International Journal of Phytomedicine 9 (2017) 501-510
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Chemical composition, antioxidant and antimicrobial activities of stem barks of *Englerina gabonensis* Englerand *Sterculia tragacantha* Lindlfrom Gabon

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Received: 02 June 2017
Accepted: 09 August 2017
Published: 02 September 2017

Abstract

Aim of this work was to evaluate the phytochemical constituents, antioxidant and antimicrobial potential of water-acetone, water-ethanol and water extracts of *Englerina gabonensis* and *Sterculia tragacantha*.. Presence of phenols was evaluated to estimate the effects of plants on microbial diseases. Water-acetone, water-ethanol and water extracts were examined for antioxidant activities. All plant extracts were tested against six reference strains, eleven clinical isolates and two fungal strains.

Phenolic content were highest in the water-acetone and water-ethanol extracts from *Englerina gabonensis* in comparison with *Sterculia tragacantha*.. The AAI (Antioxidant Activity Index)of water-acetone and water-ethanol extracts of *Englerina gabunensis* are superiors with Plant extracts of *Sterculia tragacantha.* show weak antioxidant activity (AAI < 0.5).The aqueous extract of *Englerina gabonensis* has a bactericidal effect on *Salmonella Spp*. Water-ethanol extract is bactericidal on *Bacillus cereus* LMG 13569 BHI, *Salmonella Spp* and *Neisseria meningitides.* Water-acetone extract presents a bactericidal activity on *Enterococcus faecalis* 103907 CIP, *Escherichi coli*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Neisseria meningitides.* Our results suggest that *Englerina gabonensis* extracts contain greater antioxidant and antimicrobial properties than *Sterculia tragacantha.* extracts.

Keywords: Phytochemical, antioxidants, antimicrobial, *Englerina gabonensis, Sterculia tragacantha..*

Introduction

The use of plants in therapeutics is very old and is currently experiencing a renewed interest among the public. It is possible to use whole plants or the extracts they produce [1]. WHO has estimated that about 80% of the population in developing countries uses traditional medicine for their primary health needs. In Gabon, traditional medicine provided almost all of the population's health needs during the pre-colonial period in the absence of modern medicine. Once proscribed by the colonizer, it is regaining more and more ground. Aware of the stakes of traditional medicine, Gabon is resolutely committed to its valorization. Every year more than 3 million people die from diarrheal diseases worldwide, and Africa south of the Sahara is one of the most affected by these diseases. The most common microorganisms are: *E. coli*, *Shigella*, *Salmonella*, *Yersina* and rarely *Vibrio cholera*, which occurs only in well-defined areas [2-4]. The emergence of multi-drug resistant strains of existing drugs, the emergence of new diseases, the resurgence of certain diseases, and concerns about the harmful effects of certain chemicals have increased the renewed interest in medicinal plants. Besides microbial infections, free radicals are involved in a large number of pathologies that are now considered to be one of the major public health problems. These include asthma, rheumatism, cancers, diabetes mellitus, inflammatory lesions, immunosuppressive diseases and metabolic disorders [5]. During oxidative stress, free radicals induce tissue damage. However, plants have an antiradical potential that would enable them have a beneficial role in terms of preventive action that is very important for human and animal health [6,7]. *Englerina gabonensis* (Loranthaceae) is a semi-woody hemi parasite with inflorescences in umbels of 5-15 red and yellow flowers. Several species of *Englerina gabonensis* can be observed on avocado, cola, mandarin, orange, and coffee, except on palm trees [8]. The decoction of the leaves of *Englerina gabonensis* is used to cure rheumatism. *Englerina gabonensis* leaves are also used to heal fractures and scabies [8,9]. *Sterculia tragacantha.* (Sterculiaceae) is a large tree, in primary forest. It loses its leaves after the rains. The shoulders are thin at the base but not very high. The bark is silvery-gray and stands out in irregular scales. Bark maceration is used against bronchial pneumonia infections [8,10]. These studies

DOI:10.5138/09750185.2124

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may lead to scientific data validating the uses of these plants in traditional medicine. These results can also serve as a basis for the development of new therapeutic molecules. This could partly address the problem of drug availability and the notorious lack of health coverage in remote areas of developing countries despite governments' efforts to ensure decent health for populations [11]. In this context, this study aims at contributing in search of solutions for improvement of public health by antioxidant and antimicrobial studies of two plants used to Gabon in treatment of the bacterial diseases.

Materials and methods

Plant materials

Stem barks of *Englerina gabonensis* and *Sterculia tragacantha.* were selected according to their traditional uses. The plant samples were collected in Oyem (Northern of Gabon) in July 2014. Identification of the species was carried out at National Herbarium of IPHAMETRA, Libreville (Gabon).

Processing of the plant material

The plant samples were freeze-dried, powdered, kept at ambient temperature, and protected from light. Each sample (20 g) were mixed with 250 mL of suitable solvents [water (100%); water-acetone (30:70, v/v); water-ethanol (30:70, v/v)]. The water extracts were boiled for 60 min. All the extracts were filtered and concentrated. The concentrates were lyophilized and stored in sterile vials at 4 °C.

Chemicals, reagents and media

Quercetin and 1,1-Diphenyl-2-picryl hydrazyl (DPPH), 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS), potassium persulphate and FolinCiocalteu reagent were purchased from FlukaChemika (Switzerland). Gallic acid, Butylated Hydroxyanisole (BHA) and the other chemicals were from Sigma-Aldrich (St Louis, MO, USA).

Preliminary phytochemical screening

Each extract was then tested for the presence of flavonoids, coumarins, tannins, total phenolic, saponosids, triterpenoids, alkaloids and anthracenosids as described elsewhere [12].

Quantitative analysis of phytochemicals

Total phenolic content

The total phenolic contents of the different extracts were determined according to the Folin-Ciocalteu Method [13] with minor modifications [4] using gallic acid as standard, the absorbance was measured at 735 nm. All analyses were done in triplicate and results were expressed as gallic acid equivalent per gram of lyophilized sample.

Total flavonoids content

Total flavonoid contents were determined by the aluminum chloride ($AlCl_3$) colorimetric assay method [14], using quercetin as a standard [15]. Total flavonoid contents were expressed as quercetin equivalents in milligrams per gram sample (average of the triplicate analysis).

Total tannins content

Tannin content was determined by using Obame method [16]. Absorbance at 525 nm was recorded in a spectrophotometer within 10 min and tannins contents were expressed as mg tannic acid equivalent/g of drug.

Total proanthocyanidins

Proanthocyanidins was determined by using HCl-butanol assay [16]. Absorbance was read at 550 nm and apple procyanidin was applied as standard.

Antioxidant Activity Index (AAI)

AAI based on DPPH was estimated by the method of Scherer and Godoy [17]. A range of concentration from 0.78 to 100 µg/mL was prepared for each extract. Ascorbic acid (vitamin C) and BHA were used as controls. Each sample was prepared in triplicate. Absorbance was measured at 517 nm. Percentage inhibition was obtained by the following formula:

% Radical scavenger activity = [(Absorbance of DPPH – Absorbance of sample)/ Absorbance of DPPH] × 100

%RSA = [(A control – A sample) / A control] x 100.

The concentration of extracts reducing 50% of DPPH (IC_{50}) was determined from the curve of the percentage inhibition versus concentration of the extract. AAI was calculated using the following formula: AAI = Final concentration of DPPH/IC_{50}

According to criteria of Scherer and Godoy [17], the extracts of plants show weak antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI is between 0.5 and 1.0, strong antioxidant activity when AAI is between 1.0 and 2.0, and very strong when AAI > 2.0.

ABTS scavenging activity

ABTS assay is based on the ability of an antioxidant to stabilize $ABTS^{\bullet+}$ radical transforming it into $ABTS^{+}$[12]. Mixture of ABTS solution (7 mmol/L) and potassium per sulfate (2.4 mmol/L) was incubated for 12 h in the dark at room temperature until ABTS radical complex was formed ($ABTS^{\bullet+}$). To 60 µL of extract, 2.94 mL of $ABTS^{\bullet+}$ solution was added. The mixture was incubated at 37 C for 20 min and protected from light. Ascorbic acid (vitamin C) and BHA were used as references. After incubation the absorbance was measured by a spectrophotometer at 734 nm. The percentage inhibition was calculated by the following method:

Percentage inhibition = $[(At_0 – At_{20})/At_0] \times 100$

where, At_0 is the absorbance of $ABTS^{+}$ radical + ethanol, At_{20} is the absorbance of $ABTS^{+}$ radical + sample extract or standard.

Test microorganisms

The test microorganisms used in this investigation included bacteria *Escherichia coli* 105182 CIP(*E. coli* 105182 CIP), *Listeria innocua* LMG135668BHI (*L.innocua* LMG135668BHI), *Staphylococcus aureus* ATCC25293 BHI(*S. aureus* ATCC25293 BHI), *Enterococcus faecalis* 103907 CIP(*E. faecalis* 103907 CIP), *Bacillus cereus* LMG13569BHI(*B. cereus* LMG13569BHI), *Staphylococcus camorum* LMG 13567 BHI(*S. camorum* LMG 13567 BHI), *Shigella dysenteria*5451 CIP (*S. dysenteria* 5451 CIP), *Pseudomonas aerugenosa* (*P. aerugenosa*), *Salmonella enterica* (*S. enterica*), *Salmonella typhi* (*S. typhi*), *Neisseria gonorrhea* (*N. gonorrhea*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumonia* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii), Enterobacter aerogenes (E. aerogenes), Salmonella Spp* and *Neisseria meningitides* (*N. meningitidis).* The fungal strains were *Candida albicans* ATCC 10231 (*C. albicans* ATCC 10231), and *Candida albicans* ATCC 90028 (*C. albicans* ATCC 90028).

Positive and negative control

Gentamicin (10 µg/mL), Ampicillin (30µg/mL) and Tetracycline (30 µg/mL) were used as positive control for bacterial strains test. Sterilized distilled water and dimethyl sulfoxide were used as negative control.

Antibacterial susceptibility testing

Disc diffusion method was used to study susceptibility of bacteria against plant extracts [13]. Bacteria were grown in Muller Hinton broth (Liofilchem, Italy) for 18 to 24 h. Each culture was then suspended in a sodium chloride solution (NaCl, 0.9%) to reach turbidity equivalent to that of the 0.5 MacFarland standard [4]. Extracts were diluted in dimethyl sulfoxide (0.5%) to 100 mg/mL. Previously each extract (10 µL) was loaded onto each filter paper disc (What man No. 1). Muller Hinton agar was suspended in distilled water, heated until complete dissolution and was autoclaved at 121 C and then poured into Petri dishes. The discs were placed on cultures and antimicrobial activity was estimated after incubation at 37 C for 24 h, by measuring the diameter of inhibition zone.

Determination of relative percentage inhibition

The relative percentage inhibition of the test plant extract with respect to positive control was calculated by using the following formula [14]. Relative percentage inhibition of the test extract = 100 (X-Y) / (Z-Y) Where, X is total area of inhibition of the test extract, Y is total area of inhibition of the solvent, and Z represents total area of inhibition of the standard drug.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC were determined by micro dilution method with Muller Hinton broth [13,15]. Briefly, nutrient broth (100 µL/wells) was distributed into wells of a micro plate (Nunc). One hundred micro liters of extracts were added to first row of wells and twofold dilution was added into other wells. Ninety microliters of nutrient broth and 10 µL of inocula were added into wells. A range of concentration of extract from 0.0049 to 5 mg/mL was prepared in a total volume of 200 µL to each extract. The plates were slightly shaken and incubated at 37 C for 24 h; inhibition was assessed by observing the absence of turbidity in the wells. Wells without extract were used as negative control. To determine the MBC, 100 µL from each well demonstrating no visible growth were collected and seeded in Petri dishes containing Muller Hinton agar. The dishes were incubated at 37 C for 24 to 48 h and the number of colonies was counted [15].

Antifungal sensitivity test

Antifungal activity of extracts was evaluated by the diffusion and micro dilution methods as described above with some modifications [15]. Culture media for this study were potato dextrose broth and the potato dextrose agar.

Statistical analysis

All data were measured average value of three replicates and standard error (±). Results were subjected to Microsoft excel 2013 and kaleida graph version 4.0. $P < 0.05$ was statistically significant.

Results

Phytochemical screening

The phytochemical screening of the extracts was first performed to detect the major chemical groups present in the extracts. The results of this screening are shown in table-1. These results showed that, total phenolic, tannin, gallic and flavonoids were very abundant in the stem bark crude extracts of *Englerina gabonensis* and *Sterculia tragacantha*..

Table-1: Results of the preliminary phytochemical screening

Chemical groups	*Englerina gabonensis*			*Sterculia tragacantha.*		
	Eg WE	Eg WEE	Eg WAE	St WE	St WEE	St WAE
Saponosids	+++	-	+	+	-	-
Tanningallic	+++	+++	+++	++	++	++
Tannin catechic	++	+++	++	-	-	-
Total phenolic	+++	+++	+++	+++	+++	+++
Total flavonoids	++	++	+++	++	++	++
Reducing sugars	+++	+++	+++	-	-	-
Alkaloids	+	++	+	++	+	++
Proanthocyanidins	++	++	++	-	-	-
Anthracenosids	++	++	+++	-	-	-
Coumarins	+	+	+	-	-	-
Triterpenoids	+++	+++	+++	+	++	++

+++ = Very abundant; ++= Abundant; + = not abundant, — = Not Detected. Eg = *Englerina gabonensis*; St = *Sterculia tragacantha.*; WAE = water-acetone extract; WEE=water-ethanol extract; WE= water extract.

Totals phenolic, flavonoid, tannins and proanthocyanidins contents

The results of total phenols, total flavonoids, total tannins and total proanthocyanidins contents of *Englerina gabonensis* and *Sterculia tragacantha.* are presented in table 2. Total phenolic contents (standard curve equation: Y = 0.0012X + 0.0004, R^2 = 0.9982) from the water, water-ethanol and water-acetone extracts of the stem barks of *Englerina gabonensis* and *Sterculia tragacantha.* varied from 216.44 ± 9.26 to7 176.44 ± 3.61mg GAE/100 g of extract. In this research, the water-acetone extract of *Englerina gabonensis* (7176.44 ± 3.61mg GAE/100 g) has the highest phenolic content than other extracts. Contents of phenolic compounds of *Englerina gabonensis* extractsare significantly higher than those of *Sterculia tragacantha.* extracts ($p < 0.05$).

Total flavonoid content (standard curve equation: Y = 0.0032X + 0.0077, R^2 =1) was determined in comparison with standard quercetin and the results expressed in terms of mg QE/ 100g of extract. Total flavonoids were more abundant in water-acetone extracts of *Englerina gabonensis* (2197.88 ± 3.75 mg QE/g of extract) than other extracts of plants.

Tannins contents (standard curve equation: Y = 0.0009X + 0.2088, R^2=1) were expressed in terms of tannic acid equivalent (TAE). The amount of tannin for *Englerina gabonensis* (1 017.19 ± 7.65 ATE/ 100 g of extract) of the water-acetone extract has the highest content.

Levels of proanthocyanidins were expressed in terms of apple proanthocyanidins equivalent (APE). The expression from the calibration curve of the proanthocyanidins byHCl-butanol method gave Y = 0.000 6X + 0.002 4 with R^2= 0.986.

Table 2: Total phenolic content (TPC), Total flavonoid content (TFC) Total tannins content (TTC) and Total proanthocyanidins content (TPC) of *Englerina gabonensis* and *Sterculia tragacantha.*.

Extracts	TPC (mg GAE/ 100 g of extract)	TFC (mg QE/ 100 g of extract)	TTC (mg ATE/ 100 g of extract)	TPC (mg APE/100 g of extract)
Eg WAE	7 176.44 ± 3.61	2 197.88 ± 3.75	1 017.19 ± 7.65	572.89 ± 7.5
Eg WEE	6 217.56 ± 4.58	1 375.38 ± 5.63	683.85 ± 1.85	346.22 ± 8.33
Eg WE	3 548.67 ± 7.08	1 078.29 ± 2.01	426.07 ± 2.47	226.22 ± 0.83
St WAE	1 285.33 ± 3.33	294.54 ± 3.26	110.52 ± 2.72	161.78 ± 7.41
St WEE	216.44 ± 9.26	275.79 ± 3.19	0	115.11 ± 0.56
St WE	1 318.67 ± 5	56.21 ± 1.32	368.30 ± 16.11	95.11 ± 3.89

Data represented as Mean ± SD. SD: Standard Deviation, Eg = *Englerina gabonensis*; St = *Sterculia tragacantha.*; WAE = water-acetone extract; WEE=water-ethanol extract; WE= water extract. GAE=Gallic acid equivalent, QE=Quercetin equivalent, ATE=Acid tannic equivalent, APE=Apple procyanidins equivalent.

Antioxidants activities

The antioxidant activities of the extracts are pointed out in table 3. The AAI of the extracts from *Englerina gabonensis* ranged from 2.21 to 8.81 and can be compared to AAI of vitamin C and BHA (AAI values of 7.02 and 7.58 respectively) while those of *Sterculia tragacantha.* ranged from 0.15 to 0.16.

Table-4 showed the scavenging activity of stem bark extracts of *Englerina gabonensis* and *Sterculia tragacantha.* against ABTS radical in a concentration dependent manner. A comparable and scavenging activity was observed between the extracts and the standard drugs (vitamin C and BHA). The IC_{50} values of the standard vitamin C and BHA were 5.01 ± 0.55 and 4.26 ± 0.25 µg/mL, respectively while that water-acetone (2.78 ± 0.54 µg/mL), water-ethanol (5.09 ± 0.36 µg/mL) and water extracts (11.04 ± 0.36 µg/mL) of *Englerina gabonensis* recorded high inhibitory activities compared to the extracts of *Sterculia tragacantha..* Antioxidant activity of *Englerina gabonensis* extracts presents a strong correlation with the phenolic contents (Table-5).

Table-3: Antioxidant activity of *Englerina gabonensis* and *Sterculia tragacantha.* extracts by DPPH free radical scavenging method.

Extracts	Regression curve's equations	R^2	CI_{50} (µg.mL^{-1})	AAI
Eg WAE	Y = 1.64X + 40.76	0.994	5.67 ± 0.32	8.81
Eg WEE	Y = 1.66X + 2.53	0.971	22.56 ± 0.65	2.21
Eg WE	Y = 1.1X + 16.94	0.991	29.78 ± 0.78	1.67
St WAE	Y = 0.21X + 2.55	0.998	220.65 ± 0.96	0.22
St WEE	Y = 0.15X – 0.79	0.991	328.07 ± 0.65	0.15
St WE	Y = 0.15X + 0.2	0.995	311.25 ± 0.25	0.16
Vit C	Y = 6.76X + 2.03	0.985	7.12 ± 0.6	7.02
BHA	Y = 3.32X + 28.12	0.950	6.59 ± 0.3	7.58

Eg = *Englerina gabonensis;* St = *Sterculia tragacantha.;* WAE = water-acetone extract; WEE=water-ethanol extract; WE= water extract.

Table-4: Analysis of ABTS radical scavenging activity of stem bark extracts of *Englerina gabonensis* and *Sterculia tragacantha.*

Extracts	Regression curve's equations	R^2	CI_{50} (µg.mL^{-1})
Eg WAE	Y = 17.57X + 1.09	0.996	2.78 ± 0.54
Eg WEE	Y = 5.76X + 20.67	0.994	5.09 ± 0.36
Eg WE	Y = 2.91X + 17.83	0.993	11.04 ± 0.36
St WAE	Y = 0.99 – 0.78	0.985	50.83 ± 0.43
St WEE	Y = 0.24X + 2.87	0.996	198.86 ± 0.7
St WE	Y = 0.42X + 20.81	0.958	111.54 ± 1
Vit C	Y = 6.76X + 2.03	0.989	5.01 ± 0.55
BHA	Y = 3.32X + 28.12	0.950	4.26 ± 0.25

Eg = *Englerina gabonensis;* St = *Sterculia tragacantha.*; WAE = water-acetone extract; WEE=water-ethanol extract; WE= water extract.

Table-5: Correlation between antioxidant activity (inhibitory effect of DPPH and DPPH) and total phenolic content

R^2	*Englerina gabonensis*		*Sterculia tragacantha.*	
	DPPH	ABTS	DPPH	ABTS
Total phenolic content	0.949	0.939	0.359	0.813

Relative percentage inhibition of crudes extracts

Tetracycline was used to determine the relative percentage inhibition of antibacterial activity of *Englerina gabonensis* and *Sterculia tragacantha.* stems barks extract in different solvents (Figure-1). The water-acetone extracts exhibited maximum relative percentage inhibition against *Escherichia coli* 105182 CIP (58.82%), *Listeria innocua* LMG135668BHI (69.23%), *Staphylococcus aureus* ATCC25293 BHI (60%), *Enterococcus faecalis*103907 CIP (33.33%), *Bacillus cereus* LMG13569BHI (84.61%), *Staphylococcus camorum* LMG 13567 BHI (33.33%), *Shigella dysenteria* 5451 CIP (45%), *Pseudomonas aeruginosa* (32.14%), *Salmonella enterica* (50%), *Salmonella typhi* (63.63%), *Neisseria gonorrhea* (62.5%), *Escherichia coli* (62.5%), *Staphylococcus aureus* (55.55%), *Klebsiella pneumoniae* (50%), *Acinetobacter baumannii* (50%), *Enterobacter aerogenes* (56.25%), *Salmonella Spp* (44%) and *Neisseria meningitides* (50%).

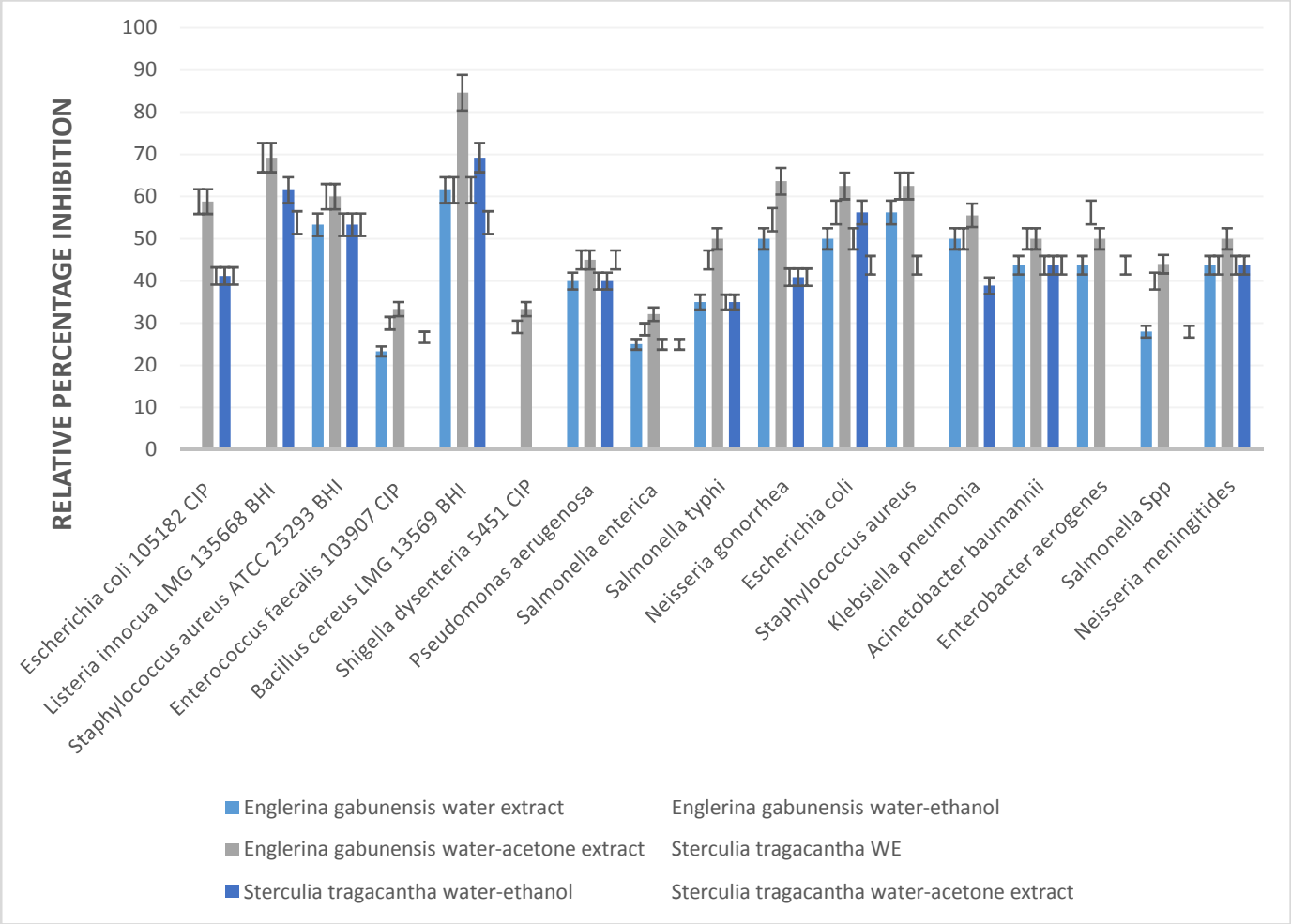

Figure-1: Determination of relative percentage inhibition of water, water ethanol and water acetone extracts from stem bark of *Englerina gabonensis and Sterculia tragacantha.* compared to standard antibiotic (Tetracycline).

Antimicrobial susceptibility testing

Antimicrobial activities of extracts varied according to the species tested. *Englerina gabonensis* extracts produced at least one zone of inhibition greater than 10 mm against at least one species. The most active extracts were water-acetone extracts from *Englerina gabonensis*. In *Sterculia tragacantha.* extracts, no inhibitory zone was found against *Shigella dysenteria* 5451 CIP, *Enterococcus faecalis* 103907 CIP, *Enterobacter aerogenes*, *Staphylococcus aureus, Salmonella Spp* and *Candida albicans* ATCC 10231 (Table-6).

Table-6: Inhibition zone diameters (mm) produced by the extracts from *Englerina gabonensis* and *Sterculia tragacantha*..

	Inhibition zone diameters (mm)								
	Extracts						Standards		
	Eg WE	Eg WEE	Eg WAE	StWE	St WEE	St WAE	Gen	Am	Te
Bacteria Reference strains									
Escherichia coli 105182 CIP	7 ± 1	10 ± 0	10 ± 1	7 ± 1	7 ± 1	7 ± 0	17 ± 1	0	0
Listeria innocua LMG 135668 BHI	7 ± 0.5	9 ± 0	9 ± 0	7 ± 0	8 ± 1	7 ± 0	13 ± 0	7 ± 1	14 ± 0
Staphylococcus aureus ATCC 25293 BHI	8 ± 1	9 ± 1	9 ± 1.5	8 ± 0	8 ± 1	8 ± 0	15 ± 0.3	0	17 ± 0.6
Enterococcus faecalis 103907 CIP	7 ± 0.5	9 ± 0	10 ± 0	0	0	8 ± 1	30 ± 0	7 ± 1	19 ± 0
Bacillus cereus LMG13569 BHI	8 ± 0	8 ± 1	11 ± 0.5	8 ± 0	9 ± 1.5	7 ± 0	13 ± 0.5	0	18 ± 0.6
Shigella dysenteria 5451 CIP	7 ± 0	7 ± 0	8 ± 0	0	0	0	24 ± 0.5	0	16 ± 0
Clinical isolates									
Pseudomonas aerugenosa	8 ± 0.5	9 ± 1	9 ± 1	8 ± 0	8 ± 1	9 ± 0	20 ± 0	7 ± 1	21 ± 1
Salmonella enterica	7 ± 1	8 ± 0.5	9 ± 0	7 ± 1	7 ± 0	7 ± 1	28 ± 1	7 ± 1	16 ± 0.3
Salmonella typhi	7 ± 0	9 ± 1.5	10 ± 0	7 ± 1	7 ± 1.5	7 ± 1	20 ± 0.5	7 ± 0	15 ± 0.5
Neisseria gonorrhea	11 ± 1	12 ± 1	14 ± 1	9 ± 0	9 ± 0	9 ± 0,5	22 ± 1.2	7 ± 1	10 ± 1
Escherichia coli	8 ± 1	9 ± 1	10 ± 1	8 ± 0	9 ± 1	7 ± 1	16 ± 1	7 ± 0	9 ± 1
Staphylococcus aureus	9 ± 0	10 ± 0	10 ± 1	0	0	0	16 ± 1	7 ± 0	8 ± 1
Klebsiella pneumonia	9 ± 0	9 ± 1	10 ± 0	7 ± 1	7 ± 1	7 ± 1	18 ± 1	7 ± 0	0
Acinetobacter baumannii	7 ± 1	8 ± 1	8 ± 1	7 ± 1	7 ± 1	7 ± 0	16 ± 0.5	0	10 ± 2
Enterobacter aerogenes	7 ± 1	9 ± 1	8 ± 0	0	0	7 ± 0	16 ± 0	7 ± 1	10 ± 0.6
Salmonella Spp	7 ± 1	10 ± 2	11 ± 2	0	0	7 ± 1	25 ± 0	7 ± 1	14 ± 1.5
Neisseria meningitides	7±1	7±1	8±1	7 ± 1	7 ± 1	7 ± 1	16±0	7±1	0
Fungi									
Candida albicans ATCC 10231	8 ± 1	9 ± 0	9 ± 1	0	0	0	0	0	0
Candida albicans ATCC 90028	7 ± 1	9 ± 2	11 ± 0	7 ± 1	8 ± 0	9 ± 1	0	7 ± 1	0

Gen=Gentamicin (10 µg/mL), Te = Tetracycline (30 µg/mL); Am = Ampicillin (30 µg/mL); Eg = *Englerina gabonensis*; St = *Sterculia tragacantha.*; WAE = water-acetone extract; WEE=water-ethanol extract; WE= water extract.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration or Minimum Fungicidal Concentration of crudes extracts

Values of MIC and MBC of extracts of *Englerina gabonensis* and *Sterculia tragacantha.* are reported in table 7. Extracts were considered as microbicides, those whose ratios MBC/MIC are equivalent to one. The MIC and MBC vary from one bacterium to another. The aqueous extract of *Englerina gabonensis* has a bactericidal effect on *Salmonella Spp;* and bacteriostatic action on *Neisseria gonorrhea* and *Neisseria meningitides.* The other microorganisms don't present definite activities. Water-ethanol extract of *Englerina gabonensis* is bacteriostatic on *Escherichia coli* 105182 CIP, *Neisseria gonorrhea, Escherichia coli, Staphylococcus aureus* and *Acinetobacter baumannii.* Water-ethanol extract is bactericidal on *Bacillus cereus* LMG 13569 BHI, *Salmonella Spp* and *Neisseria meningitides.* Water-acetone extract presents a bactericidal property on *Enterococcus faecalis* 103907 CIP, *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Neisseria meningitides*. Extracts of *Sterculia tragacantha.* don't present bactericidal or bacteriostatic activities definite.

Table-7: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or fungicidal concentration (MFC) of plants extracts from *Englerina gabonensis* and *Sterculia tragacantha*..

	MIC and MBC (mg/mL)											
	Eg WE		Eg WEE		Eg WAE		St WE		St WEE		St WAE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacteria Reference strains												
Escherichia coli 105182 CIP	Nd	Nd	2.5	5	2.5	5	>5	>5	>5	>5	>5	>5
Listeria innocua LMG 135668 BHI	Nd	Nd	5	>5	5	>5	Nd	Nd	5	>5	>5	>5
Staphylococcus aureus ATCC 25293 BHI	5	>5	5	>5	5	>5	5	>5	5	>5	5	>5
Enterococcus faecalis 103907 CIP	5	>5	5	>5	5	5	Nd	Nd	Nd	Nd	5	>5
Bacillus cereus LMG 13569 BHI	5	>5	5	5	2.5	5	>5	>5	5	>5	>5	>5
Shigella dysenteria 5451 CIP	Nd	Nd	5	>5	5	>5	Nd	Nd	Nd	Nd	Nd	Nd
Clinical isolates												
Pseudomonas aerugenosa	5	>5	5	>5	5	>5	5	>5	5	>5	5	>5
Salmonella enterica	>5	>5	5	>5	5	>5	>5	>5	Nd	Nd	>5	>5
Salmonella typhi	>5	>5	5	>5	5	>5	>5	>5	>5	>5	Nd	Nd
Neisseria gonorrhea	2.5	5	2.5	5	2.5	5	>5	>5	>5	>5	>5	>5
Escherichia coli	5	>5	2.5	5	2.5	2.5	>5	>5	>5	>5	>5	>5
Staphylococcus aureus	5	>5	2.5	5	2.5	2.5	>5	>5	>5	>5	>5	>5
Klebsiella pneumonia	2.5	>5	2.5	>5	1.25	2.5	>5	>5	>5	>5	>5	>5
Acinetobacter baumannii	5	>5	2.5	5	5	5	>5	>5	>5	>5	>5	>5
Enterobacter aerogenes	5	>5	5	>5	2.5	5	>5	>5	>5	>5	>5	>5
Salmonella Spp	5	5	2.5	2.5	2.5	5	>5	>5	>5	>5	>5	>5
Neisseria meningitidis	2.5	5	5	5	5	5	5	>5	5	>5	5	>5
Fungi	CMI	CMF	CMI	CMF	CMI	CMF	CMI	CMF	CMI	CMF	CMI	CMF
Candida albicans ATCC 10231	5	>5	5	>5	5	>5	Nd	Nd	Nd	Nd	Nd	Nd
Candida albicans ATCC 90028	Nd	Nd	5	>5	2.5	5	Nd	Nd	>5	>5	>5	>5

Nd = not determinated; Eg = *Englerina gabonensis;* St = *Sterculia tragacantha.;* WAE = water-acetone extract; WEE=water-ethanol extract; WE= water extract.

Discussion

Phytochemical screening of all extracts of *Englerina gabonensis* and *Sterculia tragacantha.* showed the presence of all flavonoids, phenols, tannin gallic and triterpenoids many of which have been reported to have health protective efficacy. Phenols, tannins, flavonoids and Proanthocyanidins contents are larger ($P < 0.05$) in the water-acetone extract of *Englerinagabonesis* compared with *Sterculia tragacantha.* extracts. Phenolic compounds are known to have antimicrobial properties. This abundance of the phenolic compounds of *Englerinagabonesis* confirms therapeutic properties which one assigns in ethnopharmacology [10]. Plant extracts of *Sterculia tragacantha.* show weak antioxidant activity (AAI<0.5). The AAI of water-acetone and water-ethanol extracts *of Englerina gabonensis* are superiors with 2;this plant presents a very strong antioxidant activity. These extracts have a potential antioxidant which would enable them to have a beneficial role in terms of very significant preventive actions for human and animal health [6]. Determination of the antioxidant activity of water, water-ethanol and water-acetone extracts of *Englerina gabonensis* and *Sterculia tragacantha.* by ABTS method corroborates with the results of DPPH. The antioxidant activity of *Englerina gabonensis* should be at least partially justified by the presence of totals phenolic highlighted by the phytochemical study [18-20]. Antimicrobial activity showed that the different extracts of *Englerina gabonensis* inhibited the growth of several microorganisms. Antimicrobial study of *Englerina gabonensis* and *Sterculia tragacantha.* showed that the water-acetone extract of *Englerina gabonensis* presents highest activity against the employed bacteria and showed also the highest antioxidant activity. These antibacterial actions could be related to their chemical components in the crude extracts [21]. Quantitative study of compounds showed that the antioxidant and antibacterial activities of the crude extracts of *Englerina gabonensis* depend on the presence of phenolic content.

Conclusion

As conclusion, this study confirm the multiple uses of *Englerina gabonensis* for the treatment of many infectious diseases and place them as candidate for further investigations for Enhanced Traditional Drug utilizable as Complementary and Alternative Medicines development and new active compounds discovery.

Author's contribution

SIMA OBIANG Cédric is the main author. Rick-LéonidNgoua-Meye-Misso revised the protocols, the manuscript and provided reference bacterial strains. NDONG ATOME Guy-Roger participated to all experiments. ONDO Joseph-Privat provided the material aids and advised in manuscript. OBAME ENGONGA Louis-Clément contributed to protocols elaboration and advised in manuscript preparation. NSI EMVO Edouard is the Responsible of LAREBIO, supervisor of this work, provided partially financial support

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

We are very grateful to local informants and healers who shared their knowledge on the use of medicinal plants with us. The authors are very much thankful to Shell Gabon for the financial support of materials in Laboratory of Research in Biochemistry of USTM (Grant No. SG/CIS/SDM/SA/sa n 77).

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