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Phytochemical screening, evaluation of antioxidant and antimicrobial properties of *Erythrophleum ivorense* A. Chev (Leguminosae) and *Megaphrynium macrostachyum* Benth (Marantaceae), medicinal plants from Gabon

Sima Obiang Cédric[1,2,3], Ondo Joseph-Privat[1,2*], Obame Engonga Louis-Clément[1,2], Padzys Guy-Stéphane[1,4], Zongo Cheikna[3], BONGUI Jean-Bernard[1,2], Nsi Emvo Edouard[1], Traore Alfred[3]

[1]*Laboratoire de Recherches en Biochimie (LAREBIO), Université des Sciences et Techniques de Masuku, Franceville, Gabon*

[2]*Laboratoire de Substances Naturelles et de Synthèses Organométalliques (LASNSOM), Université des Sciences et Techniques de Masuku, Franceville, Gabon*

[3]*Laboratoires de Biochimie et de Microbiologie, Département de Biochimie-Microbiologie, Université de Ouagadougou, Ouagadougou, Burkina Faso*

[4]*Laboratoire de Physiologie Animale: Electrophysiologie-Pharmacologie-URAB (Abréviation du labo), Université des Sciences et Techniques de Masuku, Franceville, Gabon*

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Abstract

Medicinal plants are used worldwide as an alternative and/or a complementary medicine, thus the valorization of the medicinal plants of our country and determination of their impact on health due to their abundance of substances with various pharmacological effects are our principal objective. The water, water-ethanol and ethanol extracts of *Erythrophleum ivorense* and *Megaphrynium macrostachyum,* were evaluated for antimicrobial and antioxidant activities. The powdered plants samples were analyzed for their phytochemical screening using standard laboratory methods. The total phenols, flavonoid and antioxidant activities were evaluated with methods of the Folin-Ciocalteu, aluminum chloride and Antioxidant Activity Index (AAI) assay, respectively. The antimicrobial activity was evaluated by analyses of diffusion and micro dilution. The phytochemical analysis highlights the presence of total polyphenols and the flavonoids in the extracts of *Erythrophleum ivorense* compared to the extracts of *Megaphrynium macrostachyum*. Water, water-ethanol and ethanol extracts of *Erythropleum ivorense* showed a strong antioxidant activity (AAI_{WE}=1.58; AAI_{WEE} = 2.87; AAI_{EE} = 2.38). The extracts of *Megaphrynium macrostachyum* have antimicrobial and antioxidant activities weak, compared to the extracts of *Erythrophleum ivorense,* Vitamin C and BHA. The use of these plants in traditional medicine is justified and they constitute a source for other traditional investigations.

* **Corresponding Author:** Ondo Joseph-Privat ✉ wansbop70@yahoo.fr

Introduction

In the world, most of the populations in developing countries still rely on traditional medicine practitioners and local medicinal plants for primary health care (WHO, 1995). According to the World Health Organization, traditional medicine is defined as the whole of all practical knowledge to diagnose or eliminate a physical, mental imbalance exclusively while being based on the lived experiment and the observation, transmitted from generation to generation (Adjanohoun *et al.*, 2001). Medicinal plants are plants in which one or more of its organs contain substances that can be used for therapeutic purposes (Daniyan *et al.*, 2011).

The importance of protective defence systems in living cells, against damages caused by reactive oxygen, is well known. Free radicals and other oxidants involvement in the aging process and diseases has been documented (Bruneton, 2009). On the other hand, infectious diseases account for approximately one-half of all deaths in tropical countries (Iwu *et al.*, 1999). Nevertheless, the screening of medicinal plants extracts for antimicrobial and antioxidant activities has shown that higher plants represent potential sources of novel antimicrobial and antioxidant agents (Bruneton, 2009).

Gabon is rich in rare and useful herbs from which medicines can be prepared. Indeed, with roughly 77% of the territory covered by the forest, has one of the largest intact blocks of forest in Africa with an exceptional biodiversity partially described and little or not of study. That constitutes a vast tank of the potential active molecules still unknown if it is considered that a species can produce only hundreds of molecules (Krief *et al.,* 2011). With the aim to study the antimicrobial and antioxidant potentials of the flora in Gabon, ethnopharmacologal approach was carried out. Two plants were selected for this study, among which the bark of *Erythrophleum ivorense* and fruits of *Megaphrinium macrostachyum*. The barks of *Erythrophleum ivorense* are used in traditional medicine for the treatment of chicken pox, of the cutaneous diseases, the washing of the ulcers (Richter and Dallwitz, 2000). In small quantity, these barks of *Erythrophleum ivorense* with the palm oil, give pomade employed against scale (Raponda-Walker and Sillans, 1961; White *et al.*, 1996). Previously, our laboratory have report the antiradical activity of *Megaphrynium macrostachyum* (fruits) through *in vitro* free radicals scavenging assays (Ondo *et al.*, 2013). The crude ethanolic extract show best DPPH free radical-scavenging activity in relation to hydro-ethanolic extract.

The present study was conducted to investigate the antimicrobial property of extracts of *E. ivorense* (leaves) and *M. macrostachyum* (fruits) against eight pathogenic species and their antioxidant property toward the DPPH free radical-scavenging activity, also to determine the classes of chemical constituents of the extracts.

Material and methods

Plant material

The leaves of *Erythrophleum ivorense* and the fruits of *Megaphrynium macrostachyum* were collected in Franceville (Gabon) in June 2013; the plants were then air-dried at room temperature. Identification of the species was carried out at the National Herbarium of IPHAMETRA, Libreville (Gabon). Voucher specimens have been deposited in the Herbarium of IPHAMETRA and at Laboratory of Natural Substances (LASUNA) at the Department of Chemistry-Biochemistry, Faculty of Sciences of USTM in Franceville.

Preparation of plant extract

Water extract, water-ethanol (50/50, v/v) extract, and ethanol extract were prepared from dry powder. 25 g of powder from each sample were soaked with 250 mL of the appropriate solvent mixture and left under shaking conditions at room temperature (25 to 30°C) for 24 h. Each extract was filtered using Whatman N°1 filter paper and solvents were completely removed at low pressure with a rotary evaporator (Büch, Labortechnik, Switzerland). The extracts were then concentrated, freeze-dried and stored at 4°C until analysis.

Phytochemical screening

Each extract was then tested for the presence of flavonoids, coumarins, tannins, total phenolic, saponosids, triterpenoids, alkaloids and anthracenosids as described elsewhere (Culei, 1982; Harbone, 1984; Sofowora, 1993; Trease et Evans, 2002).

Phenolic Content

The total phenolic contents of the different extracts were determined according to the Folin-Ciocalteu Method (Vernon *et al.*, 1999) with minor modifications as described by Zongo *et al.*, (2010) using gallic acid as standard. The absorbance was measured at 735 nm using a multiwell plate reader (μQuant Bio-Tek Instrument, Inc, USA). All analyses were done in triplicate and results (average of triplicate analysis) were expressed as gallic acid equivalent per gram of lyophilized sample.

Flavonoid Content

Total flavonoid contents were determined by the aluminum chloride ($AlCl_3$) colorimetric assay method (Quettier-Deleu *et al.*, 2000) adapted to 96 well-plate, using quercetin as a standard (Zongo *et al.*, 2010). The total flavonoid contents (average of the triplicate analysis) were expressed as Quercetin equivalents in milligrams per gram sample.

Antioxidant Activity Index

The Antioxidant Activity Index (AAI) was assessed according to the method described by Scherer and Godoy. This method is based on the DPPH radical test. Briefly, the working reagent was prepared by dissolving 10 mg of DPPH in 100 mL ethanol. Graded concentrations of extracts ranging from 0.781 to 100 μg/mL obtained by two-fold dilutions were prepared and 100 μL of each dilution were mixed with 100 μL of the working solution of DPPH in a 96-well plate. Absorbencies were measured at 517 nm after 15 min incubation at room temperature in the dark. Ascorbic acid (Vitamin C) and Butylated Hydroxyanisole (BHA) were used as references. The ability to scavenge DPPH radical was calculated by the following equation:

%RSA = [(A control – A sample) / A control] x 100. A = Absorbance at 517 nm.

The IC_{50} (concentration providing 50% inhibition) of extracts and standards was determinate using regression curves in the linear range of concentrations. The AAI was then calculated as follows: AAI = [DPPH] ($\mu g.ml^{-1}$) / IC_{50} ($\mu g.ml^{-1}$).

[DPPH] is the final concentration of DPPH. We considered criteria of Scherer and Godoy according to which plant extracts show poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI between 0.5 and 1.0, strong antioxidant activity when AAI between 1.0 and 2.0, and very strong when AAI > 2.0.

Microorganisms, antibiotics and media

Commercially available antibiotics discs, Nystatin (100 IU) and Kanamycin 10 μg were purchased from Beckton Dickinson. All media used were from Oxoid. Microorganisms included reference strains and fresh clinical isolates. The selection of clinical microorganisms depended on their availability, thus microorganisms that have been reported to be the most frequently implicated in infectious diseases in tropical areas were well represented (Arvouet-Grant *et al.*, 1994; Koudou *et al.*, 2008).

Clinical isolates were *Pseudomonas aerugenosa*, *Shigella flexneri* and *Candida albicans*. All these strains were isolated from clinical samples at Laboratory of Biochemistry of the Medical Center of USTM, Franceville. The microorganisms were identified by the use of their biochemical profiles as recommended by the manual "Bactériologie Médicale" (Le Minor and Véron, 1982). The reference strains were *E. coli* ATCC O157, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* ATCC 25293, *Enterococcus faecalis* CIP 103.907, *E. coli* CIP 25922.

Antibacterial assays

Agar-well diffusion

The assay was conducted as described by Perez *et al.*, (1991). Briefly, microorganisms from growth on

nutrient agar incubated at 37 °C for 18 h were suspended in saline solution 0.9% NaCl and adjusted to a turbidity of 0.5 Mac Farland standards (10^8 CFU/ml) (Lennette *et al.*, 1981). The suspension was used to inoculate 90 mm diameter Petri plates with a sterile nontoxic cotton swab on a wooden applicator. Six millimeters diameter wells were punched in the agar and filled with 50 µL of 2000 µg/mL plant extract. The dissolution of the extract was added by 0.5% (v/v) DMSO which did not affect microorganism growth, according to our control experiments. Commercial antibiotics were used as positive reference standard to determine the sensitivity of the strains. Discs were directly placed onto the bacterial culture. Plates were incubated aerobically at 30 or 37°C for 24 h. Antimicrobial activities were evaluated by measuring inhibition zone diameters (IZD).

Broth microdilution assay

Broth microdilution method was used to determine minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of the extract against the test microorganisms as recommended by the National Committee for Clinical Laboratory Standards (Performance Standards for Antimicrobial Susceptibility Test, 1999; Methods for dilution, 2000). The tests were performed in 96 well-plates. Extracts were dissolved in 0.5% DMSO was transferred in plates to obtain a two-fold serial dilutions ranging from 4.8 to 2500 µg/mL. Then plates were inoculated with microbial suspensions diluted from the same 0.5 Mac Farland standards to have 10^8 CFU/mL in each well (Lennette *et al.*, 1981). The final volume in wells was 200 µL. After 24 h incubation in air at 37°C, MIC was recorded as a lowest extract concentration demonstrating no visible growth in the broth. MBC was recorded as a lowest extract concentration that kills 99.9% of bacterial inocula. MBC values were determined by removing 100 µL of bacterial suspension for subculture demonstrating no visible growth and by inoculating nutrient agar plates. Plates were incubated aerobically at 37 °C for a total period of 48 h (Koudou *et al.*, 2008).

Statistical analysis

Experimental results were expressed as mean ± standard deviation. All measurements were duplicated three times. The IC_{50} values were calculated using linear regression analysis from the graph of scavenging effect percentage against extract concentration.

Results

Phytochemical screening

The phytochemical screening made it possible to obtain the various types of natural substances coming from *Erythrophleum ivorense* and *Megaphrynium macrostachyum* **(**Table 1). The phytochemical sifting certifies the presence of several secondary metabolites in the extracts.

Table 1. Phytochemical screening of *Erythrophloeum ivorense* and *Megaphrynium macrostachyum*.

Chemical constituents		*E. ivorense*			*M. macrostachyum*		
		Water extract	Water-ethanol extract	Ethanol extract	Water extract	Water-ethanol extract	Ethanol extract
Flavonoids		++	++	++	++	++	-
Tannins	Gallic	++	++	+	-	-	-
	Catechin	-	-	-	++	+	+
Coumarins		+	-	-	++	++	+
Total phenolic		++	++	++	++	+	+
Anthracenosides		++	++	+	-	-	-
Saponosids		++	+	-	++	++	-
Triterpenoids		++	++	+	++	++	+
Alkaloids		++	+	-	+	-	-

++ =Abundant; + = not abundant; — = Not Detected.

Total phenolic and total flavonoid contents

The contents of total phenolic, and total flavonoids, total of extracts from *Erythrophleum ivorense* and *Megaphrynium macrostachyum* are presented in table 2. Levels of phenolic content were expressed in terms of gallic acid equivalent (GAE). The equation of the righth and side of the proportioning of total phenolic content by the method of Folin-Ciocalteu gave Y = 0.0038X – 0.0026 with R^2 = 1. It appeare that water-ethanol extract of *Erythrophleum ivorense* had the highest content of phenolic compounds (1434.6 ± 0.6 mg GAE/10 g of extract) and Ethanol extract of *Megaphrynium macrostachyum* had the lowest content (66.2 ± 0.1 mg GAE/10 g of extract). Water and water-ethanol extracts from *Megaphrynium macrostachyum* show intermediate phenolic content followed by water and ethanol extracts of *Erythrophleum ivorense* with 119 ± 0.5, 171.4 ± 0.2, 119 ± 0.23 and 434.6 ± 1 mg GAE/10 g of extract, respectively. Total flavonoid contents were determined by the aluminium chloride ($AlCl_3$) colorimetric assay method. The equation of the right-hand side of the proportioning of the flavonoid gave Y = 0.0005X + 0.016 with R^2 = 0.969. Among extracts, flavonoids contents had ranged between 8.4 ± 0.2 and 156 ± 0.3 mg QE/10 g of extract (Table 2).

Table 2. Total phenolic content (TPC) and Total flavonoid content (TFC) of extracts from *Erythrophleum ivorense* and *Megaphrynium macrostachyum*.

Plants	Extracts	TPC (mg GAE/10 g)	TFC (mg QE/10 g)
E. ivorense	WE	119 ± 0.23	16.8 ± 0.2
	WEE	1434.6 ± 0.6	143.8 ± 0.3
	EE	434.6 ± 1	156 ± 0.3
M. macrostachyum	WE	119 ± 0.5	86.8 ± 0.8
	WEE	171.4 ± 0.2	92.2 ± 0.1
	EE	66.2 ± 0.1	8.4 ±0.2

EE = Ethanol extract; WEE = water-ethanol extract; WE = water extract.

Erythrophleum ivorense contain the highest amount of flavonoids (156 ± 0.3 and 143.8 ± 0.3 mg QE/10 g of extract, respectively for ethanol and water-ethanol extracts) and *Megaphrynium macrostachyum* had revealed weak flavonoids contents (92.2 ± 0.1 and 86.8 ± 0.8 mg QE/10 g of extract, respectively for water-ethanol and water extracts).

Antioxidant Activity Index

The results of the antioxidant activity of the water, water-ethanol and ethanol extracts of *Erythrophleum ivorense* and *Megaphrynium macrostachyum* are presented by table 3. The AAI of the extracts from *Erythrophleum ivorense* ranged from 1.58 to 2.87 and can be compared to AAI of Vitamin C and BHA (AAI values of 5.42 and 3.76 respectively) while those of *Megaphrynium macrostachyum* ranged from 0.45 to 1.05.

Table 3. Antioxidant activity of *Erythrophleum ivorense* and *Megaphrynium macrostachyum* extracts by DPPH free radical scavenging method.

Drugs	Extracts	Regression curve's equations	R^2	IC_{50} (µg.mL^{-1})	AAI
E. ivorense	WE	Y=1.67X – 0.43	0.99	31.51 ± 0.1	1.58
	WEE	Y= 2.58X + 5.01	0.98	17.43 ± 0.25	2.87
	EE	Y=2.12X + 3.91	0.98	21.04 ± 0.9	2.38
M. macrostachyum	WE	Y= 1.05X – 0.43	0.95	47.75 ± 1.1	1.05
	WEE	Y=0.46X + 0.17	0.95	109.4 ± 0.8	0.45
	EE	Y=0.7X + 0.52	0.93	71.65 ± 0.45	0.69
Standards	Vit C	Y=5.24X + 1.68	0.98	9.22 ± 0.5	5.42
	BHA	Y =3.74X + 0.29	0.99	13.29 ± 0.2	3.76

EE = Ethanol extract; WEE = water-ethanol extract; WE = water extract.

Inhibition zone diameters by disc assay of Erythrophleum ivorense and Megaphrynium macrostachyum

The inhibition zone diameters (IZD) obtained in the antimicrobial susceptibility assays for the plants extracts and standard antimicrobial drug discs are presented in Table 4. The highest IZD was recorded with the ethanol extract of *Erythrophleum ivorense* (EiEE) against *Shigella flexneri* (16 mm) followed by water-ethanol extract (EiWEE) against the same microorganism (14 mm). With the exception of *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *E. coli* CIP 25922, all tested bacteria showed sensitivity to both plants extracts but the efficiency of the extracts in inhibition was varied from one microorganism to another and also depends on type of extract and plant. *Megaphrynium macrostachyum* extracts exhibited lesser inhibitory effect comparatively to those of *Erythrophleum ivorense*. *M. macrostachyum* and *E. ivorense* extracts show antifungal activity.

Table 4. Inhibition zone diameters (mm) produced by the extracts from *Erythrophleum ivorense* and *Megaphrynium macrostachyum* in disc diffusion.

Bacteria	Inhibition zone diameters (mm)							
	E. ivorense			*M. macrostachyum*			Standards	
	Extracts							
	WE	WEE	EE	WE	WEE	EE	Kana	Nyst
E. coli ATCC 0157	10	15	15	7	7	7	25	Nd
E. coli CIP 25922	8	8	8	Nd	Nd	Nd	20	Nd
Shigella dysenteriae CIP 5451	7	8	10	Nd	Nd	Nd	25	Nd
Shigella flexneri	8	14	16	7	7	7	27	Nd
Staphylococcus aureus ATCC 9144	8	11	14	Nd	7	7	30	Nd
Enterococcus faecalis CIP 10907	10	11	11	7	7	7	32	Nd
Pseudomonas aeruginosa ATCC 19249	9	10	11	Nd	Nd	Nd	15	Nd
Fungi								
Candida albicans	8	8	7	7	7	7	20	20

Nd = not determinated; Kana = Kanamycin (10 µg); Nyst = Nystatin (100IU); EE = Ethanol extract; WEE = water-ethanol extract; WE = water extract.

Broth microdilution assay

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values summarized in Table 4 confirmed the antibacterial and antifungal activity of the plants. The lowest MIC (0.625 mg/mL) was recorded with the water extract of *E. ivorense* on *Pseudomonas aeruginosa*, *Candida albicans;* the water-ethanol extract of *E. ivorense* on *Shigella flexneri*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Enterococcus faecalis* CIP 10907, *Pseudomonas aeruginosa* ATCC 19249, *Candida albicans* and ethanol extract (EiEE) on *E. coli* ATCC 0157, *Staphylococcus aureus*, *Enterococcus faecalis* CIP 10907, *Pseudomonas aeruginosa* ATCC 19249. The lowest MBC (1.25 mg/mL) was observed with EiWE on *Pseudomonas aeruginosa* ATCC 19249; EIWEE on *E. coli* ATCC 0157, *Enterococcus faecalis* CIP 10907, *Pseudomonas aeruginosa* ATCC 19249 and EIEE on *E. coli* ATCC 0157, *Enterococcus faecalis* CIP 10907, *Pseudomonas aeruginosa* ATCC 19249.

Discussion

Phytochemical screening

The phytochemical screening of the extracts was first performed to detect the major chemical groups occurring in the extracts. In view of the results in table 1, it appears that two plants studied *E. ivorense* (stem bark) and *M. macrostachyum* (fruit) contain tannins, terpenoids, flavonoids and polyphenols. In

addition to these compounds, *E. ivorense* contains anthracenosids and alkaloids in water and water-ethanol extracts, abundance of compounds polyphenols, flavonoids, tannins gallic and triterpenoids justifies the use of these plants in Gabonese traditional medicine (Mandalari *et al.*, 2007; Andzi *et al.*, 2015). While the fruits of *M. macrostachyum* show a strong presence of saponins in the water and water-ethanol extracts. The results corroborate with those of Ondo *et al.*, (2013) on the screening of *M. macrostachyum*. All of these bioactive secondary metabolites identified in the various drugs have many pharmacological properties assigned to them (Bruneton, 2009). These properties from compounds found in the extracts of the two plants suggest that they can be used in pharmaceuticals.

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or fungicidal concentration (MFC) in mg/ml obtained by microdilution method.

Bacteria	MIC and MBC (mg/mL)											
	E. ivorense						*M. macrostachyum*					
	Extracts											
	WE		WEE		EE		WE		WEE		EE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli ATCC 0157	1.25	2.5	1.25	2.5	0.625	1.25	1.25	>2.5	1.25	>2.5	1.25	>2.5
Shigella flexneri	1.25	>2.5	0.625	2.5	1.25	>2.5	1.25	>2.5	1.25	>2.5	1.25	>2.5
Shigella dysenteriae CIP 5451	1.25	>2.5	0.625	2.5	1.25	2.5	1.25	nd	1.25	nd	2.5	nd
E. coli CIP 25922	1.25	>2.5	1.25	>2.5	1.25	>2.5	nd	nd	nd	nd	nd	nd
Staphylococcus aureus ATCC 9144	1.25	>2.5	0.625	2.5	0.625	2.5	1.25	>2.5	1.25	>2.5	1.25	>2.5
Enterococcus faecalis CIP 10907	1.25	2.5	0.625	1.25	0.625	1.25	nd	nd	nd	nd	nd	nd
Pseudomonas aeruginosa ATCC 19249	0.625	1.25	0.625	1.25	0.625	1.25	nd	nd	nd	nd	nd	nd
Fungi	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Candida albicans	0.625	2.5	0.625	2.5	1.25	>2.5	1.25	>2.5	1.25	>2.5	1.25	>2.5

Nd = not determinated; EE = ethanol extract; WEE = water-ethanol extract; WE = water extract.

Total phenolic and total flavonoid contents

Levels of phenolic content were expressed in terms of gallic acid equivalent (GAE). The equation of the right and side of the proportioning of total phenolic content by the method of Folin-Ciocalteu gave: y = 0.0038x - 0.0026, R^2 = 1. It appeared that water-ethanol extract (WEE) of *Erythrophleum ivorense* had the highest content of phenolic compounds (1434.6 ± 0.6 mg GAE/10 g) and water–ethanol extract (WEE) of *Megaphrynium macrostachyum* had the lowest content (66.2 ± 0.1 mg GAE/10 g). The phytochemical screening had indicated that the extracts of *Erythrophleum ivorense* had a total polyphenol abundance compared to the extracts of *Megaphrynium macrostachyum*, the study quantitative of polyphenols confirm them results. Phenolic substances have been suggested to have a preventive role in the development of chronic diseases such as cancer and heart disease (Njintang *et al.*, 2012; Sima *et al.*, 2015). They are also known to possess antibacterial, antiviral, antimutagenic and anticarcinogenic properties (Moure *et al.*, 2001; Manach *et al.*, 2004; Feuya *et al.*, 2015). Totals flavonoids (standard curve equation: y = 0.0005x + 0.016, R^2 = 0.969) were more abundant in ethanol (156 ± 0.3 mg QE/10 g) and water-ethanol extracts (143.8 ± 0.3 mg QE/10 g) of *Erythrophleum ivorense* than water (86.8 ± 0.87 mg QE/10 g) and water-

ethanol extracts (92.2 ± 0.1 mg QE/10 g) of *Megaphrynium macrostachyum*.

Antioxidant Activity Index

Water, water-ethanol and ethanol extracts of *Erythrophleum ivorense* have a strong antioxidant activity whose AAI are respectively 1.58; 2.87; 2.38. Vitamin C and BHT are the antioxidant of references. These extracts have a potential antioxidant which would enable them to play a beneficial role in terms of very significant preventive actions for human and animal health (Sabu and Kattan, 2002). Antioxidant activity of the plant should be at least partially justified by the presence of phenolic and the flavonoids highlighted by the phytochemical study (Yokozawa *et al*., 1999; Bors *et al*., 1990; Andzi *et al*., 2015). Water extract of *Megaphrynium macrostachyum* has a strong antioxidant activity whose AAI is 1.05 whereas its ethanol extract (AAI = 0.69) presents a weak antioxidant activity. It should be noted that water-ethanol extract of *Megaphrynium macrostachyum* (AAI = 0.45) has the weakest antioxidant activity compared to other extracts. More recently, some scientific studies on the biological activities of *Megaphrynium macrostachyum* were carried out. Our results corroborate with those of Ondo (2013) on antioxidant activities of these extracts of plants.

Inhibition zone diameters of by disc assay of Erythrophleum ivorense and Megaphrynium macrostachyum

Water-ethanol extract of *Erythrophleum ivorense* has a great inhibiting activity on the stocks of *E. coli* ATCC 0157 (15 mm), of *Shigella flexneri* (14 mm), *of Staphylococcus aureus* ATCC 9144 (11 mm), *Enterococcus faecalis* CIP 10907 (11 mm*)* and of *Pseudomonas aerugenosa* ATCC 19249 (10 mm). Antibacterial activities were also observed with *Shigella dysenteria* CIP 5451 (8 mm) and *E. coli* CIP 25922 (8 mm). Water-ethanol extract has a weak antifungal activity on *Candida albicans* (8 mm). Ethanol extract of *Erythrophleum ivorense* presented greatest inhibitions on *E. coli* ATCC 0157 (15 mm), *Shigella flexneri* (16 mm), *Staphylococcus aureus* ATCC 9144 (14 mm), *Pseudomonas aerugenosa* ATCC 19249 (11 mm). The weakest antibacterial activities and antifungal of the extract were successively observed on *E. coli* CIP 25922 (8 mm) and on *Candida albicans* (7 mm). However, water extract of this plant is active only on *E. coli* ATCC 0157 (10 mm) and on *Enterococcus faecalis* CIP 10907 (10 mm). This study proves that these extracts of plants prevent the growth of several of the micro-organisms used, that testifies the presence to the antimicrobial compounds to these plants.

Broth microdilution assay

Water extract of *Erythrophleum ivorense* has an effect bacteriostatic on *E. coli* ATCC 0157, *Enterococcus faecalis* CIP 10907 and on *Pseudomonas aerugenosa* ATCC 19249. This water extract has an effect on the fungi stock of *Candida albicans*. Other stocks like *Shigella flexneri*, *Staphylococcus aureus* ATCC 9144, *Shigella dysenteria* CIP 5451 and *E. coli* CIP 25922 are resistant to water extract of *Erythropleum ivorense*. Ethanol extract of the latter presents an effect bacteriostatic on *E. coli* ATCC 0157, *Shigella dysenteria* CIP 5451, *Enterococcus faecalis* CIP 10907 and on *Pseudomonas aerugenosa* ATCC 19249. The stocks *Staphylococcus aureus* ATCC 9144 and *Enterococcus faecalis* CIP 10907 have an effect of resistance on ethanol extract. Water-ethanol extract has a bacteriostatic action on *E. coli* ATCC 0157, *Enterococcus faecalis* CIP 10907 and *Pseudomonas aerugenosa* ATCC 19249.

The other stocks are resistant to the water-ethanol extract. *Candida albicans* has resistances on the ethanol and water-ethanol extracts of *Erythropleum ivorense*. This whole of results militates in favor of the use of *Erythrophleum ivorense* into traditherapeutic traditional in the treatment of the diarrheal diseases and other bacterial infections. Extracts of *Megaphrynium macrostachyum* do not have considerable effects on the stocks not highlighted. Several studies often bind the biological activities of the plants to the phenolic compounds which they contain, (Cicerale *et al*., 2010; Xia *et al*.,

2010) our results prove that the extracts with the high level of the phenolic compounds were also more active against micro-organisms where an antioxidant activity is high.

Conclusion

With the resulting one from the investigations on the two medicinal plants selected on the basis of their traditional use, it arises that these plants have antimicrobial and antioxidant activities, in vitro. *Erythrophleum ivorense* has more antibacterial activities, antifungal and antioxidant that *Megaphrynium macrostachyum* these activities are correlated with the presence of the contents of made up phenolic. More especially as we did not detect alkaloids necessary in these plants.

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