Journal of
Pharmacognosy & Natural Products

Tchouya et al., J Pharmacogn Nat Prod 2015, 1:1
DOI: 10.4172/2472-0992.1000111

Research Article Open Access

Phytochemical Analysis, Antioxidant and Antimicrobial Properties of the Leaves and Stem Bark of *Scyphocephalium ochocoa* Warb (Myristicaceae)

Feuya Tchouya GR[1,2]*, Foundikou H[1], Lebibi J[1], Choudhary MI[2], Menkem EZ[3], Nantia EA[4], Kezetas JJB[5] and Tchouankeu JC[6]

[1]*Department of Chemistry, Faculty of Science, Scientific and Technical University of Masuku, Box. 223(Potos), Franceville, Gabon*
[2]*International Center for Chemical and Biological Sciences, H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*
[3]*Department of Biochemistry, Faculty of Science, University of Yaounde 1, Box. 812, Yaounde, Cameroon*
[4]*Department of Biological Science, Faculty of Science, University of Bamenda, Box. 39, Bambili, Cameroon*
[5]*Department of Chemistry, Faculty of Science, University of Bamenda, Box. 39, Bambili, Cameroon*
[6]*Department of Organic Chemistry, Faculty of Science, University of Yaounde 1, Box. 812, Yaounde, Cameroon*

Abstract

Aim: This study aimed to evaluate the pharmacological importance of *Scyphocephalium ochocoa* Warb. (Myristicaceae), through screening of phytochemical constituents and isolation of biomolecules, *in vitro* antioxidant and antimicrobial activities of the aqueous alcohol extracts of the stem bark and leaves of this species.

Method: The phytochemical constituents were identified in the stem bark and leaves extracts of *S. ochocoa* using standard procedures described in the literature. Subsequently, the stem bark extract was fractionated by means of silica gel column chromatography, and the isolated biomolecule characterized through extensive spectroscopic analyses. Antimicrobial activities were determined using both disc diffusion and broth micro dilution methods against different bacteria and fungi. The free radical scavenging activity and the total phenolic content were determined using the DPPH free radical and the Folin-Ciocalteu assays respectively.

Results: Phytochemical analysis revealed the presence of flavonoids and tannins in both parts of the plant. Coumarins and terpenoids were present only in leaves while anthocyanins, saponosides and sterols were found in stem bark. *S. ochocoa* stem bark and leaves extracts showed significant 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (IC_{50}=0.169 ± 0.019 µg/ml) and polyphenol content (153.57 ± 0.63 mg/g of extract). *S. ochocoa* extracts significantly inhibited microbial growth of *Echerichia coli* and a gentamycin resistant Staphylococcus aureus strains. The chromatographic separation of the stem bark afforded the bioactive compound isopregomisin.

Conclusion: This study showed that *S. ochocoa* stem bark and leaves extracts contain various phytochemicals, with important amount of phenolic compounds, and possess antioxidant and antimicrobial activities. Isopregomisin can be considered as the antioxidant active principle of *S. ochocoa*.

Keywords: *Scyphocephalium ochocoa*; Phytochemistry; Antimicrobial activity; Antioxydant activity; Isopregomisin

Introduction

Antimicrobial resistance (AMR) within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors. Increasingly, governments around the world are beginning to pay attention to a problem so serious that it threatens the achievements of modern medicine. A post-antibiotic era - in which common infections and minor injuries can kill - far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century [1]. With such impact in the use of available antimicrobials, it is essential to look for new sources of cheaper and efficient drugs with broad spectrum of action. One strategy of this research is to explore the plants used in traditional medicine. Indeed, the use of therapeutic plants (herbal medicine) is very old and currently WHO estimates that for the fight against many diseases, about 80% of the African population still uses traditional medicine for which the majority of therapies involve the usage of medicinal plants extractives [2]. On the other hand, plant species with great importance for the health of populations should be subjected to scientific evaluation in order to ensure their proper use. Oxidative stress is a process through which free radical and reactive oxygen species (ROS) can damage the cell ultrastructure. Antioxidants can act to prevent or delay the process of oxidative stress and thereby, reducing the risk for chronic diseases. In fact, antioxidants are substances that can terminate the chain reactions of free radical and reactive oxygen species (ROS) before they cause damage. In addition, antioxidant compounds can scavenge free radicals and protect the cells by delaying the process of lipid peroxidation [3]. Many fresh fruits, vegetables and herbal plants have been found to contain a lot of antioxidants and they can be categorized as primary sources of antioxidants. In our search for antimicrobials and antioxidants from plants sources, investigations we conducted lead to the identification of several species used by traditional rulers in the fight against some pathogenic infections, and degenerative diseases. *Scyphocephalium ochocoa* is one of the identified species, widely used in the central African traditional medicine, especially in Gabon in the treatment of Abscess, anemia - general fatigue, respiratory tract infections and blennorrhoea [4]. The seeds of *S. ochocoa* are edible and used for seasoning. This work aimed to evaluate the phytochemical analysis, antioxidant and antimicrobial properties of the stem bark and leaves of this species, and also, to identify the active principles of the plant.

***Corresponding author:** Guy Raymond Feuya Tchouya, Department of Chemistry, Faculty of Science, Scientific and Technical University of Masuku, Box. 223(Potos), Franceville, Gabon, Tel: (241) 06043682 / 03107733; Fax: (241) 01677578; E-mail: gfeuya@yahoo.fr

Received September 30, 2015; **Accepted** October 23, 2015; **Published** October 26, 2015

Citation: Tchouya GRF, Foundikou H, Lebibi J, Choudhary MI, Menkem EZ, et al. (2015) Phytochemical Analysis, Antioxidant and Antimicrobial Properties of the Leaves and Stem Bark of *Scyphocephalium ochocoa* Warb (Myristicaceae). J Pharmacogn Nat Prod 1: 111. doi:10.4172/2472-0992.1000111

Materials and Method

Materials

Plant material: Stem bark and leaves of *Scyphocephalium ochocoa* (2 kg each) were collected in May 2012 near Franceville, in the Haut Ogooue Province, south-east of Gabon and identified by Mr Yves ISSEMBE, a botanist of the National Herbarium of Gabon. A specimen was kept in the Scientific and Technical University of Masuku, Franceville, Gabon (N° So 083/UM). The plant materials were then dried for two and four days respectively for leaves and stem bark at room temperature and finely powdered. Powders obtained were extracted aqueous alcohol solution (ethanol/water: 1/1) for two days and the extracts were freeze-dried to yield 56 g and 75 g of stem bark and leaves aqueous alcohol extracts respectively.

Microbial strains: The bacterial strains including Gram-positive bacteria *Staphylococcus aureus* (SA) and Gram-negatives, *Escherichia coli* ATCC 25922 (EC) and *Klebsiella pneumoniae* (KP) were obtained from the Central Hospital of Yaoundé (Cameroon). The strains were grown and maintained on Mueller Hinton Agar slants at 35°C until used. Four *Candida albicans* strains ATCCL26 (CA1), ATCC12C (CA2), ATCCP37039 (CA3), and ATCCP37037 (CA4) were obtained from BEI Resources, NAID, NIH. Yeasts were grown and maintained on Sabouraud Dextrose Agar slants at 37°C until used. Before any test, yeasts were cultured 48 hours in Sabouraud Dextrose Agar.

Method

Phytochemical screening: The plant extracts were screened for their qualitative chemical composition, using standard methods described in the literature [4-6]. The identification of the following groups was considered: alkaloids, anthocyanins, coumarins, flavonoids, reducing sugars, saponosides, sterols-triterpenes and tannins.

Alkaloids: 0.5 g of each extract was agitated with 5 ml of hydrochloric acid in a steam bath, then 1 ml aliquots of filtrate were treated with a few drops of Mayer's reagent or Dragendorff's reagent. The presence of a precipitate after treatment with either reagent was a preliminary indicator of the presence of alkaloids. To remove non-alkaloid compounds that could lead to false-positive reactions, part of the extract was alkalinized with 40% ammonia solution then treated twice with chloroform. The second chloroform extract was concentrated and then retested with the Mayer and Dragendorff reagents.

Anthocyanins: 1 ml of each extract was dissolved in 1 ml of water contained in a test tube, then, a few drops of a NaOH solution was added in the mixture. The appearance of a blue green color, that eventually fades, indicates the presence of anthocyanins

Coumarins: Examined in ultraviolet light, the TLC of drugs with coumarins present spots whose colouring, in presence of ammonia atmosphere, varies from blue to yellow and purple.

Flavonoids: Flavonoids were detected by using the Shibata reaction or cyanide test. Briefly, 3 ml of extract was evaporated and the residue was dissolved in 2 ml of 50% methanol, then a few magnesium shavings and a few drops of concentrated hydrochloric acid were added. The development of a red-orange or purplish color indicates the presence of flavones aglycones.

Reducing sugars: One milliliter of extract was dissolved in 2 ml of distilled water and 1 ml of Fehling liquor and boiled for 30 min. The formation of a brick-red precipitate indicates the presence of reducing sugars. Saponosides: 1% of each sample decoction was returned gradually in 10 ml test tubes for a final volume of 10 ml. After two vigorous shakes, the tubes were left to stand for 15 min and the height of foam was measured. The tube in which the height of the foam was at least 1 cm, showed the presence of saponosides. However, the height of the foam indicated the value of the foam index.

Sterols and triterpenes: These families of compounds were identified by using the Lieberman-Burchard reaction. Briefly, 0.5 g of extract was dissolved in 0.5 ml of chloroform with 0.5 ml of acetic anhydride, and cooled on ice before carefully adding sulfuric acid. A change in color from purple to blue indicates the presence of sterols, while a green or purple-red color indicates the presence of triterpenes.

Tannins: Initially, the Styasny reagent was used to detect the presence of tannins: A drop of the extract was placed on a slab of silica gel and eluted in an atmosphere saturated with chloroform/acetic acid/formic acid (5:4:1), thereafter, the plates were sprayed with 10 ml of a methanol solution at 5% nitrous acid and heated in an oven at 80°C for 10 min. The presence of tannins was revealed by the appearance of blue spots. For the classes of tannins, boiled aqueous extract (1 ml) was mixed with 1% ferric chloride. A black-blue color indicated the presence of gallic tannins and a dark green color, condensed tannins.

For all the samples tested, according to the precipitation or color intensity of each tube, following evaluations were given: (+++); (++); (+).

Determination of free radical scavenging activity: The antiradical activity of *S. ochocoa* extracts was determined according to the method described by Nantia et al. (2013) [7]. Briefly 980 µl of freshly prepared DPPH solution (40 µg/ml) was introduced in tubes and the extract or standard vitamin C (0.02, 0.2, 2, 20, and 200 µg/ml) were added. After 30 min, the change from the radical to the non-radical form leads to the disappearance of the purple coloration of DPPH, which was recorded by spectrophotometry at 517 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The inhibitory potential of extracts was expressed through their inhibitory concentration fifty (IC_{50}).

Determination of total phenolic content: The amount of total phenolics in *S. ochocoa* extracts was determined with Folin-Ciocalteu reagent according to the method of Singleton and Rossi [8] with slight modification using gallic acid as a standard. Briefly, in 200 µl of extract (2 µg/ml) was added 500 µl of 1/10 diluted Folin reagent and 20% Na_2CO_3. The mixture was allowed to stand for 30 min with intermittent shaking, and the absorbance was measured at 730 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as mg of gallic acid equivalent per gram of plant extracts using an equation obtained from the standard gallic acid calibration graph.

Antimicrobial activity

Inoculum preparation: Before any test, bacteria strains were subcultured on Mueller Hinton agar slants at 35°C for 18 h. Mature colonies were collected with inoculating loop and introduce in a tube with 5 ml of sterile saline (0.9% NaCl) and homogenize. The turbidity of the solution was adjusted at 0.5 McFarland standards [9]. A stock inoculum suspension of yeast was prepared from a 2 days old culture on Sabouraud Dextrose Agar at 37°C. The colonies were collected with inoculating loop and introduced in a tube with 5 ml sterile normal saline. The suspension was quantified using the Malassez counting chamber under a microscope and adjusted to 2.5 x 10^4 cells/ ml using sterile 0.9% sodium chloride (normal saline) solution [9].

Disc diffusion method: *In vitro* antibacterial and antifungal activity were screened by disc diffusion method on Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) respectively. The MHA and SDA plates were prepared by pouring 15 ml of molten media into sterile petri dishes (90 mm). The plates were allowed to solidify for 5 min and 0.1 ml of inoculum suspension was poured and the inoculum was allowed to dry for 5 min. The different extracts at 20 mg/disc were loaded on 5 mm sterile individual discs. The loaded discs were placed on the surface of medium, then the extracts were allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 hours and 48 hours for bacteria and fungi respectively. Negative control was prepared using respective solvent of dilution of extracts (10% DMSO). Amoxicillin, ampicillin, chloramphenicol and gentamycin (2 mg/disc) were used as positive control for bacteria and fluconazole and nystatin (2 mg/disc) were used for fungi. At the end of incubation, inhibition zones formed around the disc were measured with Vernier Calliper in millimeter. These studies were performed in triplicate [9].

Microbroth dilution method: The Minimum Inhibitory Concentration (MIC) was determined according to M38-A and M27-A3 protocols for bacteria and yeast respectively [10]. This method was applied on extracts that showed some efficacy against microorganisms by the agar well diffusion method (inhibition diameters above or equal to 7 mm). In the well of the first line, 100 µl of culture medium (Mueller Hinton Broth for bacteria and Sabouraud broth for fungi) was introduced and 100 µl in the remaining wells of the plates. Later on, 100 µl of stock solution of crude extracts and reference antibiotics at 20 mg/ml and 2 mg/ml respectively was added to the first well. The medium and sample in the first well were mixed thoroughly before transferring 100 µl of the resultant mixture to the well of the second line. Ten two-fold serial dilutions of the test samples were made from line 1 until line 11 and 20 µl of inoculum standardized at 0.5 McFarland standards for bacteria and 2.5 x 10^3 cells/ml for yeast were introduced in the entire well containing the test substances except the columns of blank (column C and F) which constitute the sterility control. The concentration range was 0.0081 to 8.3 mg/ml for extracts. In each microtiter plate, a column with reference drugs (Chloramphenicol and Gentamycin for bacteria and fluconazole and nystatin for yeast) with the concentration range from 0.83 to 0.00081 mg/ml was used as positive control. After incubation period of 24 hours and 48 hours for bacteria and yeasts respectively (at 37°C), turbidity was observed as indication of growth. Thus the lowest concentration inhibiting the growth of bacteria or yeasts was considered as the Minimum Inhibitory Concentration (MIC).

Phytochemical constituents	Plant parts extracts	
	Stem bark	leaves
Alkaloids	-	-
Anthocyanin	+++	-
Coumarins	-	+++
Flavonoids	+++	+++
Reducing sugars	-	-
Saponosides	+	-
Sterols/Triterpenes	++ (sterols)	++ (triterpenes)
Tannins	+++ (gallic)	+++ (gallic)

+++ Very intense, ++ intense, + weak, − absent

Table 1: Qualitative analysis of phytochemical constituents of the stem barks and leaves of *S. ochocoa*.

Inhibition diameters (mm)							
Microbial strains / Extracts and antibiotics	SA	EC	KP	CA1	CA2	CA3	CA4
S. ochochoa (B)[a]	7	6	-	-	7	-	6
S. ochochoa (L)[b]	7	6	6	6	-	-	-
Nystatin				20	30	20	15
Fluconazol				33	37	32	32
Chloramphenicol	21	24	22	/	/	/	/
Ampicillin	-	-	-	/	/	/	/
Gentamycin	11	21	21	/	/	/	/
Amoxicillin	6	6	-	/	/	/	/

[a]B: barks; [b]L: leaves. - No inhibition observed. SA: *Staphylococcus aureus*, EC: *Escherichia coli* ATCC 25922, KP: *Klebsiella pneumoniae*, CA1: *Candida albicans* ATCCL26, CA2: *Candida albicans* ATCC12C, CA3: *Candida albicans* ATCCP37039, CA4: *Candida albicans* ATCCP37037.

Table 2: Inhibition diameters of the plant parts and reference antibiotics.

Minimum Inhibitory Concentrations (mg/ml)							
Microbial strains / Extracts and antibiotics	SA	EC	KP	CA1	CA2	CA3	CA4
S. ochochoa (B)[a]	8.3	8.3	8.3	>8.3	>8.3	>8.3	>8.3
S. ochochoa (L)[b]	2.075	2.075	4.15	ND[c]	ND	ND	ND
Nystatin				0.01295	0.01295	0.01295	0.01295
Fluconazol				0.01295	0.01295	0.01295	0.01295
Chloramphenicol	>0.83	>0.83	0.415				
Gentamycin	ND	0.01295	0.01295				

[a]B: barks; [b]L: leaves. [c]ND: Not Determined.
SA: *Staphylococcus aureus*, EC: *Escherichia coli* ATCC 25922, KP: *Klebsiella pneumoniae*, CA1: *Candida albicans* ATCCL26, CA2: *Candida albicans* ATCC12C, CA3: *Candida albicans* ATCCP37039, CA4: *Candida albicans* ATCCP37037

Table 3: Minimum Inhibitory Concentration of the plant parts and reference antibiotics.

Isolation and identification of active substance: 30 g of the stem bark aqueous alcohol extract obtained (56 g, 2.8%) was submitted to flash chromatography on a 70-230 mesh silica gel column (400 g) with stepwise gradient elution by CH_2Cl_2/MeOH mixtures (100:0; 98:2; 95:5; 90:10; 80:20; 0:100). Seventy column fractions, each containing 300 mL, were collected and combined according to their TLC profiles on pre-coated silica gel 60 F_{254} plates developed with *n*-hexane/EtOAc and CH_2Cl_2/MeOH mixtures to give 6 groups of fractions: F_1 (1-10); F_2 (11-22); F_3 (23-40); F_4 (41-50); F_5 (51-63) and F_6 (64-70). Among the six fractions obtained, Fraction F3, after evaporation under reduced pressure and dissolution in an *n*-hexane/EtOAc mixture (95:05), showed a precipitate that was filtered under vacuum to give a pinkish powder. The recrystallization of the obtained powder in an *n*-hexane/OAcEt mixture afforded a beige crystalline solid that, after spectroscopic analysis and comparison of its spectroscopic and physical data with those reported in the literature, was identified to isopregomisin [11].

Data analyses

Data were expressed in mean ± standard deviation. For the anti-scavenging activity, the inhibitory concentration fifty (IC_{50} value is the amount of the antioxidant required to decrease the initial DPPH radical concentration to 50%) of extract was determined using Graph Pad Prism software. Differences between fractions were assessed by one factor ANOVA followed by the Student-Newman-Keuls test.

Results

Phytochemical screening

Phytochemical screening using qualitative analysis on aqueous alcoholic extracts from different parts of *S. ochocoa* showed the presence of following constituents: flavonoids and tannins present in both parts of the plant; coumarins and terpenoids in leaves; and anthocyanins, saponosins and sterols only in the stem bark (Table 1).

Antimicrobial analysis

The antimicrobial activities of *S. ochocoa* extracts were evaluated on some pathogenic strains using the disc diffusion and the microbroth dilution methods (Tables 2 and 3). In general, activities exhibited by the extracts were lower than those of reference antibiotics. Leaves extract was more active than the stem bark extract, especially against bacteria. Compared to other microbial strain, *S aureus* was more sensitive to both bark and leaves extracts of *S. ochocoa* (Table 2). However the leaves extract of *S. ochocoa* showed the lowest Minimum inhibitory concentrations (Table 3) for *S. aureus* and *E. coli* compared to the stem bark extract.

Total phenolic content and radical scavenging effect

The total phenolic content of the aqueous alcohol extract of *S. ochocoa* leaves (153.57 ± 0.63 mg/g extract) was significantly higher (P=0.0008) than the stem bark extract (102.01 ± 2.84 mg/g extract). Both extracts displayed comparable DPPH radical scavenging activity (IC_{50}=0.169 ± 0.019 µg/ml and 0.175 ± 0.020 µg/ml for leaves and bark extracts, respectively) which was significantly higher (P=0.0012) than the reference compound vitamin C (IC_{50}=0.267 ± 0.009 µg/ml).

Identification of the active substance

The chromatographic separation of the stem bark aqueous alcohol extract of *S. ochocoa* afforded the known biomolecule isopregomisin. Isopregomisin, $C_{22}H_{30}O_6$ (0.0047 % from the starting material), beige crystals in hexane-EtOAc (v/v 95:05), mp 111-113°C (litt. 110-112°C), was identified on the basis of its NMR spectra [Figures 1-3] and compared with reported data in the literature [11]. The isopregomisin structure is shown in Figure 4. NMR spectra were recorded on a Brucker-500 spectrometer. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.83 (d, *J*=6.4 Hz, 6 H, 2 x CH_3), 1.75 (d, *J*=6.4 Hz, 2 H, 2 x CH), 2.3±2.7 (m, 4 H, 2 x CH_2), 3.80 (s, 12 H, 4 x OCH_3), 6.33 (s, 4 H, ArH). ^{13}C NMR (125 MHz, $CDCl_3$): δ 146.82 (C); 132.87 (C); 132.72 (C); 105.59 (CH); 56.25 (OCH_3); 39.40 (CH_2); 39.05 (CH_2); 16.23 (CH_3).

Discussion

The phytochemical screening of *S. ochocoa* revealed molecules that may potentially be active against some bacterial and fungal pathogenic strains. These included flavonoids, tannins and triterpenes that have been shown to be active against diarrhea, dermal ulcers, skin rashes and abdominal pains [12-15]. Tannins also act as antifungals [16]. Similarly, alkaloids are stimulants, antibiotics, antifungals and pest-destroying [16]. The saponins have antitussive, expectorant, analgesic, immunomodulatory and cytoprotective properties [16]. The presence of these phytochemicals in *S. ochocoa* extracts sustains the common usage of this plant in traditional medicine for the treatment of several diseases. From the compared analysis of Tables 1 and 3, we realize that there is accordance between the chemical composition of the extracts and their antimicrobial activities. Hence, the leaves of *S. ochocoa* which are rich in flavonoids, tannins and triterpenes that have been shown to be active against diarrhea and skin rashes among others, are active against

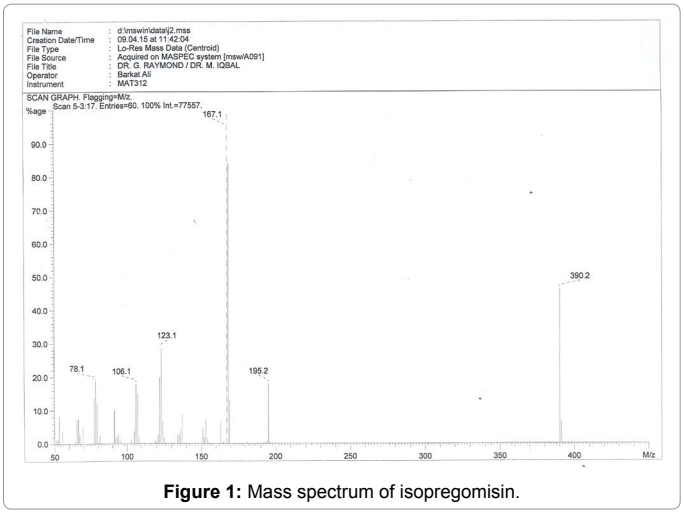

Figure 1: Mass spectrum of isopregomisin.

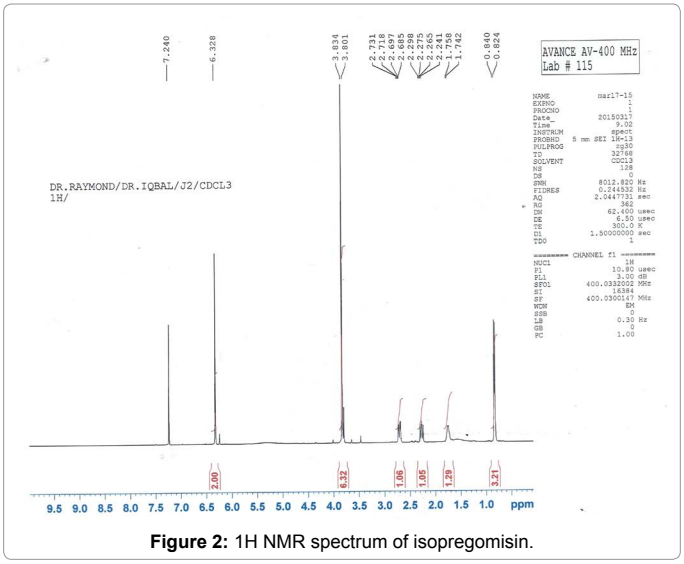

Figure 2: 1H NMR spectrum of isopregomisin.

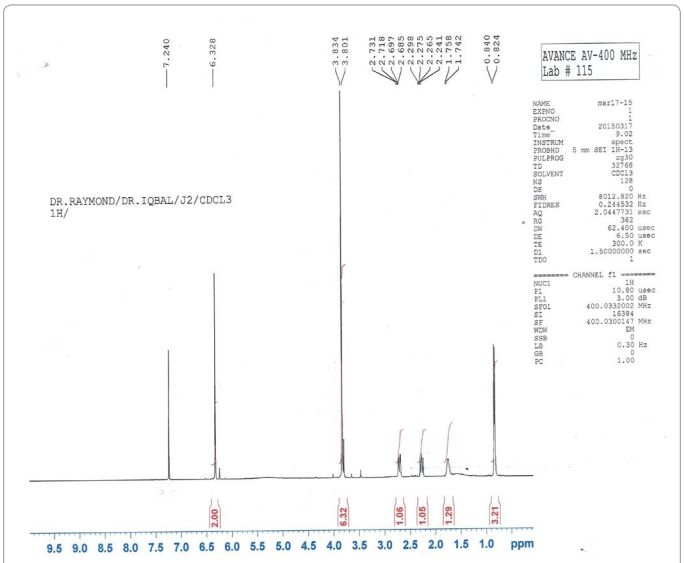

Figure 3: 13C NMR spectrum of isopregomisin.

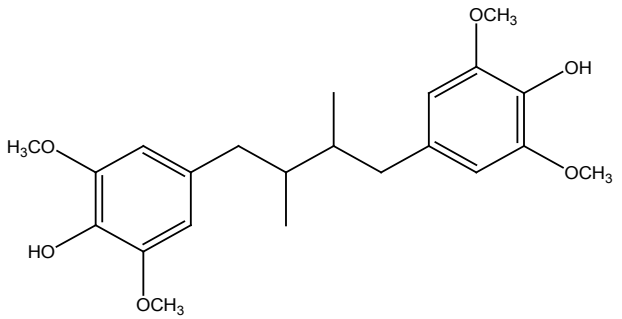

Figure 4: Chemical structure of isopregomisin.

S. aureus and *E. coli* which are among microbial species responsible of diarrhea, vomiting and stomach ache [12-16]. Nevertheless, the stem bark of *S. ochocoa* that is also rich in flavonoids and triterpenes did not exhibit any interesting activity. This could be due to the concentration and the chemical structures of the classes of chemicals present in each plant part. In this case, a complete quantitative analysis of extracts would have been important to understand the exact amount of the different substances. Results obtained during our study are in agreement with antimicrobial activities of many other plant extracts that essentially show Minimum Inhibitory Activities ranging between 1 and >8 mg/ml [17,18]. In all the studies, a consistency appeared for the leaves as the most active part of the plant. This justifies the fact that leaves are the plant part mostly used for medicinal purposes [19-21]. Phenolic compounds have also been reported as antioxidant agents [22]. The present study on *S. ochocoa* extracts showed significant phenolic contents similar to *Distemonanthus benthamianus* ethanolic extracts (102.8 ± 0.57 mg/g extract) [23]. Phenols are known to act as antioxidants, which can neutralize unstable and reactive molecules. They could protect membrane lipids from oxidation [24]. The presence of various phenolic compounds in *S. ochocoa* extracts is consistent with the free radical scavenging activity of this plant as demonstrated in different studies [7,24,25]. This is consistent with the isolation from *S. ochocoa* of isopregomisin, a phenolic compound that have been shown to exhibit strong antioxidant activity *in vitro* [26]. On the other hand, microbial metabolism can generate free radical compounds [27]. However, the inhibitory activity of *S. ochocoa* leaves is in accordance with previous work on this plant part which resulted in the isolation of ocholignan A, a compound found to possess significant *in vitro* antibacterial activity against Gram-positive bacteria methicillin-resistant *S. aureus* ATCC 33591 and *S. aureus* 78-13607A [28]. The antiradical activity of *S. ochocoa* extracts can also be considered as one mode of action of the antimicrobial activity of this plant as demonstrated in many studies [29,30].

Conclusion

These findings demonstrated the presence of various phytochemicals including phenolics in S. ochocoa extracts. Among phenolic compounds, lignans, especially isopregomisin was isolated from the stem bark aqueous alcohol extract and identified through its Mass and NMR spectral data. These extracts also possessed antiradical activities above that of the reference compound vitamin C, and inhibited microbial growth of some microorganisms, including E. coli and a gentamycin resistant S. aureus strains. This study supports the use of S. ochocoa in the central African traditional medicine for the treatment of infectious diseases, and also justifies the predominance of the use of leaves in traditional medicine due to their interesting biological activities.

Acknowledgement

This work was supported by the financial assistance of the International Foundation for Science (IFS) through the grant N°F-4738 “1 and 2” (Dr FEUYA TCHOUYA). The authors thank Mr Yves ISSEMBE, at the National Herbarium of Gabon, Libreville, Gabon. We are also grateful to the “Centre Pasteur du Cameroun” for the supply of microbial strains. We are indebted to the International Center for Chemical and Biological Sciences, University of Karachi, for recording the ^{1}H and ^{13}C NMR spectra. The research stay in Pakistan was sponsored through a TWAS-UNESCO Associateship Scheme fellowship (Dr. FEUYA TCHOUYA).

References

1. World Health Organization (2014) Antimicrobial Resistance: Global Report on Surveillance. Geneva.
2. World Health Organization (2002) Traditional Medicine Strategy 2002-2005. Geneva.
3. Gülçin I, Elmastaş M, Aboul-Enein HY (2007) Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family Lamiaceae) assayed by different methodologies. Phytother Res 21: 354-361.
4. Tchouya GR, Souza A, Tchouankeu JC, Yala JF, Boukandou M, et al. (2015) Ethnopharmacological surveys and pharmacological studies of plants used in traditional medicine in the treatment of HIV/AIDS opportunistic diseases in Gabon. J Ethnopharmacol 162: 306-316.
5. Line-Edwige M, Raymond Ft, François E, Edouard NE (2009) Antiproliferative effect of alcoholic extracts of some Gabonese medicinal plants on human colonic cancer cells. Afr J Tradit Complement Altern Med 6: 112-117.
6. Mengome LE, Akue JP, Souza A, Feuya Tchoua GR, Nsi Emvo E (2010) In vitro activities of plant extracts on human Loa loa isolates and cytotoxicity for eukaryotic cells. Parasitol Res 107: 643-650.
7. Nantia EA, Manfo FPT, Beboy NSE, Moundipa PF (2013) In vitro antioxidant activity of the methanol extract of *Basella alba* L. (Basellaceae) in rat testicular homogenate. Oxid Antioxid Med Sci 2: 131-136.
8. Singleton VL, Rossi JA (1965) Colorimetry of Total Phenolics with Phosphomolybdic Phosphotungstic Acid Reagents. Am J Enol Vitic 16: 144-158.
9. Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493-496.
10. Clinical and Laboratory Standards Institute (2008) Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard. (3rd edn) Wayne, PA.
11. Mingyi W, Hongfang Y, Anxin W, Xinfu P (1999) Facile Total Synthesis of Isopregomisin. J Chem Res (S) 158-159.
12. Rwangabo PC, Claeys M, Pieters L, Corthout J, Vanden Berghe DA, et al. (1988) Umuhengerin, a new antimicrobially active flavonoid from Lantana trifolia. J Nat Prod 51: 966-968.
13. Mona H (1997) Health Benefits Of Morinda Citrifolia: Tahitian Noni Juice. Consumer Health.
14. Okoli AS, Okeke MI, Iroegbu CU, Ebo PU (2002) Antibacterial activity of Harungana madagascariensis leaf extracts. Phytother Res 16: 174-179.
15. Repetto MG, Llesuy SF (2002) Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz J Med Biol Res 35: 523-534.
16. Bruneton J (1999) Pharmacognosie, Phytochimie des Plantes Médicinales. (3rd edn) TEC & DOC, Paris.
17. Kouitcheu Mabeku LB, Kouam J, Penlap BV, Ngadjui BT, Fomum ZT, et al. (2006) Evaluation of antimicrobial activity of the stem bark of Cylicodiscus gabunensis (Mimosaceae). Afr J Tradit Complement Altern Med 4: 87-93.
18. Baba H, Onanuga A (2011) Preliminary phytochemical screening and antimicrobial evaluation of three medicinal plants used in Nigeria. Afr J Tradit Complement Altern Med 8: 387-390.
19. Lamorde M, Tabuti JR, Obua C, Kukunda-Byobona C, Lanyero H, et al. (2010) Medicinal plants used by traditional medicine practitioners for the treatment of HIV/AIDS and related conditions in Uganda. J Ethnopharmacol 130: 43-53.
20. Mugisha MK, Asiimwe S, Namutebi A, Borg-Karlson AK, Kakudidi EK (2014) Ethnobotanical study of indigenous knowledge on medicinal and nutritious plants used to manage opportunistic infections associated with HIV/AIDS in western Uganda. J Ethnopharmacol 155: 194-202.

21. Razafindraibe M, Kuhlman AR, Rabarison H, Rakotoarimanana V, Rajeriarison C, et al. (2013) Medicinal plants used by women from Agnalazaha littoral forest (Southeastern Madagascar). J Ethnobiol Ethnomed 9: 73.

22. Aziz AFA, Iqbal M (2013) Antioxidant activity and phytochemical composition of Cynometra cauliflora. J Exp Integr Med 3: 337-341.

23. Konan K, David NJ, Souleymane M, Ahoua Y, Félix YH, et al. (2013) In vitro antioxidant activity and phenolic contents of the leaves of Olax subscorpioidea and Distemonanthus benthamianus. Res J Pharm Biol Chem Sc 4: 1419-1430.

24. Castro V, Aires A, Dias A (2014) Phytochemical characterization and screening of *in vitro* antioxidant proprieties of extracts obtained from plants of Caatinga Biome (Brazil). Planta Med.

25. Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS (2009) Antioxidant activity of plants methanolic extracts containing phenolic compounds. Afr J Biotechnol 8: 484-489.

26. Faurb M, Lissi E, Torres R, Videla LA (1990) Antioxidant activities of lignans and flavonoids. Phytochemistry 29: 3773-3775.

27. Cabiscol E, Tamarit J, Ros J (2000) Oxidative stress in bacteria and protein damage by reactive oxygen species. Int Microbiol 3: 3-8.

28. Hu JF, Garo E, Yoo HD, Cremin PA, Goering MG, et al. (2005) Cyclolignans from *Scyphocephalium ochocoa* via high-throughput natural product chemistry methods. Phytochemistry 66: 1077-1082.

29. Ali NA, Sharopov FS, Al-Kaf AG, Hill GM, Arnold N, et al. (2014) Composition of essential oil from Tagetes minuta and its cytotoxic, antioxidant and antimicrobial activities. Nat Prod Commun 9: 265-268.

30. Nowak R, Olech M, Pecio L, Oleszek W, Los R, et al. (2014) Cytotoxic, antioxidant, antimicrobial properties and chemical composition of rose petals. J Sci Food Agric 94: 560-567.