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IN VITRO EVALUATION OF THE ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *ALSTONIA BOONEI* AND *GAMBEYA AFRICANA* MEDICINAL PLANTS

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ABSTRACT: Plants have always been considered as an important source of medicine for human and phytomedicine has been an integral part of traditional health care system in most parts of the world for thousands of years. Research has shown the potential exploitation of plant products as a source of new bioactive compounds. Chloroform, methanol and water extracts of *Alstonia boonie* and *Gambeya africana* were evaluated for phytochemical screening, antioxidant and antimicrobial activities. Antioxidant Activity Index (AAI) was determined for antioxidant activity evaluation. The antimicrobial activity was evaluated by disc diffusion and microdilution assays. The result revealed that the extracts of *Gambeya africana*, are rich in bioactive compounds. Flavonoids, proanthocyanidins and anthocyanins are abundant in all extracts. Water extract of *Alstonia boonei* and methanol extract of *Gambeya africana* exhibited very strong antioxidant activities. *Alstonia boonie* and *Gambeya africana* show a well-defined inhibition on strains tested. The extracts are active on Gram positive and negative bacteria. The aqueous extract of *Alstonia bonnei* has been very active and has bactericidal activity on *Staphylococcus aureus*, *Staphylococcus camorum*, *Streptococcus pyogenes*, *Pseudomonas aerugenosa* and *Salmonella enterica*. The study confirms the multiple uses of *Alstonia boonie* and *Gambeya africana* for the treatment of many infectious diseases and place them as candidate for further investigations for traditional drug utilizable as complementary and alternative medicines development and new active compounds.

Keywords: Medicinal plants, *Alstonia boonie*, *Gambeya africana*, antioxidant and antimicrobial activities.

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1.INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases and continue to do important services at human being. Plants have been an increased interest in the use of natural antimicrobial agents thus the use of these combinations is strategies to control food-borne bacteria and other pathogenic microorganisms [1]. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties but recent research demonstrates that many phytochemicals can protect humans against diseases. Despite the development of the pharmaceutical industry, more than 80% of the world's populations continue to use herbal remedies to treat common diseases [2]. These plants have served as a very important replacement for the resistant strains. Hence the useful achieve the phytochemical studies of medicinal plants for discovery of news bioactive compounds. As such the WHO is currently supporting clinical validation of some traditional medicines. *Alstonia boonei* De Wild (Ekouk in Fang) is a large tree with rough bark, uncommon outside the forested Guinea. The greyish bark is thick and rough with an abundant white latex, bitter taste that coagulates giving a resin, hardening very quickly. Single leaves are whole, whorled and whitish. The large flowers with green corolla are prolonged by 5 yellow lobes. The seeds are provided at both ends of egrets. The wood is white, yellowish and light. The wood of this species is used for making combs, masks, harps, seats and drums. The latex makes it possible to obtain a very active purgative [3]. The latex resin is used against yaws. This latex is used as a poison. Latex is dangerous to the eyes and causes blindness. The bark is an effective remedy against gonorrhea. It is also used for its galactogenic properties. Bark infusion treats stomach upset, fever and malaria [4]. *Gambeya africana* Pierre (Abam in Fang) is located in tropical Africa (West and East Africa), occurs in primary or virgin forest. It is a large tree with gray bark, streaked with shallow wrinkles. The underside of the leaves is rubiginous. The flowers are yellowish. The heartwood is brown or red-gray. The color of the sapwood is different from the wood. The wall fibers are of medium thickness. The axial parenchyma is present in lines. The rays are composed of two types of square cells. The disjointed walls of ray cells are clearly visible. The fruit is the shape and size of a big orange. The acidulated fruits are eaten when they are ripe. The seeds are oleaginous [3]. Phytochemical work by Wandji et *al*. isolated five new fatty acid esters of erythodiol [5]. The present work reports results of phytochemical screening, antioxidant and

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antimicrobial proprieties of *Alstonia boonei* and *Gambeya africana*, medicinal plants from Gabon. Such a study may help in the contribution of the ongoing search for beneficial uses of these plants to eradicate various multi-resistance infectious diseases.

2. MATERIALS AND METHODS

Plant materials

The bark of *Alstonia boonei* and *Gambeya africana* were collected in June 2017 from Mebane Endama village, in Oyem (Northern of Gabon). Identification of the species was carried out at National Herbarium of IPHAMETRA, Libreville (Gabon). Voucher specimens have been deposited in Herbarium of IPHAMETRA (Gabon checklist: *Alstonia boonei* De Wild. Lit.: Jong, B.H.J. de 1979. Meded. Landbouwhogeschool Wageningen 79–13: 5. Prov.: ES, NG, OI, OL, WN. Alt.: 200–500 m) and inLaboratory of Research in Biochemistry (LAREBIO) at Department of Chemistry, Faculty of Sciences of USTM in Franceville-Gabon.

Compoud extraction and qualitative analysis

The harvest samples were dried in the laboratory at room temperature, afterwards they were pulverized with a mechanical crusher. The powder was used for several extractions. For water extraction, 50 g of powder were percolated for 24 h in 500 mL water. After cooling at room temperature (25°C), the extract was filtred and lyophilised. For methanol extraction, 50 g of powder were percolate in 500 mL of methanol for 24 h. For chloroform extract, 50 g of powder were percolated with 500 mL of chloroform for 24 h. Methanol and chloroform were evaporated with a rotary evaporator. The extract was then washed with hexane in order to eliminate the chlorophyll and other pigments. The solvent were evaporated and the extract was finally lyophilised. For the test, aqueous extract was dissolved in distilled water. Chloroform and methanol extracts were dissolved in dimethyl sulfoxide (DMSO) and a serial dilution was made to have a final concentration of 0.5% DMSO to obtain a twofold serial dilutions ranging from 0.10 to 1000 mg/mL. Qualitative tests were performed on each extract for chemical groups such as carotenoids, flavonoids, reducing compounds, saponosids, sterols, tannins, triterpens, phenolic compounds, proanthocyanidines and anthracenoside detection as described by Ciulei [6].

Quantitative analysis of phytochemicals

Phenolic content: The Folin-Ciocalteu method was used to measure total amount of phenolic content [7]. The original assay was adapted to a microtiter 96-wells plate system. Gallic acid (3, 4, 5-trihydroxybenzoic acid) was used as standard. To 20 µL of beverage, 80 µL of Folin-Ciocalteu reagent were added. After 5 min incubation at room temperature (25°C), 80 µL of 20% (w/v) sodium carbonate solution was added and incubated. After 30 min of incubation, the absorbances were read at 760 nm. All tests were carried out in triplicate and results were expressed as gallic acid equivalent (GAE).

Tannins: The reference method of European communauty was used to measure total amount of

tanninsin 1994 [8].

Proanthocyanidins: The method was quantified with an adaptation to a 96-well plate assay [9]. It involved the hydrolysis of proanthocyanidins in a hot acid-alcohol medium into anthocyanidins. This method allows taking into account all the units of flavan-3-ol constituting the polymers. The heating step destroys the anthocyanidins pigments generated by flavan-4-ol and eliminates part of the chlorophyll pigments. The routine assay is performed by mixing 50 µL of the extract with 700 µL of 30% HCl-butanol solution (v/v). The mixture was put in tightly closed 1.5 mL Eppendorf tube and vortexed for 1 min. Subsequently, the tube was heated at 100°C for 2 h and after cooling, 200 µL aliquots were put in triplicate into a 96 multiwell plate and the absorbances were read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as aforementioned were used as a standard. Results were expressed as apple procyanidins equivalent (APE) [10].

Flavonoid: The Dowd method was used to measure total amount of flavonoids [11]. The original assay was adapted to a microtiter 96-wells plate system. Quercetin was used as standard. 100 µL of $AlCl_3$ 2% in methanol were added to 100 µL of extract. After 10 min incubation at room temperature (25°C), the absorbances were read at 415 nm. All tests were carried out in triplicate and results were expressed as qercetin equivalent (QE) [9].

Microorganisms and antibiotics

The reference strains were *Escherichia coli* CIP 105182, *Enterococcus faecalis* CIP 103907, *Bacillus cereus* LMG 13569 BHI, *Listeria innocua* LMG 135668 BHI, *Staphylococcus aureus* ATCC 25293 BHI, *Staphylococcus camorum* LMG 13567 BHI, *Proteus mirabolis* CIP 104588, *Shigella dysenteria* CIP 5451 and *Staphylococcus aureus* ATCC 9144. Clinical isolates were *Staphylococcus aureus* (n = 5), *Enterococcus faecalis* (n = 5), *Pseudomonas aerugenosa* (n = 10), *Salmonella enterica* (n = 5) and *Streptococcus pyogenes* (n = 10). All these strains were isolated from clinical samples at Laboratory of Research in Biochemistry of USTM, Franceville Gabon. Commercially available antibiotics discs, Penicillin (10 IU/IE/UI) and Tetracyclin (30 µg) were purchased from Beckton Dickinson.

Determination of antioxidant activities

Determination of Antioxidant Activity Index (AAI): The Antioxidant Activity Index was assessed according to the method described by Scherer and Godoy in 2009 [12]. This method is based on 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:

$\%RSA = [(A_{control} - A_{sample}) / A_{control}] \times 100$.

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 in 2009, 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 [12].

Determination of Relative Antioxidant Activity (RAA%): The antioxidant capacity of the extracts was determined according to method described by Dapkevicus [13]. 0.5 mg ofβ-carotene was dissolved in 1 mL of chloroform (HPLC grade); 25 µL of linoleic acid and 200 mg of tween 40 were added as emulsifier because β-carotene is not water soluble. Chloroform was completely evapored using a vacuum evaporator. Then, 100 mL of distilled water saturated with oxygen was added with vigorous shaking at a rate of 100 mL/min for 30 min; 2500 µL of this reaction mixture was dispersed to test tubes, and 350 µL portions of extracts, prepared in 2 mg/mL concentrations, were added. The emulsion system was incubated for up to 48 h at laboratory temperature. The same procedure was repeated with a positive control BHA and a blank. After this incubation time, the absorbance of the mixture was measured at 490 nm. Antioxidant capacities of extracts were compared with those at BHA and blank. Tests were carried out in triplicate. The relative antioxidant activity (RAA %) of the extracts was calculated from the equation: $RAA (\%) = (A_{Sample} / A_{BHA}) \times 100$. Where A_{BHT} is the absorbance of the positive control BHA and A_{Sample} is the absorbance of the extract.

Antibacterial assays

Agar-well diffusion: The assay was conducted as described by Perez et *al*. in 1991 [14]. 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 (10^8CFU/mL). 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 extract. The dissolution of the extract was aided 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 in air at 37°C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters (IZD). The experiments were conducted twice [15; 16].

Broth microdilution assay: Broth microdilution method was used to determine minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of extract against the test microorganisms as recommended by National Committee for Clinical Laboratory Standards [17]. The tests were performed in 96 well-plates. Extract dissolved in 0.5% DMSO was transferred in plates to obtain a twofold serial dilutions ranging from 0.25 to 400 µg/mL. Then plates were inoculated with microbial suspensions diluted from the same 0.5 Mac Farland standards to have 10^8CFU/mL in each well. The final volumes in wells were 200 µL. After 24 h incubation in air at 37°C, MIC was recorded as a lowest extract concentration demonstrating no visible growth in broth. MBC was recorded as a lowest extract concentration killing 99.9% of bacterial inocula [18; 19]. MBC values were determined by removing 100 µL of bacterial suspension from subculture demonstrating no visible growth and inoculating nutrient agar plates. Plates were incubated at 37°C for a total period of 48 h [20; 21].

Statistical analysis

Data were expressed as mean ± SEM. A one way variance was used to analyse data. $P< 0.01$ represented significant difference between means (Duncan's multiple range test).

3. RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of the extracts was first performed to detect the major chemical groups. The results of screening are shown in table 1.

Table 1. Phytochemical screening of *Alstonia boonei* and *Gambeya africana*

Chemical	*Alstonia boonei*			*Gambeya africana*		
Groups	Chloroform	Methanol	Water	Chloroform	Methanol	Water
Anthracenosids	+	+	++	++	+	++
Anthocyanes	-	-	-	++	++	++
Alkaloids	+	+	+	++	++	+++
Carotenoids	++	++	++	+	+	++
Coumarins	+++	++	+++	++	++	+++
Flavonoids	+++	++	++	+++	+++	+++
Tanningallic	+	+	+	+	+++	++
Tannin catechic	+	+	-	+	++	++
Total phenol	++	+++	+++	+	++	+++
Total flavonoids	+++	++	+	+++	+++	++
Saponosids	-	-	+++	-	-	+++
Sterol and Triterpenoids	+	-	-	+	++	+++
Proanthocyanidin	+	+	+	++	+++	+++

Reducing compounds	+++	-	++	+++	-	++

+++ = Very abundant; ++= Abundant; + = Rare; — = Not Detected.

It appears that two plants studied *Alstonia boonei* and *Gambeya africana* contain phenolic compounds and triterpenoids. The extracts of *Gambeya africana* are rich in biactive compounds. Flavonoids, proanthocyanans and anthocyanins are abundant in all extracts. The aqueous and methanolic extracts are rich in tannins, reducing compounds and phenolic compounds. The aqueous extract is rich in saponosides. Chloroform extract of *Alstonia boonei* is rich in flavonoids and reducing compounds. Saponosids are abundant in aqueous extract.Several works have demonstrated that phenolic compounds confer to the plant several biologic activities. This abundance in phenol compounds would confirm the therapeutic properties that there are assigned in ethnotherapy. All of these bioactive secondary metabolites identified in the various drugs have many pharmacological properties. These properties from compounds found in the extracts of these plants suggest that they can be used in pharmaceuticals.

Phenolic content

The results show that the bark of *Alstonia boonei* and *Gambeya africana* contains extractable polar compounds superior to non-polar compounds (Table 2).

Table 2. Total phenolic content (TPC), total flavonoid content (TFC), total tannins content (TTC) and total proanthocyanidins content (TPC) from *Alstonia boonei* and *Gambeya africana*

Extracts	TPC (mg GAE/ 100 g of extract)	TFC (mg QE/ 100 g of extract)	TTC (mg TAE/ 100 g of extract)	TPC (mg APE/100 g of extract)
Ab WE	Nd	462.40±6.78	255.78±27.77	175.43±11.67
Ab CE	Nd	1092.71±7.34	1835.78±11.66	556.83±13
Ab ME	442.17±2.50	783.23±3.26	139.11±18.06	198.77±26.17
Ga WE	2206.75±3.25	572.50±4.65	1187.63±9.70	1379.90±11.31
Ga CE	Nd	1159.90±9.90	538.00±6.71	1331.33±15.81
Ga ME	1477.17±2.08	1033.96±3.19	3457.27±8.14	5189.33±13.11

Ab = *Alstonia boonei*; Ga = *Gambeya africana*; WE = Water extract; ME=Methanol extract; CE= Chloroform extract; Nd = Non determined.

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 Folin-Ciocalteu method gave $Y = 0.0012 X – 0.0004$ with $R^2 = 0.9902$ [22]. Total flavonoid content (standard curve equation: $Y = 0.0032 X + 0.007 7$, $R^2 = 1$) was determined in comparison with standard quercetin and the results expressed in terms of mg QE/100 g of extract. The HCl/butanol assay used here for the determination of proanthocyanidins is more specific than many other tests such as vanillin assay.

Levels of proanthocyanidins were expressed in terms of apple procyanidins equivalent (APE) [23; 24]. The equation of the right-hand side of the proportioning of proanthocyanidins by HCl-Butanol method gave Y = 0.0006 X + 0.0024 with R^2 = 0.9869 [22].The results reveal that nonpolar extractable compounds are predominant in the bark of *Alstonia boonei*. The concentration of proanthocyanidines (556.83 mg APE/100 g of extract), flavonoids (1092.71 mg QE/ 100 g of extract) and tannins (1835.78 mg TAE/ 100 g of extract) is greater in chloroform extract whereas those of total phenols are greater in aqueous and methanolic extracts. The yield of water, chloroform and methanol of *Gambeya africana* were 4.43% (v/v), 1.49% (v/v) and 2.76% (v/v), respectively. The results reveal that the bark of *Gambeya africana* contains a strong presence of polar extractable compounds superior to nonpolar ones. The concentration of proanthocyanidines (1477.17 mg APE/100 g of extract) and tannins (3457.27 mg TAE/ 100 g of extract) is much greater in methanolic extract whereas those of total phenols (2206.75 mg GAE/ 100 g of extract) and flavonoids (1159.90 mg QE/ 100 g of extract) are greater in water and chloroform extracts, respectively.

Antioxidant activity

The antioxidant activities of the extracts are provided in table 3.

Table 3. Antioxidant activities of *Alstonia boonei* and *Gambeya africana* extracts

	Antioxidant activity		Extracts	
			Water	Methanol
Alstonia boonei	DPPH	IC_{50} (µg/mL)	47.43±0.15	49.00±0.19
		AAI	2.31	1.60
		Activity	Very strong	Strong
	β-carotene	RAA (%)	41.20±0.05	44.40±0.05
	DPPH	IC_{50} (µg/mL)	23.80±0.50	9.22±0.02
Gambeya africana		AAI	1.61	8.54
		Activity	Strong	Very strong
	β-carotene	RAA (%)	54.10±0.05	74.60±0.05
Vitamin C		IC_{50} (µg/mL)	7.12 ± 0.60	
		AAI	7.02	
		Activity	Very strong	
BHA		IC_{50} (µg/mL)	6.59 ± 0.30	
		AAI	7.58	
		Activity	Very strong	

AAI=Antioxidant activity index; BHA= Butylated hydroxyanisole; IC_{50}=Concentration providing 50% inhibition; RAA=Relative antioxidant activity; Nd = Non determined.

The AAI of the extracts from *Alstonia boonei* ranged from1.60 to 2.31 and can be compared to AAI of vitamin C (7.02) and BHA (7.58) while those of *Gambeya africana* ranged from 1.61 to 8.54. The extracts of *Alstonia boonei* reduce the concentration of free radical DPPH, the IC_{50} value is 47.43 ± 0.15 µg/mL. Water and methanol extracts exhibited very strong and strong antioxidant activities, respectively. The antioxidant capacity of the extracts is lower than that of the positive control BHT ($p > 0.01$) but much higher than that of the negative control. Forβ-carotene / linoleic acid test, the oxidation was weakly inhibited by methanolic and aqueous extracts of *Alstonia boonei* (RAA = 44.40 and 41.20 ± 0.05%). The capacity of methanol extract to reduce β-carotene is greater than that of aqueous extract and does not exceed 50%. The antioxidant capacity of β-carotene is higher than that observed by DPPH. The antioxidant activity follows a non-radical process. This low activity is due to the chemical composition of low-hydroxylated polyphenol-rich extracts. These extracts make low potential antioxidant agents. The extracts reduce the concentration of the free radical DPPH. The antioxidant capacity of the extracts is lower than that of positive control BHT but much higher than that of negative control. Water and methanol extracts of *Gambeya africana* exhibited strong and very strong antioxidant activities, respectively. The IC_{50} value is 23.80 ± 0.15%, the oxidation of β-carotene / linoleic acid is weakly inhibited by methanolic and aqueous extracts of *Gambeya africana* (74.60 and 54.10 ± 0.05%). The capacity of methanolic extract to reduce β-carotene is greater than that of aqueous extract and exceeds 50%. The antioxidant capacity follows a radical process because it contains compounds such as the polyphenols which has long been known for its antiradical activity [25; 26; 27]. The antioxidant capacity by β-carotene is higher compared to the trapping of the radical DPPH. This activity is due to the chemical composition of methanol extract rich in polyphenols, more recently, several scientific studies of their biological activity were performed [20; 28; 29]. As it can be seen, the AAI of extracts ranged from 1.60 to 8.54.The crude extract of *Alstonia boonei* and *Gambeya africana* showed a strong activity. These plants have a potential antioxidant which would enable them to play a beneficial role in terms of very significant preventive actions for human health.

Antimicrobial activity

The results of susceptibility testing with extracts show that they exhibit significant antibacterial activity of the strains tested. Extracts of *Alstonia boonei* and *Gambeya africana* recorded inhibition diameters contained in table 4. The antimicrobial activity of the extracts of *Alstonia boonei* shows that some have a well-defined inhibition on strains tested. The extracts are active on Gram positive and negative bacteria. Water and methanol extracts gave a high activity with inhibition diameters ranging from 9 to 29 mm on the microbial strains.

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Table 4. Results of susceptibility testing

		Inhibition zone diameters IZD (mm)							
		Alstonia boonei			*Gambeya africana*			Te	P
	Gram	WE	CE	ME	WE	CE	ME		
Escherichiacoli CIP 105182	-	15	10	15	25	20	27	11	Nd
Enterococcus faecalis CIP 103907	+	Nd	13	12	30	22	37	12	19
Bacillus cereus LMG 13569 BHI	+	29	0	0	27	14	21	11	18
Listeria innocua LMG 135668 BHI	+	20	Nd	26	28	Nd	30	12	14
Staphylococcus aureus ATCC 25293 BHI	+	15	Nd	9	21	Nd	25	12	26
Proteus mirabolis CIP 104588	-	10	Nd	26	15	Nd	19	13	15
Shigella dysenteria CIP 5451	+	25	Nd	15	31	Nd	37	23	16
Staphylococcus aureus ATCC 9144	-	13	Nd	9	21	Nd	25	5	26
Staphylococcus camorum LMG 13567BHI	+	20	Nd	17	30	Nd	27	15	21
Staphylococcus aureus (n=5)	+	Nd	Nd	9±4	21±3	Nd	25±3	0±0	39
Salmonella enterica (n=5)	+	Nd	Nd	22±2	40±5	Nd	35±5	12±3	16
Streptococcus pyogenes (n=10)	+	10±2	Nd	16±3	27±2	Nd	22±3	13±2	21
Enterococcus faecalis (n=5)	-	Nd	Nd	20±5	25±2	Nd	20±5	13±2	17
Pseudomonas aerugenosa (n=10)	-	12±2	Nd	22±2	21±3	Nd	23±5	13±2	21

WE= Water extract; CE = Chloroform extract; ME= Methanol extract; P = Penicillin (10 IU/IE/UI); Te = Tetracycline (30 µg); Nd = Non determined.

The most sensitive strains to water extract are *Bacillus cereus* LMG 13569 BHI (29 mm), *Shigella dysenteria* 5451 CIP (25 mm), *Staphylococcus camorum* LMG 13567 BHI and *Listeria innocua* LMG 135668 BHI (20 mm). The aqueous extract gave an intermediate antibacterial activity with zones of inhibition of 12 to 15 mm on *Staphylococcus aureus* ATCC 25293 BHI (15 mm), *Staphylococcus aureus* ATCC 9144 (13 mm) and *Pseudomonas aerugenosa* (12 mm).Methanol extract gave a high antibacterial activity and a notable inhibition on all the tested strains with diameters of inhibition ranging from 0 to 26 mm. The extract was very active on *Proteus mirabolis* 104588 CIP, *Listeria innocua* LMG 135668 BHI (26 mm), *Pseudomonas aerugenosa*, *Salmonella enterica* (22 mm) and *Enterococcus faecalis* (20 mm). Methanol extract gave an intermediate antibacterial activity with inhibition diameters of 12 to 17 mm on *Staphylococcus camorum* LMG 13567 BHI (17 mm), *E. coli* 105182 CIP, *Shigella dysenteria* 5451 CIP (15 mm) and *Enterococcus faecalis* 103907 CIP (12 mm). *Proteus mirabolis* 104588 CIP, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Salmonella enterica* exhibited low sensitivity to

the aqueous extract while *Bacillus cereus* strains LMG 13569 BHI, *Staphylococcus aureus* ATCC 25293 BHI, *Staphylococcus aureus* ATCC 9144 and *Staphylococcus aureus* were have been resistant to methanolic extract. The antimicrobial activity of *Gambeya africana* extracts shows that some have inhibitory potential. Water and methanol extracts are active on both Gram positive and negative bacteria. They gave high antimicrobial activity and marked inhibition with diameters ranging from 15 to 40 mm. The strains most sensitive to aqueous extract were *Salmonella enterica* (40 mm), *Shigella dysenteria* 5451 CIP (31 mm), *Staphylococcus camorum* LMG 13567 BHI, *Enterococcus faecalis* 103907 CIP (30 mm), *Listeria innocua* LMG 135668 BHI (28 mm) and *Bacillus cereus* LMG 13569 BHI (27 mm). The other strains are sensitive to aqueous extract with inhibition diameters ranging from 15 to 25 mm. The methanol extracts gave a high antibacterial activity on all the strains tested with inhibition diameters ranging from 20 to 37 mm. This extract was very active on *Enterococcus faecalis* 103907 CIP, *Shigella dysenteria* 5451 CIP (37 mm), *Salmonella enterica* (35 mm), *Listeria innocua* LMG 135668 BHI (30 mm), *E. coli* 105182 CIP, *Staphylococcus camorum* LMG 13567 BHI (27 mm), *Staphylococcus aureus* ATCC 25293 BHI, *Staphylococcus aureus* ATCC 9144 and *Staphylococcus aureus* (25 mm), *Pseudomonas aerugenosa* (23 mm), *Streptococcus pyogenes* (22 mm) and*Bacillus cereus* LMG 13569 BHI (21 mm). The methanol extract gave an intermediate antibacterial activity with inhibition diameters of 19 and 20 mm on *Proteus mirabolis* 104588 CIP and *Enterococcus faecalis*. No strain exhibited resistance to methanol extract.

MIC and MBC recorded in microdilution assay

The results of MIC and MBC of *Alstonia boonei* and *Gambeya africana* gave values in table 5.

Table 5. MIC and MBC from extract of *Alstonia boonei* and *Gambeya africana* (mg/mL).

Extracts	*Alstonia boonei*						*Gambeya africana*					
	Water		Chloroform		Methanol		Water		Chloroform		Methanol	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli 105.182 CIP	>100	Nd	>100	Nd	100	100	>200	Nd	>200	Nd	200	200
*Enterococcus faecalis*103.907 CIP	50	100	Nd	Nd	>200	Nd	>200	Nd	>200	Nd	200	200
Bacillus cereus LMG 13569 BHI	>100	Nd	>100	Nd	>200	Nd	100	100	100	Nd	200	200
Listeria innocua LMG 135668 BHI	>100	Nd	>100	Nd	100	100	200	200	200	Nd	200	200

Staphylococcus aureus ATCC 25293 BHI	100	100	Nd	Nd	>200	Nd	200	200	200	Nd	100	100
Proteus mirabolis 104588 CIP	50	100	Nd	Nd	25	25	>200	Nd	>200	Nd	200	200
Staphylococcus camorum LMG 13567 BHI	100	100	Nd	Nd	50	100	50	50	50	Nd	200	200
Shigella dysenteria 5451 CIP	100	100	Nd	Nd	50	50	200	200	200	Nd	200	200
Staphylococcus aureus ATCC 9144	100	100	Nd	Nd	>200	Nd	200	200	200	Nd	100	100
Staphylococcus aureus	12.5	50	Nd	Nd	>200	Nd	200	200	200	Nd	100	100
Streptococcus pyogenes	100	100	Nd	Nd	100	100	100	100	100	Nd	200	200
Enterococcus faecalis	>100	Nd	>100	Nd	>200	Nd	100	100	100	Nd	>200	Nd
Pseudomonas aerugenosa	100	100	Nd	Nd	100	100	>200	Nd	>200	Nd	200	200
Salmonella enterica	100	100	Nd	Nd	25	25	50	50	50	Nd	200	200

Water extract of *Alstonia bonnei* has been very active and has bactericidal activity on *Staphylococcus aureus* ATCC 25293 BHI, *Staphylococcus camorum* LMG 13567 BHI, *Staphylococcus aureus* ATCC 9144, *Streptococcus pyogenes*, *Pseudomonas aerugenosa* and *Salmonella enterica*. It has a bacteriostatic effect on *Staphylococcus aureus*, *Proteus mirabolis* 104588 CIP and *Enterococcus faecalis* 103907 CIP. The methanol extract of *Alstonia bonnei* is active and has a bactericidal activity on *E. coli* 105182CIP, *Listeria innocua* LMG 135668 BHI, *Proteus mirabolis* 104588 CIP, *Shigella dysenteria* 5451 CIP for reference strains and *Streptococcus pyogenes*, *Pseudomonas aerugenosa* and *Salmonella enterica* for hospital strains. It has a bacteriostatic effect on *Staphylococcuscamorum* LMG 13567 BHI. The chloroform extract is less active than the other two. It is neither bactericidal nor bacteriostatic on all strains studied. This set of results still militates in favor of the use of *Alstonia boonei* in traditional traditherapy in the treatment of bacterial infections. Methanol extract of *Gambeya africana* is active and contains

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bactericidal properties with MICs and CMBs equal on *E. coli* 105182 CIP, *Enterococcus faecalis* 103907 CIP, *Bacillus cereus* LMG 13569 BHI, *Listeria innocua* LMG 135668 BHI, *Proteus mirabolis* 104588 CIP, *Staphylococcus camorum* LMG 13567 BHI, *Shigella dysenteria* 5451 CIP, *Streptococcus pyogenes*, *Pseudomonas aerugenosa* and *Salmonella enterica*. The highest bactericidal activity (MIC and CMB = 100 mg/mL) of methanolic extract was recorded on *Staphylococcus aureus* ATCC 25293 BHI, *Staphylococcus aureus* ATCC 9144 and *Staphylococcus aureus*. Chloroform extract of *Gambeya africana* has no antimicrobial activity on all the strains studied. The aqueous extract has bactericidal properties (MIC and CMB equal to 200 mg/mL) on *Listeria innocua* LMG 135668 BHI, *Staphylococcus aureus* ATCC 25293 BHI, *Staphylococcus aureus* ATCC 9144,*Staphylococcus aureus*and*Shigella dysenteria* 5451 CIP. It is bactericidal with MICs and CMBs equal to 100 mg/mL on *Bacillus cereus* LMG 13569 BHI, *Enterococcus faecalis* and *Streptococcus pyogenes*. The highest bactericidal activity of aqueous extract with MICs and CMBs equal to 50 mg/mL, was observed on *Staphylococcus camorum* LMG 13567 BHI and *Salmonella enterica*. This set of results argues for the use of *Gambeya africana* in traditional treatment of diarrhea and other bacterial infections [4].The result showed that different extracts of *Alstonia bonnei* and *Gambeya africana* inhibited the growth of nearly all microorganisms used in the essay, indicating the presence of antimicrobial compounds in these plants. It can be inferred that the presence and the quantity of antimicrobial compounds in *Alstonia boonei* and *Gambeya africana* could justify the observed results. *Alstonia boonei* and *Gambeya africana* are used in folk medicine for the treatment of many diseases including bacterial diseases, gonorrhea, syphilis, parasitic diseases and diarrhea. Plants are the richest sources of multiple drugs and used of traditional medicines to cure microbial and non-microbial diseases [30; 31]. Various species have been known to display antimicrobial properties by acting against foodborne pathogens and spoilage bacteria and be used as sources of natural antimicrobial substances for the treatment of infectious diseases [15; 18; 21; 30; 31].

4. CONCLUSION

Medicinal plants are the local heritage with global importance and world is endowed with a rich wealth. *Alstonia boonei* and *Gambeya africana,* Gabonese medicinal plants, are riches in phenolic compounds and antimicrobial activity against several microorganisms. The plants showed antioxidant and DPPH radical scavenging activities, and they displayed the inhibition of lipid peroxidation. The results of the present study support the traditional medicinal use and suggest that a great attention should be paid to these plants which are found to have many pharmacological properties. The study confirms the multiple uses of *Alstonia boonei* and *Gambeya africana* for the treatment of many infectious diseases including bacterial diseases, gonorrhea, syphilis, parasitic diseases, diarrhea, stomach upset, fever and malaria. The result place them as candidate for further investigations for traditional drug utilizable as complementary and alternative medicines

development and new active compounds.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest. All the authors read and approved the final version.

REFERENCES

1. Rath SK, Mohapatra N, Dubey D, Panda SK, Thatoi HN and Dutta SK. Antimicrobial activity of *Diospyros melanoxylon* bark from similipal biosphere reserve, Orissa, India. Afr J Biotechnol. 2009; 8 (9): 1924-1928.
2. Zirihi GN, N'guessan K, Dibié ET and Grellier P. Ethnopharmacological study of plants used to treat malaria, in traditional medicine, by Bete Populations of Issia (Côte d'Ivoire). J Pharm Sci Res. 2010; 2 (4): 216-227.
3. Raponda WA and Sillans R. Les plantes utiles du Gabon. Paul Lechevalier, Paris, France. 1961 ; 1 (1) : 614 p.
4. Burkill HM. "The usefull plants of West Tropical Africa" (Vol. 1: Families A-D). 101 noms vernaculaires, et de nombreuses indications sur l'utilisation des plantes. 1985 ; 960 p.
5. Wandji J, Tillequin F, Mulholland DA, Wansi JD, Fomum TZ, Fuendjiep V, Libot F, Tsabang W. Fatty acid esters of triterpenoids and sterol glycosides from *Gambeya africana*. Planta Medica. 2002; 68(9): 822-826.
6. Ciulei I. Pratical manual on the industrial utilization of chemical and aromatic plants methology for analisis of vegetable drugs 1st Edn., Ministry of Chemical Industry, Bucharest. 1982; 1-62.

7. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth Enzymol. 1999; 299: 152-179.
8. The reference method of european communauty was used to measure total amount of Tannins, 1994; 49:462–468.
9. Prigent S. Interactions of phenolics compounds with globular proteins and their effects on food related functional properties. PhD Thesis, Wageningen University, Wageningen. Netherlands. 2005; 131–133.
10. Arvouet-Grant A, Vennat B, Pourrat A and Legret P. Standardisation d'un extrait de propolis et identification des principaux constituants. J Pharmacol Belge. 1994; 49 (6): 462-468.
11. Scherer R and Godoy HT. Antioxidant activity index (AAI) by 2,2-diphenyl-1- picrylhydrazyl method. Food Chem. 2009; 112: 654-658.
12. Dapkevicius A, Venskutonis R, Van Beek TA and Linssen JPH. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J Sci Food Agric. 1998; **77**: 140-146.
13. Pérez FJ and Ormeno-Nunez J. Root exudates of wild oats: Allelopathic effect on spring wheat. Phytochem.1991; 30: 2199-2202.
14. Sima OC, Ondo JP, Ndong AGR, Obame ELC, Djoba SJF, Nsi EE. Phytochemical screening, antioxidant and antimicrobial potential of stem barks of *Coula edulis* Baill. *Pseudospondias longifolia* Engl. and *Carapa klaineana* Pierre from Gabon. Asian Pac J Trop Disease. 2016; 6 (7): 557-563.
15. Obame ELC, Sima OC, Ngoua MMRL, Orango BJO, Ondo JP, Ndong AGR, Koudou J and Traoré SA. Larvicidal and ovicidal properties against *Anopheles gambiae,* antioxidant and antibacterial activities of the combination of essential oils *Eucalyptus citriodora, Cymbopogon giganteus* and *Cymbopogon nardus* from Gabon. JMEST. 2017; 4 (8): 7887-7894.
16. NCCLS (National Committee for Clinical Laboratory Standards). Methods for dilution, antimicrobial susceptibility tests for bacteria that grow aerobically, 5th edition volume 17, Approuved standards-M7-A4. NCCLS document M7-A4. National Committee for Clinical Laboratory Standard Wayen Pa. Nikaido H, Vaara M. 2000.
17. Obame-Engonga LC, Abdoul-Latif FM, Ondo JP, Sima OC, Ngoua MMRL, Traoré SA and Koudou J. Phytochemical screening, antioxidant and antibacterial activities of *Guibourtia ehie* and *Syzygium rowlandii* medicinal plants from Gabon. Int J Current Research. 2017; 9 (8): 56354-56360.
18. Fatouma MAL, Nabil M, Edou P, Adwa AA, Samatar OD, Obame LC, Bassolé IHN and Dicko MH. Antimicrobial and antioxidant activities of essential oil and methanol extract of *Matricaria chamomilla* L. from Djibouti. J Med Plants Res. 2011; 5(9): 1512-1517.

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19. Sima OC, Ondo JP, Obame ELC, Padzys GS, Zongo C, Bongui JB, Nsi EE, Traoré SA. Phytochemical screening, evaluation of antioxidant and antimicrobial properties of *Erythrophleum ivorense* A. Chev (Leguminosae) and *Megaphrynium macrostachyum* Benth (Marantaceae), medicinal plants from Gabon. Int J Biosciences. 2016; 8 (6): 43-53.
20. Obame ELC, Bongui JB, Andzi BT, Ondo JP, Edou EP, Koudou J. Antifungal and antibacterial activities of *Aucoumea klaineana* Pierre essential oil from Gabon. VRI Phytomedicine.2014; 2 (1): 17-21.
21. Ngoua-Meye-Misso RL, Ndong JDLC, Sima-Obiang C, Ondo JP, Ndong-Atome GR, Ovono-Abessolo F and Obame-Engonga LC. Phytochemical studies, antiangiogenic, anti-inflammatory and antioxidant activities of *Scyphocephalium ochocoa* Warb. (Myristicaceae), medicinal plant from Gabon. Clinic Phytosci. 2018; 4:15, 1-13.
22. Makkar HPS. Quantification of tannins in tree foliage–A laboratory manual.FAO/IAEA Working Document, Vienna, Austria. 2000.
23. Santos-Buelga C and Scalbert A. Proanthocyanidins and tannin-like compounds– nature, occurrence, dietary intake and effects on nutrition and health. J Sci Food Agric. 2000; 80 (7): 1094-1117.
24. Palic R, Stojanovic G, Alagic S, Nikolic M, Lepojevic Z. Composition chimique et activité antimicrobienne de l'huile essentielle et des extraits du tabac d'Orient, Prilep. Flav Fragr J. 2002; 17 (5): 323-326.
25. Ngoua MMRL, Ondo JP, Assam EJN, Orango BJO, Sima OC, Ndong JC, Ovono AF, Obame ELC. Phytochemical screening, antioxidant and antiangiogenic properties of *Oncoba welwitschii* (Oliv.) Gilgn. and *Tetrorchidium oppositifolium* (Pax. and Khoffm.), medicinal plants from Gabon. Int J Innov Res Sci Engin Techn. 2017; 6 (1): 1-10.
26. Andzi BT, Massala KK, Obame ELC, Lebibi J. Phytochemical studies, total phenolic and flavonoids content and evaluation of antiradical activity of the extracts of the leaves from *Dischistocalyx* sp. (Acanthaceae). JPP. 2015; 3 (6): 174-178.
27. Obame-Engonga LC, Ondo JP, Padzys GS, Andzi BT, Kounga T, Bongui JB, Ndong MT, Zongo C and Traoré AS. Ovicidal and larvicidal activities against *Anopheles gambiae*, antioxidant and antibacterial proprieties of *Aucoumea klaineana* Pierre, *Canarium schweinfurthii* Engl and *Dacryodes edulis* (G. Don) H. J. Lam essential oils from Gabon. Int J Pharmacol Res. 2016; 6 (1): 68-75.
28. Ngoua-Meye-Misso RL, Sima OC, Ndong GR, Ondo JP, Ovono AF, Obame ELC. Phytochemical screening, antioxidant, anti-inflammatory and antiangiogenic activities of *Lophira procera* A. Chev. (Ochnaceae) medicinal plant from Gabon. Egypt J Basic Appl Sci. 2018; 5: 80-86.

29. Obame ELC, Andzi BT, Ondo JP, Mewono L, Ndong AGR, Abdoul-Latif F, Bassolé IHN. Total phenolic composition, antibacterial and antioxidant activities of *Fagara heitzii* Aubr & Pellegr medicinal plant of Gabon. VRI Phytomedicine. 2013; 1 (3): 63-69.
30. Sima-Obian C, Ngoua-Meye-Misso RL, Ndong-Atome GR, Ondo JP, Ovono AF, Obame-Engonga LC and Nsi-Emvo E. Phytochemical screening, phenolic compounds content, antioxidant, anti-inflammatory and antimicrobial properties of *Pachylobus balsamifera* Guillaum from Gabon. Phyto. 2018; 0059.

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