease in the absorbance of the mixture was spectrophotometrically read at λ = 517 nm. Trolox was used as standard (0.125 mg/mL), and the radical scavenging activity was expressed as the percentage of inhibition based on equation (4):

DPPH $_{scavenging\ activity}$ (%) = [(Abs $_{Control}$ − Abs $_{Sample\ or\ Trolox}$)/Abs $_{Control}$] x 100 (4)

where Abs$_{Control}$ is the OD value of the DPPH solution only, Abs$_{Sample\ or\ Trolox}$ is the OD value of the DPPH solution mixed either with extracts or Trolox solution.

Antimicrobial activity of the plant extracts

Test microorganisms

The following strains were used as test organisms: *Bacillus subtilis* MIUG B1; *Bacillus cereus* MIUG B2; *Lactobacillus plantarum* MIUG BL3; *Listeria monocytogenes* EGD-e and *Staphylococcus aureus* ATCC 25923; *Salmonella agona* MIUG BP1; *Saccharomyces* spp. MIUG D8; *Penicillium expansum* MIUG M11; *Aspergillus niger* MIUG M64; *Kluyveromyces* spp. MIUG M26. The strains

were obtained from the collection of Integrated Center for Research, Expertise and Technological Transfer in Food Industry BioalimentTehnIA, "Dunarea de Jos" University of Galati, Romania.

Antimicrobial screening

Screening of extracts was determined using a modified agar well-diffusion technique as described by Zhang *et al.* (2020). Before use, bacteria were cultivated on Muller Hinton Broth at 35°C until was reached 0.5 McFarland (~10^8 CFU/mL) (Kirby-Lee, 2019). The spores of *Bacillus* strains were produced on sporulation agar as described by Bayoï *et al.* (2014) and heat-activated (80°C for 10 min), the final spore concentration was adjusted at 10^8 spores/ mL. The molds spores were collected from 7 days-old cultures in 10 mL of sterile distilled water, and the final concentration was adjusted to 10^7 spores/ mL. The yeasts were subculture onto Sabouraud dextrose agar at 35°C ($\pm$2°C) for 24h and the yeast inoculum was prepared by picking five distinct colonies and suspended in 5 mL of sterile saline water. The resulting suspension is vortexed for 15 seconds, and it is adjusted to obtain a yeast stock suspension of 10^7 cells per mL (Espinel-Ingroff, 2007). For the agar well-diffusion technique, one hundred microliters of each microbial suspension were spread on nutrient plates with formed wells of 9 mm diameter, and after that, each well was filled with 100 μL of the different extracts prepared at 10 mg/L concentration. Tween 80 (0.2%) and sterile water were used as negative controls and to dissolve hexane/acetone and ethanolic extracts. Gentamycin and Nystatin (1 mg/mL) were used as a positive control for bacteria and fungi species, respectively. After a pre-diffusion of 20 minutes at room temperature, the plates were incubated at 37°C for 24 h for bacteria and 25°C for 72 hours for fungi. The antimicrobial activity was assessed by measuring the inhibition zone (IZ) diameter (mm) around the well using a caliper. This screening test was carried out to select the most active extracts against the test microorganisms.

Microbial susceptibility test

This test was used to determine the minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and the minimum bactericidal concentration (MBC) of extracts using the protocol of Andrews (2001) with minor modifications. Firstly, each extract was serially diluted to obtain concentrations ranging from 10 mg/L to 0.156 mg/L. Secondly, 1 mL of extract and dilutions was mixed with 1 mL of the overnight culture of Muller Hinton Broth, corresponding to 0.5 McFarland standard. After incubation at 35$\pm$2°C for 18-20h, the tubes were observed for growth, and MIC value was recorded as the lowest concentration where visible growth was not observed. The minimum concentration for the inhibition of spore germination (MICg) was determined as described by Voundi *et al.* (2015). Briefly, the spore suspensions (10^6 spores/mL) were inoculated in 2 mL of nutrient broth at a low concentration of extracts to avoid its effect on vegetative growth. Tubes were incubated for 24 h at the optimal growth temperature. The MBCs were determined by inoculation of 2-3 μL of the culture corresponding to the minimum inhibitory concentration and spread on a nutrient plate and incubated at the optimal growth temperature for 24 hours. The minimum fungicidal

concentration (MFC) was obtained by spreading 10 ml of each serial dilution on Sabouraud dextrose agar. Plates were incubated at 28°C for 72 h. The MFC was defined as the lowest extract concentration that yielded three or fewer colonies (Silveira et al., 2009). The extract was microbiocidal when MB(F)C:MIC ratio was between 1 and 2, and microbiostatic when the ratio was superior to 2 (Breda *et al.,* 2016).

HPLC analysis of individual phenolic and pigments

The analyses of phenolic compounds of ethanolic extract and pigments of hexane/acetone extracts were performed using HPLC-DAD (Shimadzu Co, Japan), equipped with an autosampler model SIL-20A, a Shimadzu pump (LC-20AT), diode array detector model SPD-M20A. The separation was carried out in the C18 column (150 mm × 4 mm, 5 μm). The elution solvents were 6:3 methanol/sodium acetate (A) and 2.3% methanol (B). Total run time was 30 min for pigments analysis with the gradient of 15% (B) for 3 min changing to 50% (B) in 7 min, and reaching at 100% (B) in 20 min. Concerning the analysis of the phenolic compounds, the entire run was 60 min with the elution programmed as follow: 10% (B) for 2 min; 30% (B) until 10 min; regulated rise to 80% (B) at a rate of 10%/8 min for 40 min and finally 100% (B) at a rate of 10%/5 min for 10 min. The flow rate was 1 mL/min, and the volume injected was 20 μL. Identification of compounds was performed based on their retention time with those of referenced standards. When standards were not available, identification was based on DAD spectra and data reported in the literature.

Data analysis

The data were mean of triplicate analysis ± standard deviation. The parameters evaluated were subjected to one-way ANOVA to determine the mean differences among the extracts. When significant differences in ANOVA ($p < 0.05$) were detected, the HSD Tukey's multiple range test was applied at $p < 0.05$. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were performed to analyze the correlation between the quantitative variables evaluated from extracts, and the relationship between the plant extracts, using SPSS Statistical program (SPSS version 20, IBM Inc., Armonk, New York, USA).

Results and discussion

Phytochemical characterization of the extracts

Table 2 presents Chl a, Chl b, carotenoids, phenols, and flavonoid contents of ethanolic and hexane/acetone extracts. Phenols and flavonoids contents in ethanolic extracts were higher than those in hexane/acetone extracts. The extraction of phenols and flavonoids was significantly ($p < 0.05$) higher in ethanolic extracts for the *L. camara* leaves (110±0.5 mg GAE/g and 158±1.6 mg CE/g, respectively). The hexane/acetone extraction revealed a higher content of phenols in the *A. melegueta* seeds and for the flavonoids in the *L. camara* leaves (50±0.1 mg CE/g).

Table 2. Contents of Chl a, Chl b, carotenoids, phenols, flavonoids of ethanolic, and hexane/acetone extracts.

Plants	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Carotenoids (mg/g)	Phenols (mg GAE/g)	Flavonoids (mg CE/g)
			Ethanolic extracts		
A. melegueta	12±0.08[a]	22±4.3[a]	23.2±0.2[a]	91±6.3[a]	107±3.6[a]
A. conyzoides	229±1.1[b]	14±2.5[a]	19±0.1[b]	6.9 ± 0.9[b]	7.2±0.7[b]
C. citratus	104±0.7[c]	125±8.9[b]	15±0.8[c]	9.1±0.3[c]	10.7±0.9[c]
G. kola	16±0.1[d]	37±3.2[c]	6.8±0.3[d]	15 ± 0.3[d]	9 ± 1.1[c]
L. camara (leaves)	219±1.1[e]	65±5.4[d]	12.5±0.7[e]	110 ±0.5[e]	158±1.6[d]
L. camara (rods)	68±0.4[f]	46±4.2[e]	5.2±0.4[f]	31.7±2.2[f]	23.8±2.8[e]
N. diderrichii	6±0.05[g]	2±0.1[f]	9±0.5[g]	32±2.4[f]	34.6±3.5[f]
V. amygdalina	148±0.8[h]	214±12.9[g]	8.4±0.7[h]	4.8±0.2[g]	6±0.4[g]
			Hexane/Acetone (1:3, v/v) extracts		
A. melegueta	34±0.34[a]	23.5±0.4[a]	116±0.6[a]	30±0.4[a]	40±0.5[a]
A. conyzoides	656.5±6.5[b]	479.5±4.8[b]	95±0.5[b]	2±0.8[b]	2±0.3[b]
C. citratus	280.6±2.8[c]	309±3.1[c]	77±0.4[c]	5±0.7[c]	6±0.2[c]
G. kola Heckel	63±0.63[d]	99±0.9[d]	7.6±0.04[d]	9.7±1.1[d]	4.2±0.3[d]
L. camara (leaves)	639±6.4[e]	328.5±3.3[e]	156±0.3[e]	20±0.1[e]	50±0.1[e]
L. camara (rods)	89.5±1.4[f]	67±0.8[f]	6±1.7[d]	5±0.3[b]	3±0.5[b]
N. diderrichii	24±0.4[g]	19±0.3[g]	36±0.3[f]	8±1.4[d]	7±0.8[c]
V. amygdalina	346.8±3.5[h]	478±4.8[h]	54±0.3[g]	1±0.2[b]	2±0.1[b]

Mean values (n = 3 repetitions) preceded by at least one letter common in the same column are not significantly different ($p < 0.05$) for the same type of extract.

Chl a, Chl b, and carotenoids were better extracted in hexane/acetone extracts than in ethanolic extracts. The Chl a contents were significantly important in hexane/acetone extracts of *A. conyzoides* (656.5±6.5 mg/g) and *L. camara* leaves (639±6.4 mg/g). The highest contents of Chl b were found in hexane/acetone extracts of *A. conyzoides* (479.5±4.8 mg/g) and *V. amygdalina leaves* (478±4.8).

The differences between the major chlorophyll types (Chl b becoming higher than Chl a) can be explained by several stressed conditions induced to plants (Idhan et al., 2018). Moreover, a Chl a and Chl b decreased ratio indicates that photosystem II (PSII) reaction centers can be more influenced than light-harvesting complexes (LHC), Chl b being only in LHC, whereas Ca are part of PSII (Contreras et al., 2013). The highest contents of carotenoids were found in hexane/acetone extracts of *L. camara* leaves (156±0.3 mg/g), almost ten times higher than in the ethanolic extracts.

The antioxidant potential of extracts is shown in Figure 1. The results reveal that all the extracts tested exhibited an antioxidant activity with significant differences (p<0.05) between ethanolic and hexane/acetone extracts. The scavenging activity of ethanolic extracts was significantly higher (p<0.05) than hexane/acetone extracts. The ethanolic extracts from *A. melegueta* seeds (83.81%), *L. camara* leaves (84.4%), and *G. kola* fruits (63.6%) had the highest scavenging activity values. Furthermore, the scavenging activities of the same extracts were significantly higher (p<0.05) than the activity of Trolox (53.75%) used as standard. Among hexane/acetone extracts, the fruit of *G. kola* recorded the best scavenging activity (47.6%).
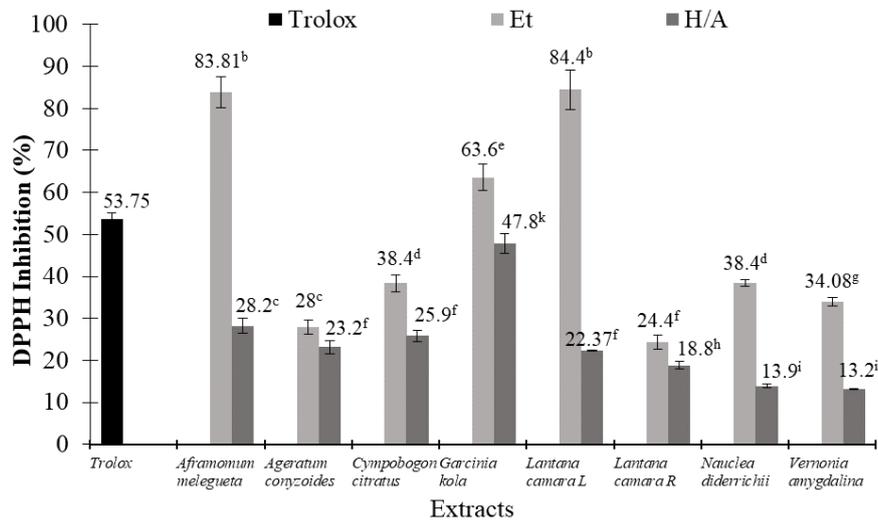

Figure 1. DPPH scavenging inhibition of the plant extracts. Trolox has been used at the concentration of 0.125 mg/mL. Mean values that do not share the same superscript letters are significantly different at *p* < 0.05. Et – Ethanolic extracts; HA - Hexane/Acetone

Ethanolic extracts displayed higher phenolic contents than hexane/acetone (1:3, v/v) extracts. This might be linked to the polarity of the solvent. It has been reported that solvent polarity increases phenolic compounds' solubility (Złotek *et al.,* 2016). Therefore, adding a small quantity of water in an organic solvent increases the relative polarity of the solvent leading to the mass transfer process and enhancing solubility of the phenolic compounds from the plant matrix (Dahmoune *et al.,* 2014). The high contents in natural pigments associated with hexane/acetone extracts were also due to the polarity of solvent used. Hexane and acetone are both non-polar solvents that can easily solubilize lipid-soluble pigments such as chlorophylls and carotenoids. At the same time, water-soluble molecules such as phenolic compounds were hardly solubilized by the same non-polar solvent, justifying the lowest contents of phenolics in hexane/acetone extracts.

Similarly, previous studies revealed that the phenolic content of plant extract is highly correlated to antioxidant activity (Prakash *et al.,* 2016). Meanwhile, according to the scavenging test, three ethanolic extracts were more antioxidants than Trolox. Among these extracts, *L. camara* leaves have been identified as one of the most antioxidant extracts. The phytochemical composition of this plant might explain this result.

Phenolic and pigments composition of L. camara

Based on the high phytochemical content coupled to the best antioxidant and antimicrobial activities, HPLC-DAD of ethanolic extract of *L. camara* leaves was carried out and led to the identification of seven known phenolic compounds, mainly phenolic acids (Figure 2).
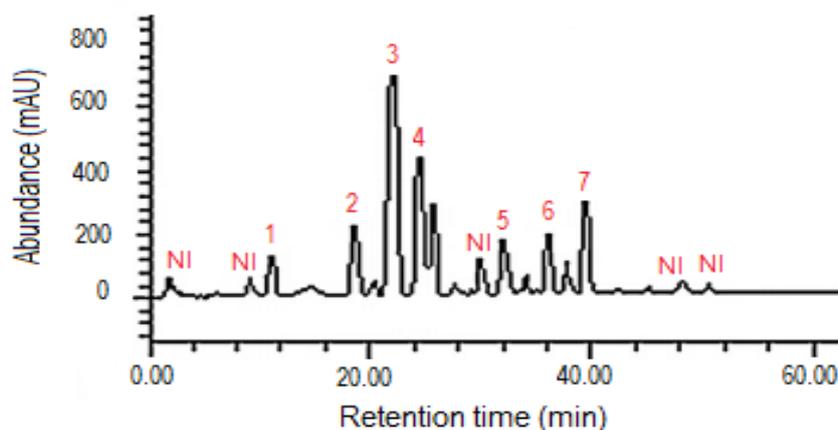

Figure 2. HPLC chromatogram of phenols and flavonoids of ethanolic extract from *Lantana camara*. NI.: No Identified.

The identified compounds in the ethanolic extract of *L. camara* were gallic acid (peak 1, RT: 14.92 min), catechin (peak 2, RT: 19.39 min), ellagic acid (peak 5,

RT: 32.05 min). Chlorogenic (peak 3, RT: 22.67 min) and caffeic (peak 4, RT: 25.24 min) acids were found as the most abundant phenolic acids. In addition, two flavonoids, rutin (peak 6, RT: 36.03 min) and kaempferol (peak 7, 39.87 min), were also found.

HPLC analysis of an ethanolic extract of this plant showed five major phenolics with caffeic and chlorogenic acids as primary phenolic acids. Both compounds have been reported as major phenolic compounds in various plants like *Hypericum perforatum*, *Salvia officinalis*, *Linum usitatissimum*, *Punica granatum* (Apak *et al.,* 2007). The scavenging activity of caffeic acid and standards had been compared in a study conducted by Gülçin (2006). It was found that the DPPH scavenging effect of caffeic acid (93.9%) was by far higher than Trolox (14.3%) at the concentration of 20 mg/mL. According to many scientists, the antioxidant capacity of phenolic acids depends on the degree of hydroxylation and the position of hydroxyl groups (Rice-Evans *et al.,* 1996).

The pigment composition in Table 3 showed that the hexane/acetone extract of *L. camara* leaves was made up of five unidentified chlorophylls, which accounted for more than 80% of the total pigment present in the extracts. In addition, three known carotenoids, zeaxanthin (1.35%), ß-carotene (2.17%), and lutein (1.15%), were also identified. Furthermore, two other unidentified carotenoids were found at the percentage of 3.18% and 8.35%.

Table 3. The major pigments detected in the hexane/acetone extracts of *Lantana camara* leaves.

Peak	Retention time (min)	λ_{max} (nm)	Compounds	Abundance (%)
1	6.57	580, 622, 690	NI Chlorophyll	21.34
2	8.76	546, 612, 634	NI Chlorophyll	5.15
3	9.54	378, 435, 495	NI Carotenoid	3.18
4	9.96	270, 418, 482	NI Carotenoid	8.35
5	12.54	444, 448, 476	Zeaxanthin	1.53
6	13.79	594, 618, 646	NI Chlorophyll	11.2
7	14.80	424, 449, 468	ß-Carotene	2.17
8	17.79	398, 435, 465	Lutein	1.15
9	24.12	528, 603, 668	NI Chlorophyll	25.77
10	28.07	534, 618, 644	NI Chlorophyll	20.16

Fruits, vegetables, and edible plants with a high content of coloring principles are involved in producing "coloring foods". However, orange carrots, sweet potatoes, tomatoes, and spirulina are just a few of many edibles and deeply colored raw materials used (Stich, 2016). *V. amygdalina*, one of the selected plants for this investigation, is an edible green plant very appreciated by Cameroonians because of its use for cooking of delicious local dish called "ndolé". *C. citratus*, another

plant used, is an edible and medicinal green plant. The leaf extracts of this plant are widely used as relaxing herbal tea. *A. conyzoides* and *L. camara* are medicinal green plants, and they are usually used to treat malaria and toothache. All these plants displayed extracts with high chlorophyll contents (*A. conyzoides* and *L. camara*) are associated with carotenoids which could serve as a food-grade dye. Indeed, Martins *et al.* (2016) reported that chlorophyll (E 140) from *Spinacea oleracea* leaves and *Spirulina pratensis* (algae) are already used as a natural coloring agent for beverages, fruit juices, and dairy products.

Antimicrobial activity of the plant extracts

The results from Table 4 show that the inhibition diameters of the active plant extracts were significantly different (p<0.05) and varied from 10 mm to 26 mm for the vegetative cells and 13.5 mm to 22 mm for bacterial spores. Extracts of *Aframomum melegueta* seeds (IZ = 26±1.4 mm; IZ = 10.8±0.4 mm), *Lantana camara* leaves (IZ = 24.8±0.8 mm; IZ = 27.5±1.6 mm), and *Nauclea diderrichii* stem bark (IZ = 10.5±0.71 mm; IZ = 11.1±1.4 mm) were active against *Salmonella agona*. Hexane/acetone extract of *L. camara* leaves was also more effective than gentamycin (IZ = 26 mm) against *S. agona*. Except for the extracts from *L. camara* rods, at least one extract from the remaining plant extracts was efficient against *Staphylococcus aureus*. The efficiency of hexane/acetone extracts of *Garcinia kola* fruits (IZ = 15.7±1.5 mm), *L. camara* leaves (IZ = 11.6±0.6 mm), *Vernonia amygdalina* leaves (IZ = 17.3±1.5 mm), and ethanol/water extract of *N. diderrichii* leaves (IZ = 15.2±0.84 mm) were shown on *L. monocytogenes*. Hexane/acetone extracts from *Ageratum conyzoides* (IZ = 15.5±2.1 mm) and *L. camara* leaves (IZ = 24.8±3.9 mm) were the most active extracts against vegetative cells of *B. cereus* and *B. subtilis,* respectively. Hexane/acetone extracts of *G. kola* seeds (IZ = 18±1.4 mm and IZ = 22±1.4 mm) and *L. camara* leaves (IZ = 18±1.4 mm and IZ = 20.5±0.7 mm) had the most significant effect on germination and outgrowth of *Bacillus cereus* and *Bacillus subtilis* spores.

Hexane/acetone extracts of *A. conyzoides* (IZ = 12±1.4 mm) and *V. amygdalina* leaves (IZ = 12±0 mm) and *L. camara* rods (IZ = 11.3±0.4) were active against *Kluyveromyces* spp. In contrast, hexane/acetone extracts of *A. conyzoides* (IZ = 13±1.4 mm), *Cymbopogon citratus* leaves (IZ = 14±1.4 mm), *G. kola* fruits (IZ = 17±1.4 mm), and *L. camara* leaves (IZ = 14±1.4 mm) were more efficient against *Aspergillus*. Furthermore, hexane/acetone extract of *G. kola* displayed higher antifungal activity on *Aspergillus* than Nystatin (IZ = 14 mm) used as a reference compound. None of the analyzed extracts exerted antimicrobial activity against *Lactobacillus plantarum* and *Saccharomyces* spp. Furthermore, no inhibition and no growth were noticed with *Penicillium expansum* for all the extracts. Except for the mentioned cases above, it has been noted that both antibiotics were more effective than extracts.

Table 4. Antimicrobial activity of ethanolic and hexane/acetone extracts (10 mg/mL) of the selected plants.

Extracts (Part used)	Solvent	G⁻ strain			G⁺ strains					Fungi	
		S. agona	*St. aureus*	*L. monocyt.*	*B. cereus*		*B. subtilis*		*Kluyv.*	*Asperg.*	*Penicill.*
					vegetative / spores		vegetative / spores				
A.Melegueta (seeds)	Et	26±1.4ᵃ	19.4±1.5ᵃ	-	14±1.4ᵃ / 13.5±0.7ᵃ		17.3±0.6ᵃᵉ / 19±1.4ᵃ		-	-	+
	HA	10.8±0.4ᵇ	10.2±1.4ᵇ	-	- / -		10.5±0.9ᵃ / 11.2±1.1ᵃ		-	-	-
A.conyzoides	Et	-	-	-	- / -		- / -		-	-	+
L (entire plant)	HA	-	13±1ᵇ	-	15.5±2.1ᵃ / 15.5±2.1ᵃ		12.5±1.3ᵇ / 19.5±0.7ᵃ		12±1.4ᵃ	13±1.4ᵃ	+
C.citratus (leaves)	Et	-	-	-	- / -		- / -		-	-	+
(leaves)	HA	-	13.7±0.6ᵇ	-	- / 13.5±0.7ᵃ		14.5±3.5ᵃᵇ / 10.5±0.7ᵇ		-	14±1.4ᵃ	+
G. kola	Et	-	-	-	- / -		- / -		-	-	+
Heckel (fruits)	HA	-	17.7±2.08ᵃ	15.7±1.5ᵃ	12.7±0.6ᵃ / 18±1.4ᵇ		16±0ᵇ / 22±1.4ᶜ		-	17±1.4ᵇ	-
L.camara L. (leaves)	Et	24.8±0.8ᶜ	-	-	- / -		- / -		-	-	+
(leaves)	HA	27.5±1.6ᵃ	13.7±1.5ᵇ	11.6±0.6ᵇ	13.3±1.5ᵃ / 18±1.4ᵇ		24.8±3.9ᶜ / 20.5±0.7ᶜ		-	14±1.4ᵃ	-
L.camara L. (rods)	Et	-	-	-	- / -		10±0ᵈ / -		10±0ᵇ	-	+
(rods)	HA	-	-	-	- / -		13.5±1.8ᵃ / -		11.3±0.4ᵃ	-	+
N.diderrichii (stem bark)	Et	10.5±0.71ᵇ	12.4±0.55ᵇ	15.2±0.84ᵃ	- / -		- / -		-	-	+
(stem bark)	HA)	11.1±1.4ᵇ	10.3±0.4ᵇ	11.6±1.9ᵇ	- / -		- / -		-	-	-
V.amygdalina (leaves)	Et	-	-	-	- / -		- / -		-	-	+
(leaves)	HA	-	-	17.3±1.5ᵃ	12.7±2.5ᵃ / 16.5±0.7ᵇ		19.3±2.1ᵉ / 21.5±0.7ᶜ		12±0ᵃ	-	+
Gentamycin (1 mg/mL)		26	24	32	30 / NT		26 / NT		NT	NT	NT
Nystatin (1 mg/mL)		NT	NT	NT	NT / NT		NT / NT		17	14	12

IZ- Inhibition Zone; Et – Ethanolic extracts (70% v/v); HA - Hexane/Acetone (3:1, v/v); (-) - no clear zone and growth; (+) - no clear zone and growth; NT - not tested; Mean values (n = 3 repetitions) that on the same column do not share a letter (a, b, c, d, e) are significantly different at $p < 0.05$.

Table 5. Antimicrobial parameters (MIC, MICg, MBC, MFC) in mg/mL of ethanolic and hexane/acetone extracts from the plants selected.

Extracts (solvent used)	Parameters (mg/mL)	G⁻ strains			G⁺ strains				Fungi		
		S. agona	St. aureus	L. monocyt	B. cereus vegetative	B. cereus spores	B. subtilis vegetative	B. subtilis spores	Penicill. spp.	Kluyv. spp.	Asp. spp.
A.melegueta (Et)	MIC/MICg	10	10	>10	10	>10	10	0.3125	0.625	>10	>10
	MBC/MFC	-	-	-	-	-	-	-	10	-	-
A.conyzoides (H/A)	MIC/MICg	-	10	>10	-	-	0.315	-	16	-	-
	MBC/MFC	-	-	-	-	0.3125	0.625	0.156	0.3125	>10	>10
	MBC/MIC	-	-	-	-	-	2	-	1.25	-	-
C. citratus (H/A)	MIC/MICg	-	10	-	-	-	0.625	-	4	-	-
	MBC/MFC	-	-	-	-	0.625	1.25	0.3125	0.625	-	10
	MBC/MIC	-	-	-	-	-	2	-	10	-	-
G. kola Heckel (H/A)	MIC/MICg	-	10	10	0.156	-	0.625	-	16	>10	10
	MBC/MFC	-	-	-	0.3125	0.3125	0.625	0.156	0.625	>10	10
	MBC/MIC	-	-	-	2	-	1	-	10	-	-
L.camara leaves (H/A)	MIC/MICg	0.315	10	10	0.156	-	0.156	-	16	-	-
	MBC/MFC	1.25	-	-	0.625	0.156	0.156	0.156	0.625	-	10
	MBC/MIC	4	-	-	4	-	1	-	10	-	-
L.camara rods (Et)	MIC/MICg	-	-	-	-	-	10	-	-	-	-
	MBC/MFC	-	-	-	-	-	-	-	16	-	-
N.diderrichii (Et)	MIC/MICg	>10	>10	-	-	-	-	-	-	-	-
	MBC/MFC	-	-	-	-	-	-	-	-	-	-
V.amygdalina (H/A)	MIC/MICg	-	10	10	0.625	-	0.156	-	-	-	-
	MBC/MFC	-	-	-	1.25	0.3125	0.3125	0.3125	0.625	10	-
	MBC/MIC	-	-	-	2	-	2	-	10	-	-

Et – Ethanolic extracts; HA - Hexane/Acetone; MIC - minimum inhibitory concentration; MBC - minimum bactericidal concentration; MICg - minimum concentration inhibiting germination of bacterial spores; MFC - minimum fungicidal concentration; (-) - not found.

The minimum inhibitory concentration, minimum bactericide concentration, minimum concentration inhibiting bacterial spore germination, minimum fungicidal concentration, and MB(F)C/MIC of the plant extracts are shown in Table 5. It has been noted that most of the extracts displaying good antimicrobial parameters were obtained from hexane/acetone (3:1, v/v) extracts. Vegetative cells and spores of *B. cereus* and *B. subtilis* and spores of *Penicillium expansum* were found more sensitive to extracts.

Hexane/acetone extract from *G. kola* seeds was the most effective extract on the vegetative cells of *B. cereus* by exhibiting a MIC value of 0.156 mg/mL and MBC value of 0.3125 mg/mL a bactericidal activity (MBC/MIC=2). Hexane/acetone extract of *L. camara* leaves exerted bactericidal effect (MBC/MIC=1) on the vegetative cells of *B. subtilis* with MIC and MBC values of 0.156 mg/mL. Except for *N. diderrichii*, the others six plants (85.7%) exerted an inhibiting activity on spore germination. The lowest MICg values were observed with hexane/acetone extract of *L. camara* leaves (0.156 mg/mL) on *B. cereus* and *B. subtilis* spores. Among the most effective plant extracts which exerted an inhibiting effect on *Penicillium* spp., the extract of *A. conyzoides* was the one that displayed a strong fungistatic activity (MFC/MIC=4). This extract revealed MIC value of 0.312 mg/mL and MFC value of 1.25 mg/mL.

Many plant extracts possess antimicrobial activities and are proposed as food preservatives (Lyumugabe *et al.,* 2017; Hintz *et al.,* 2015; Negi, 2012) and food coloring agents (Boo *et al.,* 2012). In this investigation, except for extracts from *A. melegueta* seeds, *L. camara* leaves, and *N. diderrichii,* which were active against *Salmonella agona*, the remaining extracts were ineffective. This result disagrees with the findings of other authors on the antimicrobial activity of these plants. They reported the antibacterial activity of methanol extracts from *A. conyzoides* leaves, methanol, and water extracts from *C. citratus* leaves (Prakash *et al.,* 2016), and ethanol extracts from *V. amygdalina* and *L. camara* leaves (Lyumugabe *et al.,* 2017) against *Salmonella* spp. However, hexane/acetone extracts showed good antimicrobial activity against bacteria (*S. aureus*, *L. monocytogenes*, *B. cereus*, and *B. subtilis*), spore-forming bacteria (*B. cereus* and *B. subtilis*), and some fungi (*Penicillium* spp. and *Aspergillus* spp.). In the same line, Kumar *et al.* (2017) reported that acetone extracts of *L. camara* leave and flowers exhibited intense antimicrobial activity against *Bacillus subtilis*, *Lactobacillus* spp.*, S. aureus, Penicillium* spp.*, Aspergillus niger* which are among the most dangerous food spoilage and food poisoning microorganisms. Though the antimicrobial activity of hexane/acetone extracts seems different depending on the strains of bacteria and the fungi, it was demonstrated from the multivariate analysis that the antimicrobial efficiency of these extracts is positively correlated to their contents in chlorophyll and carotenoids. In consistence with our results, it is apparent that some plant extracts are bactericidal. This study showed that extracts from *G. kola* fruits, *L. camara,* and *V. amygdalina* leaves exerted bactericidal effects against *B. cereus* and *B. subtilis*, food poisoning, and food spoilage bacteria. In contrast, extract from *A. conyzoides* displayed a fungicidal effect on *Penicillium* spp. Our results also

revealed the inhibitory effect of some extracts on germination of *B. cereus* and *B. subtilis* spores. In agreement with the present study, Voundi *et al.* (2015) studied the anti- germinating activity of some Cameroonian plants' essential oils (EOs) on pathogenic and spoilage spores of *Bacillus* spp. They reported that EOs from *A. conyzoides* and *C. citratus* exerted an inhibitory effect on the germination of spores of *B. cereus* and *B. subtilis*. Hexane/acetone extracts were found more active on gram-positive than gram-negative bacteria. This suggests that fatty-soluble pigments present in hexane/acetone extracts might exert their antimicrobial activity by disorganization of the cell wall and leakage of cytoplasm material (Gyawali *et al.,* 2015). Even if the reason for antimicrobial activity of carotenoids is still poorly understood, previous reports suggested that ß-carotene could lead to the accumulation of lysozyme, and antibacterial immune enzyme that digests bacterial cell walls, therefore generating the antibacterial activity (Bhagavathy *et al.,* 2011). This study revealed that all extracts analyzed did not have an antifungal effect against *Saccharomyces* spp. Lyumugabe *et al.* (2017) reported a similar effect with other plant extracts. Therefore, this finding is reassuring for their potential use as preservatives in indigenous sorghum beers because of the role as a starter played by *S. cerevisiae* in the fermentation stage.

Multivariate analysis

To analyze the correlations between the measured variables during the testing of extracts, the principal component analysis (PCA) was applied to seven parameters belonging to phytochemical, antioxidant, and antimicrobial variables, as shown in Figures 3a and 3b. The variables were reduced into two principal components (PC1 and PC2) with eigenvalues more significant than 1. PC1 and PC2 accounted for 39.85% and 39.41%, respectively, and explained together 79.26% of the total variance after varimax rotation. The PCA analysis showed that antimicrobial activity, carotenoids, Chl a, and Chl b contributed positively to PC1.

A strong positive correlation between carotenoids, Chl a, Chl b contents of the extracts, and the antimicrobial activity of the plant extracts. Phenols and flavonoids were positively correlated to the scavenging activity of the extracts, being strongly loaded on the positive side of the component PC2. The scores plot displayed that ethanol/water extract of *A. melegueta* seeds had the highest phenols and flavonoids contents and the best DPPH scavenging activity (figure 3b). Furthermore, the scores plot confirmed that most hexane/acetone extracts present the best antimicrobial activity.

A cluster analysis was applied using the centroid method and squared Euclidean measurement to interpret the relationship between the different tested extracts based on their phytochemical contents and biological activity. The centered-reduced normalization was applied to improve the classification of the extracts. Figure 3c shows a dendrogram of the sixteen extracts. In figure 3, two main clusters marked down, and three major sub-clusters, I, II, and III, were revealed. The first sub-cluster was formed by all ethanolic extracts and hexane/acetone extracts of *A. melegueta* seeds, *G. kola* fruits, *L. camara* rods, *N. diderrichii* stem bark. Hexane/acetone extracts of *A. conyzoides*, *C. citratus* leaves, *L. camara*

leaves, and *V. amygdalina* leaves were classified into the second sub-cluster with a close relationship between *A. conyzoides* and *L. camara* extracts. Ethanol/water extract of *A. melegueta* seeds formed the third sub-cluster.
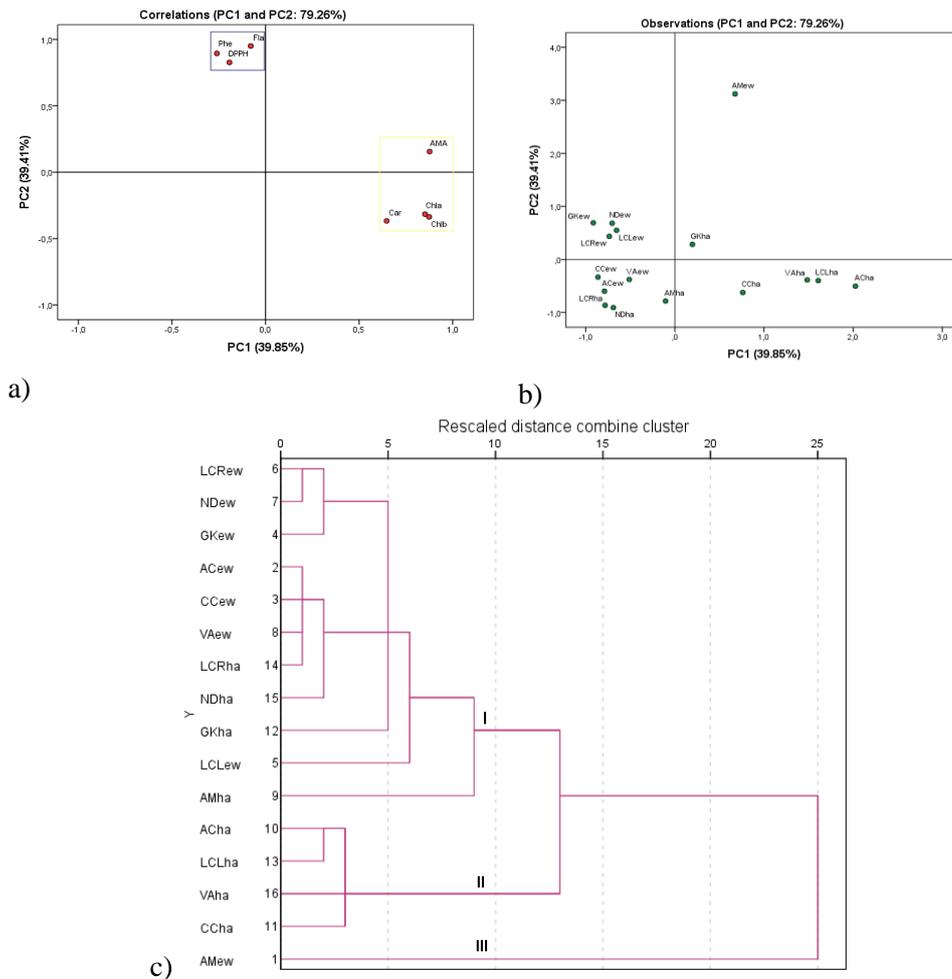

a)   b)

c)

Figure 3. Principal component analysis of correlation loadings (a) and scores (b) plots derived using phytochemical, the biological activity of extracts. Dendrogram (c) of extracts from different solvent systems was performed with the hierarchical cluster analysis using the centroid method and squared Euclidean measurement. Rotation method: varimax with Kaizer normalization (KMO = 0.753; $p < 0.001$). x-axis and y-axis mean rescaled distance cluster and the plant extracts measured. AMA - antimicrobial activity as a sum of the inhibition diameter obtained from each plant extract; Car - carotenoids; Chl a - chlorophyll-a; Chl b - chlorophyll b; DPPH - DPPH scavenging activity; Fla - flavonoids; Phe - phenols; AM - *Aframomum melegueta*; AC - *Ageratum conyzoides*; CC - *Cymbopogon citratus*; GK - *Garcinia kola*; LCL and LCR - *Lantana camara* leaves and rods; ND - *Nauclea diderrichii*; VA - *Vernonia amygdalina*; ew - ethanol/water; ha - hexane/acetone.

Excessive formation of free radicals accelerates the oxidation in foods and biological systems. Therefore, radical scavenging activity of natural compounds is significant for food quality and consumer health. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. This test is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical-scavenging ability of specific compounds or extracts. In this study, all the extracts had expressed a scavenging activity. The antioxidant potential of extracts showed the highest scavenging activity with ethanol/water extracts and the lowest for hexane/acetone extracts. Principal component analysis (PCA) revealed that the DPPH radical scavenging activity appears to depend on phenols and flavonoid contents of the extracts (figure 3). This finding agrees with what Boo et al. (2012) reported, where the antioxidant properties of thirteen kinds of natural plant pigments were evaluated using different extraction procedures. They demonstrated that the scavenging activity was correlated with the total phenols and flavonoids contents present in aqueous and ethanolic extracts.

Conclusions

In this study, seven edible and Cameroonian medicinal plants exhibited an appreciable antioxidant activity. Apart from *Saccharomyces* spp. MIUG D8 and *Lactobacillus plantarum*, all other tested microorganisms were sensitive to at least one plant extract. *B. cereus* and *Listeria monocytogenes* were affected by 31.25% of the extracts tested, while *Salmonella agona* was affected by 37.5% of the extracts. Nine extracts were active against *B. subtilis* and *S. aureus* (56.25%). The extracts inhibited the germination of food poisoning and food spoilage spores by 37.5%. *Aspergillus niger* and *Kluyveromyces* spp. MIUG M26 were sensitive to 4 (25%) different extracts. This antimicrobial activity has been related to the natural fatty-soluble pigments contained in the extracts. According to the results, some of the plant extracts might valuably increase the shelf life of foodstuffs, replace synthetic antioxidants, and prevent cellular damage, cause of aging, and human diseases. In addition, antibacterial activity and food colorant properties might be exploited to find natural preservative ingredients in the food industry. Therefore, a strong interest should be given to some selected plants because of their great potential as a natural antioxidant (*L. camara*), food preservatives (*L. camara, C. citratus*, *V. amygdalina*), and food colorant (*L. camara, A. conyzoides*). However, further evaluation of the biological activity of these extracts against a larger microbial panel and toxicity studies are needed to reinforce the potential of the candidate plant extracts for these purposes.

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