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Ethnobotanical Survey and Antibacterial Activity of African Plants used Against Diarrhea

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Results: The most represented families of plant used to treat diarrhea were *Euphorbiaceae.* Leaves and barks were the most used organs. Decoction and maceration appeared as the most common preparation methods and oral administration the most used route of administration. The EO of *Psidium guajava, Lantana camara* and *Ageratum conyzoïdes* were mainly composed of (E) -β-caryophyllene (26.5%), (E) -nerolidol (26.4%) and germacrene D (41.6%) respectively. The EO inhibited the growth of bacterial species with the smallest MIC of 6.25 obtained with *P. guajava* EO. Plant extracts were more active compared to EO, the smallest MICs of 0.19 to 6.25 mg / mL were obtained with *P. guajava* extracts

Conclusions: These results explain the use of different plants in Africa medicinal to treat diarrheal infections and present four of them as potential source of antimicrobial compounds.

Keywords: diarrheal infections; african medicine, plants extracts; essential oils, antibacterial activity.

Key Messages: A great diversity of African medicinal plants is used in the treatment of diarrhea. Some of these plants included in the study are *Psidium guajava, Lantana camara, S. acuta* and *Ageratum conyzoïdes* have shown to have antimicrobial activities on the growth of some bacterial species causing diarrhea.

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I. Introduction

Diarrheal infections are generally caused by bacteria, fungi or some viruses leading to digestive tract dysfunction. Although these infections are sometimes transient, acute forms can quickly get worse and lead to death or other severe adverse effects in some vulnerable people such as children under five, HIV/AIDS and individuals with an aging immunity [1]. To date, diarrhea is one of the most common and widespread diseases worldwide, particularly in African marginalized communities where it causes millions of deaths yearly [2]. Diarrhea is more deadly than malaria and tuberculosis. It is responsible of about 1.5 million of deaths in children each year worldwide. It is the third leading cause of death in low and middle income countries and sub-Saharan African populations are the most at risk [3]. In Gabon and Cameroon, diarrhea is the third leading cause of morbidity with a prevalence of 13 to 19 % [4].

Treatment of diarrhea involves oral rehydration as the first-line therapy (WHO and United Nations Children's Fund (UNICEF) but, in case of severe infections antibiotics are conventionally used. However, there is an upsurge of antibiotic resistance due to the loss of their efficacy resulting from inappropriate use or the poor management of infections. Besides, the high cost of antibiotics and their unavailability justify the resortment of those populations to traditional medicines [5, 6]. Use of traditional medicines is a fundamental component of African traditional health care system. About 80% of African populations rely on medicinal plants for various ailments and diarrheal infections particularly because appropriate health centers are often very far [7], [8], [9]. However, there is paucity of detailed scientific information on the composition and bactericidal effects of those plants which can constitute an alternative and or complementary method to the use of antibiotics.

Ageratum conyzoïdes, *Lantana camara*, *Psidium guajava* and *Sida acuta* are some of the plants found in Gabon and Cameroon flora. They are used in traditional pharmacopeia to treat various diseases [5, 10]. However, there are very few scientific research data on their antibacterial potential. Hence, the present work was carried out to study the growth inhibition effects of crude extracts and essential oils of *Ageratum conyzoïdes*,

Lantana camara, *Psidium guajava* and *Sida acuta* on five diarrhea-causing bacteria species as well as their phytochemical composition after an ethnobotanical survey which shows that they are traditionally used in Gabon to treat diarrheal diseases.

II. Subjects and Methods

a) Ethnobotany survey, harvest and identification of plants

The first step of the study was to carry out the ethnobotanical survey, to compile the data regarding the medicinal plants use against diarrheal infections, the mode of preparation and the route of administration. This survey was carried out in Gabon, in the Region of Woleu-Ntem, city of Medouneu with coordinates 0 ° 57'00 'North and 10 ° 47'00' 'East. The survey was conducted by administration of verbal questionnaire to 100 traditional medicinal healers locally called "Ngueguan" which means holder of ancestral medicinal knowledge. The purpose of this survey was to identify the most used plants in the treatment of diarrhea, to report the used plant part, the mode of preparation and the route of administration. The most cited plant were selected, photographed and botanically identified at the Gabonese National Herbarium at Libreville.

The four most cited plants namely *Ageratum conyzoïdes*, *Lantana camara*, *Psidiumguajava* and *Sidaacuta*were harvested from the botanical reserves of the University of Yaounde I with coordinates 3 ° 51'25 'North and 11 ° 30'05' East. Indeed, Cameroon and Gabon are two bordering countries with very close flora and culture, which explains the similarities in the treatment of diseases in their traditional pharmacopoeia. All plant harvests were made during November and December 2015, early in the morning (before 6: am) and involved the removal of leaves that were used for this study. The plant samples used for the extraction of the non-volatile compounds were dried at room temperature for two weeks and ground to obtain a powder.

b) Bacterial strains

Five bacterial species were used for antimicrobial tests. *Escherichia coli* ATCC, *Staphylococcus aureus* ATCC 13565 and *Bacillus cereus* T are referenced strains coming respectively from American Type Culture Collection (for the first two) and the Institute of Food Research in Reading. These last, are responsible for enterotoxigenic diarrhea. To these three species were added two clinical isolates including *Salmonella enteritidis* and *Shigella spp*., two bacterial species frequently responsible for entero-invasive diarrhea. These bacterial strains were kept at the Laboratory of Microbiology of the University of Yaounde I where antibacterial tests were carried out.

c) Extraction of essential oils

Extraction of the essential oils was carried out by hydrodistillation using a Clevenger type apparatus as described by Nyegue [11]. 300 g of leaves of each plant material were submitted to hydro-distillation using the Clevenger-type apparatus for 6 hours. The EO were separated from the mixture water-EO by decantation and then introduced into a dark bottle. After weighing, the obtained EO was dried over anhydrous sodium sulfate and stored at +4°C before used [12]. The extraction yields of EO expressed as a percentage (%) were calculated according to the formula Yield (%) = Mass of essential oil / Mass of raw plant material X 100.

d) Preparations of crude extracts

All the extractions were carried out by maceration using for each plant, three different solvents namely water, ethanol and the hydro-ethanol mixture (V / V). For this, 300 g of powder of leaves of each plant were immersed separately in 1500 ml of water (aqueous extract), pure ethanol (ethanolic extract) and ethanol-water mixture (V / V) (hydro ethanolic extract). The maceration was carried out for 48 hours with regular shake. At the end of this time, the solutions were filtered with hydrophilic cotton followed by Whatman paper N° 3. The filtrates collected from ethanolic extracts were evaporated at 70 ° C using a rotavapor, while the filtrates from aqueous and hydroethanolic extract were submitted to lyophilisation. The extracts obtained were kept at 4 ° C before used. The yields of the extractions were calculated according to the formula: Yield (%) = Mass of extract / Mass of raw plant material X 100 [13]

e) GC/FID and GC/MS analysis of the essential oil

The chemical composition of the EO was realized by GC/FID and GC/MS method as described by Kemegne et al. [14] GC/FID analysis was carried out using a TRACE 1300 Thermo scientific instrument equipped with two fused silica capillary columns DB-5 (30m*0.25mm*0.25μm) and DB-Wax (30m*0.25mm*0.25μm), programmed from 60-220°C at 3°C/min with a final hold time of 17 min, carrier gas Ultra High Purity N_2 at a split flow of 10 ml/min and purge flow of 5 ml/min, injector at 220°C.

GC/MS analyses was carried out using a Agilent 5977 MSD serie (Agilent technologies) apparatus equipped with two silica capillary columns HP-5MS (30m*250μm*0.25μm), HP-INNOWAX (30m*250μm*0.25μm) and interfaced with a single quatrupole detector. Column temperature 60-240°C at 3°C/min; injector temperature 240°C; carrier gas, He, at a flow rate of 0.7 ml/min; injector type split 20:1; the spectrometer was operated at 70.0 eV; mass range 33-400 and scan acquisition type.

Injections of authentic reference compounds, determination of their linear retention indices relative to the retention times of a series of n-alkanes as well as published mass spectra (9-21) and retention indices

were used as basis for the identification of the constituents, which were quantified as area percentage of total volatiles from electronic integration. Results obtained on HP-5 column permitted to the identification of components in comparison to some available data base (NIST14, NIST98, FFNSC 2.L, ESSENCES L.) and to literature data. Results obtained on Carbowax permitted to confirm one obtained on HP-5 column.

f) Phytochemical analyses

To identify the major phytochemical families of compounds present in the crude extracts, qualitative phytochemical analyses were carried out according to qualitative methods described by Odebiyi and Sofowora [15] and Harbone [16].

i. *Test of alkaloid:* 50 mg of the sample of each plant extract were dissolved in 10 mL of H_2SO_4 2%. The mixture was homogenized and boiled for 2 min before being filtered. 1 mL of the filtrate was added to five drops of Mayer's reagent. Formation of turbidity or precipitation was taken as evidence for the presence of alkaloids in the extract.

ii. *Test of phenols and polyphenols:* 50 mg of the sample of each plant extract were dissolved in 2 ml of water and this solution was added to 3 ml of fresh solution of $FeCl_2$ 5%. After homogenization, five drops of potassium ferricyanide 1.00% were added. Formation of a green or blue precipitate was considered as evidence of the presence of phenol or polyphenols respectively.

iii. *Test of flavonoids:* 50 mg of each extract were dissolved in 5 mL of methanol. To this solution were added some magnesium chips and drops of concentrated HCl. The presence of flavonoids was revealed by the appearance of orange or purple color.

iv. *Test oftriterpenes and steroids:* A vegetable preparation was obtained by dissolving 50 mg of extract in 20 ml of methylene chloride. To this solution were successively added 4 drops of acetic anhydride and sulfuric acid. The presence of triterpenes was revealed by the appearance of the purplish red color whereas the greenish blue color was considered as characteristic of steroids.

v. *Test of saponin:* 25 mg of plant extracts were solubilized in 15 ml of distilled water and the solution boiled in a water bath for 5 minutes. After cooling, the solution was mixed. The presence of persistent foam more than 1 cm thick for at least one minute indicates the presence of saponins

vi. *Test of Anthocyanin:* 50 mg of plant extract were dissolved in 15 ml of HCl 1% and the mixture was boiled for 5 minutes. The change of the color from orange-red to orange-blue reveals the presence of anthocyanins.

vii. *Test of Tannins:* To 15 ml of an alcoholic or aqueous solution of plant powder, were added a few drops of iron chloride. A color change reflects the presence of tannins. The dark coloration was characteristic of gallic tannins and the green color revealed the presence of catechism tannins

g) Evaluation of antibacterial effects

The antibacterial assay consisted of determining the sensitivity of the bacteria to the extracts and subsequently evaluating the inhibition parameters (MIC and CMB). The sensitivity of bacteria was assessed by the agar diffusion method using disks for the EO and from the wells for non-volatile extracts [17]. The MIC and MBC (Minimum Inhibitory Concentration and Minimal Bactericidal Concentration) were then determined by the micro-dilution method as described by Ateufack et al. [18] recommended in the standard protocol of CLSI-M-A9 [19].

i. *Agar diffusion method (Antibiogram)*

The test was performed by swabbing a suspension of the overnight tested microorganism load of 0.5 McFarland scale (corresponding to approximately 1.5×10^6 cells/mL standardized with a spectrophotometer) on the Muller Hinton Agar. The solutions of each extract were prepared at 100 mg / mL, using $Tween_{40}$ 5% for the EO and sterile distilled water for the nonvolatile extracts. Disks impregnated with 15 µL of solution of each EO at 1 mg / mL or gentamicin at 1 mg / mL (reference antibiotic) were then deposited on the inoculated media. Concerning the extracts, wells of 6 mm of diameter were punched on the agar medium and each well was filled with 50 µL of extract solution. After 30 minutes of pre-diffusion at room temperature, the plates were incubated at 37 ° C for 24 hours, the diameters of the microbial growth inhibition zones were measured and the averages calculated from the repetitions of three tests.

ii. *Determination of Minimal Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)*

MIC and MBC were determined by the culture broth microdilution method according to the standard protocol of CLSI-M-A9 (2012) using 96-well microplates. All the tests were performed in three replicates. In each well of the microplate, were first introduced 100 µL of Muller Hinton broth (supplemented with $Tween_{40}$ at 5% for th EO). Subsequently, 100 µL of 200 mg / mL solutions of each extract or EO were added to the first wells. The dilutions of the extracts or EO were done according to a geometric progression reason 2 until the concentration of 0.048 mg / mL. 100 µL of inoculum prepared with overnight tested microorganism were then inoculated into each well to obtain a final load of 10^6 cells / mL and the whole plate incubated at 37°C. After 24 hours of incubation, the bacterial growth was revealed by addition of 40 µL of a solution of 2,3,5-

Triphenyltetrazolium Chloride (TTC) 0.01% in each well. This was followed with 30 minutes of incubation and the appearance of a red color indicates the presence of living bacteria in the medium. The smallest concentration of extracts or EO not showing the red color represents the MIC.

The MBC were determined by inoculation in new microplates, 50 µL of inoculum (with concentrations greater than or equal to the MIC) in 150 µL of nutrient broth contained in new wells. The plates were then incubated for 48 hours at 37 ° C, before being revealed with TTC as previously described. After 30 min of incubation, all concentrations not showing red color were considered as bactericidal. The smallest of these has been noted as MBC.

The MBC / MIC ratio were calculated to determine the bactericidal effect (MBC / MIC <4) or bacteriostatic effect (MBC/ MIC ≥ 4) of the test substances [20].

iii. *Statistical analysis*

Inhibition diameter values were statistically analyzed by ANOVA at the 5% probability level ($p < 0.05$) using GraphPad prism 5.0 software. The Excel spreadsheet was used to calculate means and standard deviations.

III. Results

a) Ethnobotanical survey

The ethnobotanical survey was conducted in the locality of Medouneu (Gabon) in order to identify the commonly used plants for the treatment of diarrhea. The part of plant used, the mode of preparation and the route of administration were the main points of the survey. The results summarized in Table 1 present 24 identified main plant families namely *Anacardiaceae*, *Annonaceae*, *Apocynaceae*, *Aspleniaceae*, *Asteraceae*, *Bombacaceae*, *Caesalpinaceae*, *Canellaceae*, *Chenopodiaceae*, *Combretaceae*, *Convovulaceae*, *Ebenaceae*, *Euphorbiaceae*, *Fabaceae*, *Hypericaceae*, *Labiaceae*, *Leguminosae*, *Lycopodiaceae*, *Malvaceae*, *Mimosaceae*, *Moraceae*, *Myrtaceae*, *Sterculiaceae* and *Vernaceae*. The most represented family was that of *Euphorbiaceae* with 8 different genera and species (*Acalypha reticulate*, *Alchornea cordifolia*, *Bridelia atroviridis*, *Euphorbia hiria*, *Macaranga* spp., *Hymenocardia acida*, *Jatropha curcas* and *Uapaca densifolia*).The second most encountered family was the *Asteraceae* with 6 species (*Acanthospermum hispidum*, *Ageratum conyzoïdes*, *Apodocephala pauciflora*, *Biden spilosa*, *Vernonia cephalophora* and *Vernonia amygdalina*), followed by *Anacardiaceae* with 5 species (*Anacardium occidentale*, *Lannea acida*, *Lannea microcarpa*, *Mangifera indica* and *Sclerocarya birrea*). *Combretaceae* were also highly represented with 5 species (*Anogeissusleiocarpus*, *Combretum micranthum*, *Combretum nigricans*, *Guiera senegalensis* and *Terminalia avicenioides*). The other plant families were represented by one or two species generally belonging to different genera, except *Annona muricata* and *Annona senegalensis* both belonging to *Annonaceae* family.

Table 1 reveals a great diversity of plant organs namely barks, leaves, stem, stem bark, fruits, seed, pulp roots and entire plant (in some cases) used by the population in the treatment of diarrhea. However, the type of organs used varied according to the plant but in general the leaves and barks were the most used organs except for *Lannea microcarpa* and *Biden spilosa* where young leaves have been recommended. About the method of preparation, various methods were also recorded although decoction and maceration appeared as the most used methods. Infusion was recommended only in the case of *Apodocephala pauciflora* and *Chenopodium ambrosioides (leaves)* and the entire plant of *Ocimum sp.* Likewise, preparation by expression was mentioned only for the leaves of *Macaranga sp.* In addition, oral administration was the most common route although bath was recommended in the case of stem and leaves of *Merremia pellaia,* barks or stems of both *Parkia biglobosa* and *Ficus sycomorus*.

b) Extraction yields

After maceration and hydro distillation, the extraction yield values of different plants sample were recorded in Table 2. It appears that the extraction yields vary from one plant to another and depend on the solvent used. Overall, the lowest yields were obtained with EO, while the highest percentages were obtained with hydro ethanolic extraction. *A. conyzoïdes* leaves showed the highest EO yield of 0.21 ± 0.02%, while *S. acuta* did not have any essential oils. The highest yields were obtained from hydro ethanolic extracts of *A. conyzoïdes* (11.20%), followed by *L. camara* (10.33%) and *P. guajava* (9.74%). The highest extraction yield value for *S. acuta* (3.73%) was obtained with the aqueous extract.

c) Essential oils composition

Table 3 presents the results of the chemical composition of the essential oils (EO) of *P. guajava*, *L. camara* and *A.* conyzoïdes analyzed by Gas Chromatography- FID (GC-FID) and GC coupled to Mass Spectrometry. Analysis of *P. guajava* EO enables to identify 61 compounds representing 99% of the EO. The oil is composed by 8.9% of aliphatic compounds, 6.2% of monoterpenes and 83.9% of sesquiterpenes. *P. guajava* EO was characterized by 8 main components accounting *(E)*-*β*-caryophyllene (26.5%), *β*-bisabolol (8.9%), benzaldehyde (7.8%), *(E)*-nerolidol (7.2%), 1,8-cineole (5.7%), *β*-sesquiphellandrene (4.3%), *α*-humulene (3.9%) andisodaucene (3.7%). *(E)*-*β*-caryophyllene is therefore the main component of the oils.

In the other hand, analysis of *L. camara* EO allowed to identify 55 compounds over 61 representing 99.4% of the total EO. The EO is composed by 23.9% of monoterpenes, 73.8% of sesquiterpenes and 1.6% of diterpenes. It contains7 main components namely *(E)*-nerolidol (26.4%), *(E)*-*β*-caryophyllene (12.7%), sabinene (8.4%), 1,8-cineole (6.9%), *α*-zingiberene (5%), *α*-humulene (4.6%) and *γ*-curcumene (3.8%). Nevertheless, *(E)*-nerolidol (26.4%) and *(E)*-*β*-caryophyllene (12.7%) are the main compounds of the oils.

Chemical analyses of *A. conyzïdes* EO revealed 16 compounds representing 88.6% of the total EO. The class of hydrocarbon monoterpenes with 5.8% of the total EO is poorly represented. On the other hand, germacrene D (41.6%) and β-trans-caryophyllene (24.6%) are the major compounds of EO conferring it an important composition in sesquiterpenes.

d) Phytochemical screening of nonvolatile extracts

The results of the phytochemical screening of non-volatile extracts of each plant are presented in table 4. This results show that the plant extracts studied contain the major families of phytochemicals compounds with antibacterial properties. Anthocyanins, flavonoids and tannins are present in all the extracts. The same observation is made concerning the presence of the alkaloids, except for the ethanolic extracts of *L. camara*and ethanolic and hydro ethanolic extracts of *P. guajava* which are devoid. The polyphenols are present in the aqueous and hydro ethanolic extracts of all the plants, except all the extracts of *L. camara*. Tri terpenes on the other hand are present only in the hydro-ethanolic extracts of all the plants, as well as all the extracts of *L. camara*. However, the presence of phenols is especially noted in ethanolic extracts although their presence is observed in all the extracts of *L. camara*.

e) Antibacterial activities of essential oils and plant extracts

i. *Antibiogram*

Tables 5 and 6 respectively present the values of the inhibition zone diameters of the EO and non-volatile extracts. The analysis of Table 5 reveals that each EO inhibits the growth of at least three out of five bacterial species with overall inhibition diameters between 7.0 ± 0 and 13.6 ± 2.5 mm. The most effective EO is that of *P. guajava*, which exhibited inhibition diameters zone of 12.6 ± 0.5 and 13.6 ± 2.5 mm respectively on the growth of *S. aureus* and *S. enteritidis*. The EO of *L. camara* was also active but only on three bacterial strains (*E. coli, S. enteritidis* and *Shigella* spp.) with inhibition diameters zones between 9 ± 1 and 13 ± 2 mm. The essential oil of *A. conyzoïdes* showed extensive effects on all bacteria, although the observed inhibition zone diameters were < 9.7 ± 0.6 mm. The inhibition diameter zones of the EO of all the plants were 2 to 5 fold lower than those of Gentamicin, the reference antibiotic used.

Tables 6 shows that, the crude extracts inhibit the bacterial growth more significantly compared to the EO ($P < 0.05$) and in diverse manner (extract-dependent effect) with the values of inhibition diameter zones varying between 7 and 16 mm. Concerning *A. conyzoïdes*, only aqueous extract (AE_{AC}) was active, with effects observed on all the bacterial species tested. Values of inhibition diameter zones were ranged from 11.0 ± 1.0 (on *E. coli*) to 12.7 ± 2,1 mm (on *S. aureus*). For *L. camara* the most effects ($P < 0.05$) were obtained with hydro ethanolic extract (HEE_{Lc}) which exhibited effect on all the bacterial species with inhibition diameter zones between 11.0 ± 1.0 (on *B. cereus*) and 15.0 ± 3.5 mm (on *S. enteritidis*). Ethanolic extract (EE_{Lc}) also effective shows antibacterial effect on four out to five bacterial species, with inhibition diameter zones between 9.7 ± 0.6 (on *Shigella* spp.) to 14.3 ± 2.1 mm (on *E. coli*).Aqueous extract of *L. camara* (AE_{Lc}) presented no effect on the growth of all the bacterial species. *P. guajava* presented the same antimicrobial profile as compared to *L. camara* with the best inhibitory potential observed with ethanolic extract (EEpg) and hydro ethalolic extract (HEEpg). The first one exhibited the antibacterial effect on all the bacterial species with inhibition diameter zones ranged from 10.3 ± 0.58 (on *Shigella* spp.) to 11.7 ± 0.6 mm (on *E. coli* and *S. enteritidis*). The other one was active on four out to five bacterial species. However, the latter inhibited more efficiently the growth of *S. enteritidis* with inhibition diameter zone of 16.0 ± 3.6 mm. Regarding *S. acuta*, only hydro ethanolic extract (HEE_{Sa}) was active, with effects observed on all the bacterial species and values of inhibition diameter zones ranging from 8.7 ± 1.5 (on *S. aureus)* to 13.0 ± 2.0 mm (on *S. enteritidis*)

ii. *Determination of inhibition parameters (MIC, MBC and MBC / MIC)*

The results of the growth inhibition parameters (MIC, MBC and MBC/MIC) of the EO, the non-volatile extracts and Gentamicin on bacteria growth are summarized respectively in Tables 7 and 8. Table 7 shows that the MIC of the EO ranges from 6.25 (with EO of *P. guajava*on *Shigella* spp.) to 100 mg / mL (with EO of *A. conyzoïdes* on *S. enteritidis* and *S. aureus*).Overall, the MICs of 25 mg/mL were generally obtained from all the EO although MIC of 12.5 mg / mL was obtained with EO of *L. camara* on *Shigella* spp.

The results presented in Table 8 presented the greater antibacterial effect of the nonvolatile extracts (as compared to the EO) with MICs ranging from 0.19 (with aqueous extract of *P. guajava* on *B.cereus*) to 100 mg / mL (with aqueous extract of *A. conizoïdes* on *B. cereus*). A strong effect was observed with hydroetanolic extract of *L. camara* with MIC values of 0.39 mg/mL on *S. aureus* and 0.78 mg/mL on *B. cereus* and *E. coli.*

Similarly, the same observations were noted with the aqueous extract of *P. guajava* which presented MIC values of 0.19 mg/mL and 0.78 mg/mL on *B. cereus* and *S. aureus* respectively. Likewise, its ethanolic counterpart exhibited the same effect both on *B. cereus* and *S. aureus* with the same MIC value of 0.78 mg/mL. *S. acuta* presented the more effect with MIC value of 0.78 mg/mL with ethanolic extract on *B. cereus* and hydroethanolic extract on *S. enteritidis*. Some good activities can also be noted at MIC value of 1.56 mg/mL as observed with ethanolic extract of *L. camara* on *S. enteritidis*, *shigella spp*. and *S. aureus*; aqueous extract of *L. camara* on *S. aureus*; hydrethanolic extract of *S. acuta* on *B. cereus* and ethanolic extrac of *S. acuta* on *S. aureus*. However, as already observed with the inhibition diameters zones, the activity of the EO and the plant extracts were lower than that of Gentamicin with MIC values between 0.009 and 1.39 mg /mL. The ratio MBC/MIC < 4 is used to determine the bactericidal effect of plant extract on a given strain while, the same ratio greater than 4 is characteristic of a bacteriostatic effect [20]. Thus, the calculation of this ratio showed that most of the EOs from the plants used have a bactericidal effect on all the tested strains, except *B. cereus*, *Sighella* spp. and *S. aureus* where the effects were rather bacteriostatic (Table 7). Non volatile extract have also shown both bactericidal and bacteriostatic activities on the bacterial species tested (Table 8). However, the EO and the extracts showed in more case, bactericidal than bacteriostatic effects on the bacterial species studied.

IV. Discussion

This study allowed the identification of some plants commonly used in Gabon for the treatment of diarrhea, the part of plant used, the mode of preparation and the route of administration. The ethnobotanical survey showed that they are greatly diversify with 52 plant species belonging to 24 main families. This great diversity observed is explained by the geographical and cultural situation of Gabon. Indeed, the country is located in the equatorial zone characterized by the heavy rainfall and the presence of the forest. This forest represents an important medicinal wealth and explains the great biodiversity of plant resources. On the other hand, in Central Africa the natives possess a good knowledge of their natural environment. This knowledge is integrated in important activities of the cultural life, and applied during the rites of initiations, religious and even of cure.

In this study, the most represented families among the cited plants are *Euphorbiaceae*, *Fabaceae* and *Asteraceae*. These results are in accordance with those of Njoroge and Kibunga [21] and Njume and Goduka [22]. These authors also respectively mentioned *Asteraceae*, *Euphorbiaceae* and *Fabaceae* as the most families of plants used in the treatment of diarrhea in other African localities. The leaves and bark were the most part of plant organs used, with various methods of preparation, dominated by decoction and maceration. Oral administration as a drink was the major route of administration. This could be explained by the fact that leaves and bark are perennial parts of the plant and decoction and maceration usually allow the extraction of the majority of plant metabolites. These observations are in the accordance with other studies regarding the use of herbal medicine in the treatments of diarrheal infections[21, 22]. In addition the oral route could be the most used route of administration because it allows bringing the drug directly in the digestive tract facilitating its antidiarrheal effect as compared to the other modes of administration.

Four commonly used plants namely *Ageratum conyzoïdes*, *Lantana camara*, *Psidium guajava* and *Sida acuta* were further investigated to determine their chemical compositions and their effects on the inhibition of the growth of some bacteria responsible of diarrhea. After sample collection and extraction, the yields obtained varied according to the type of extract, plant species and the solvent of extraction. This variability is related to the chemical composition of the plant. Overall, the lowest yields are obtained with EO, while the highest percentages were obtained with hydro ethanolic extraction. This is explained by the fact that the EO are mainly composed by terpenic compounds generally represented at a low percentage in the plant composition. The highest yields obtained from hydro ethanolic extracts is due to the capacity of the solvent to extract both polar and nonpolar compounds as compared to water and ethanol which permit to extract only polar or more non polar compounds respectively.

Phytochemical screening of extracts revealed that the phytochemical components are unequally distributed according to the extract and the plant species. This variability is due to the influence of two parameters, namely the molecular composition of the plant species and the affinity of the solvent with the molecules present in the plant. Indeed, there are differences in solubilization capacity and extraction of solvents with respect to phytomolecules. It has been reported that during liquid-liquid extraction, bioactive substances are distributed among solvents according to their polarity and solubility [23].

Regarding the antibacterial tests, most of the bacterial strains tested were sensitive to the EO and crude extracts. The sensitivity of bacterial species is explained by the presence of families of phytochemicals compounds with antibacterial properties found in the different EO and plant extracts. In fact, concerning the EO, β-caryophyllene presents in all the EO (26.5% in *P. guajava* EO, 12.7% in *L. camara* and 24.6% in *A. conyzïdes*) is sesquiterpene with broad-spectrum antibacterial effects due to its hydrophobicity and ability

to cause structural and functional damage to the cell membrane [24]. Thus, many plant sources of β-caryophyllene are used for several purposes, including antimicrobial activity [25]. Xiong et al [26]. showed wide-spectrum activity of β-caryophyllene against Gram-positive bacteria including *S. aureus*(including methicillin-resistant), *S. epidermidis*, *S. auricularis*, *M. caseolyticus*, *E. faecium* and *E. faecalis* with MIC values ranging from 0.032 to 0.256 mg/mL. Also, Nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol) present in the EO of *Lantana camara* (26.4 %) and *Psidium guajava* (7.2%), is a sesquiterpene alcohol present in various plants with a floral odor. The usage of nerolidol is widespread across different industries including cosmetics and food (permitted by U.S. Food and Drug Administration as a food flavoring agent). Nerolidol already showed to possess antibacterial effect due to the disruption of cell membranes. The observed effects could be due to the presence of the long aliphatic chain in chemical structure of nerolidol [27]. The antimicrobial activities of Nerolidol on the growth of the pathogens *Escherichia coli* O157, *Clostridium difficile*, *Clostridium perfringens*, *Salmonella typhimurium* and *Salmonella enteritidis* were already highlighted [28].

Concerning nonvolatile extracts, the broad antimicrobial activity observed can be attributed to the presence of various bio-actives components such as tannins, polyphenols, alkaloids, flavonoids, steroids and saponins found in this extract. The variation observed with the diameters of inhibition zone of the bacteria tested can be attributed either to the difference of the bioactive molecules present in each extract or to their mechanism of action on Gram-positive and Gram-negative bacteria. The mechanism of action of the polyphenols, tannins and alkaloids on Gram-positive and Gram-negative bacteria was demonstrated [29].

The species *L. camara* and *P. guajava* showed strong activity on the strains tested, unlike some previous studies which showed little or no activity with the leaf extracts of *P. guajava* on *S. aureus*, *S. enteridis* and *E. coli* [30].This can be explained by the variation in concentration of active biomolecules due to the plant's harvesting locations (Cameroon) as compared to that used by these researchers. Ecosystem parameters play an important role in the plant phytochemistry responsible for antimicrobial activity in the plant [31].

V. Conclusion

This study reveals a great diversity of the plant used to treat diarrhea in the locality of Medouneu (Gabonese locality) with *Euphorbiaceae*, *Fabaceae* and *Asteraceae* as the most represented families. The leaves and bark are the most plant organs used, with various methods of preparation, dominated by decoction or maceration and oral administration was the most used route of administration. Further studies on some of listed plants namely *Ageratum conyzoïdes*, *Lantana camara*, *Psidium guajava* and *Sida acuta* show the broad antimicrobial activity of the EO and plant extracts on the inhibition of the growth of bacteria responsible of diarrhea. These results explain the use of those plants to treat diarrheal infections and show a potential source of antimicrobial compounds.

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Table 1: List of medicinal plants and their used as recorded by ethno botanical survey

Plant families	Species	Part used	préparation	Administration
Anacardiaceae	*Anacardium occidentale*	barks and leaves	decoction	drink
	Lannea acida	aerial part of plant	decoction	drink
	Lannea microcarpa	young leaves	maceration	drink after filtration
	Mangifera indica	barks or leaves	decoction / maceration	drink
		barks or stem	decoction	bath
	Sclerocarya birrea	barks	decoction	drink
Annonaceae	*Annona muricata*	leaves	decoction	drink
	Annona senegalensis	roots	decoction /maceration	drink
Apocynaceae	*Alstonia congensis*	stem barks	maceration	drink
	Mascarenhasia arborescens	leaves	decoction	drink
Aspleniaceae	*Asplenium monanthes*	fresh fruits	maceration	anal administration
Asteraceae	*Acanthospermum hispidum*	leaves and stem	decoction	drink
	Ageratum conyzoïdes	leaves	decoction	drink
	Apodocephala pauciflora	leaves	decoction / infusion	drink
	Biden spilosa	young leaves	mastication	oral administration
	Vernonia cephalophora	leaves	decoction	drink
	Vernonia amygdalina	leaves	maceration	drink
Bombacaceae	*Adansonia digitata*	Pulp or leaves	maceration or infusion	drink
	Ceiba pentandra	bark	decoction	drink
Caesalpinaceae	*Piliostigma reticulatum*	stem bark	decoction	drink
Canellaceae	*Pentadiplandra brazzeana*	roots	decoction	bath
	Cinnamosma fragrans	bark and stem	decoction	drink
Chenopodiaceae	*Chenopodium ambrosioides*	entire plant/ crush leaves	Infusion / maceration	bath
Combretaceae	*Anogeissus leiocarpus*	leaves	décoction	drink
	Combretum micranthum	leaves	décoction	
	Combretum nigricans	roots	décoction	
	Guiera senegalensis	fruit	décoction	
	Terminalia avicenioides	roots	décoction	
Convovulaceae	*Merremia pellaia*	stem and leaves	decoction	bath
Ebenaceae	*Diospyros mespiliformis*	fruits	maceration	drink
Euphorbiaceae	*Acalypha reticulata*	stem or leaves	decoction	
	Alchornea cordifolia	leaves	maceration	
	Bridelia atroviridis	leaves	decoction	
	Euphorbia hiria	entire plant	decoction	
	Macaranga sp	sap leaves	Expression	
	Hymenocardia acida	barks and roots	maceration	
	Jatropha curcas	latex of stem or leaves	dilution	spoonful
	Uapaca densifolia	barks	decoction	drink
Fabaceae	*Dalbergia melanoxylon*	leaves	decoction	drink
	Pterocarpus erinaceus	stem barks		
Hypericaceae	*Psorospermum cerasifolium*	green seeds	decoction	drink
Labiaceae	*Ocimum sp*	leaves	tea	drink

Leguminosae	*Cassia hirsuta*	leaves	decoction	drink
Lycopodiaceae	*Lycopodiella cernua*	sporange	decoction	drink
Malvaceae	*Sidaacuta*	fresh leaves	decoction or maceration	drink
	Urena lobata	barks or stems orroots	decoction	drink
Mimosaceae	*Acacia dudgeoni*	barks or stems	decoction	drink
	Parkia biglobosa	barks or stems	decoction	bath
Moraceae	*Ficus sycomorus*	barks or stems	decoction	bath
Myrtaceae	*Psidium guajava*	leaves	decoction or maceration	drink
Sterculiaceae	*Dombeya pentandra*	barks	decoction	drink
	Waltheria indica	stems and leaves	decoction	drink
Vernaceae	*Stachytarpheta indica*	leaves	maceration	drink

Table 2: Extraction yield (%) of essential oils and plant extracts

	Extraction yields (%)			
Plants	EO	AE	EE	HEE
A. conyzoides	0.21±0.02	1.3	11.20	6.64
L. camara	0.08±0.03	5.94	10.33	2.15
P. guajava	0.16±0.03	5.99	9.74	5.07
S. acuta	0.00±0.00	4.5	1.48	3.73

EO = essential oils; AE = Aqueous extract; EE = Ethanolic extract; HEE = Hydro ethanolic extract

Table 3: Quantitative and qualitative chemical composition of the essential oils of *P. guajava*, *L. camara* and *A. conyzoïdes* leaves

Compounds names	RI (HP5-apolar)	RI (Adams)	RI (Carbowax-polar)	Percentage (%)		
				P. guajava	*L. camara*	*A. conyzïdes*
Aliphatic				**8.9**	-	**2.1**
(3Z)-hexenol	595	859	-	0.5	-	-
(n)-hexanol	629	870	1349	<0.1	-	-
benzaldehyde	**966**	**960**	**1530**	**7.8**	-	-
6-methyl-Hept-5-en-2-one	984	985	1456	0.6	-	-
Methyl acetate	1287	1267	-	-	-	2.1
Monoterpenes				**6.2**	**23.9**	**5.8**
α-thujene	801	930	-	-	<0.1	-
α-pinene	938	939	1033	-	1.7	0.7
Camphene	952	954	1073	-	<0.1	1.8
Sabinene	**978**	**975**	**1134**	-	**8.4**	**-**
β-pinene	983	979	1117	-	1.3	-
Myrcene	991	990	1169	-	0.8	-
α-phellandrene	1007	1002	-	-	<0.1	0.7
δ-3-carene	1014	1011	1156	-	1.2	-
α-terpinene	1019	1017	1183	-	<0.1	0.9
*para*cymene	1026	1024	1273	-	<0.1	1.2
Myrcene	990	990	929	<0.1	-	-
Limonene	1031	1029	1202	<0.1	0.7	0.5

1,8-cineole	**1035**	**1031**	**1215**	**5.7**	**6.9**	**-**
(Z)-β-ocimene	1040	1037	1035	-	<0.1	-
(E)-β-ocimene	1050	1050	1046	<0.1	<0.1	-
γ-terpinene	1060	1059	1251	<0.1	<0.1	-
Linalool	1099	1096	1548	<0.1	-	-
cis-hydrate de sabinene	1068	1065	-	-	<0.1	-
terpinolene	1090	1088	1284	-	<0.1	-
Linalool	1101	1096	1562	-	<0.1	-
trans-hydrate de sabinene	-	1098	1556	-	<0.1	-
Camphre	1146	1146	1519	-	0.9	-
Borneol	1166	1169	1684	-	<0.1	-
terpinen-4-ol	1175	1177	-	<0.1	2	-
α-terpineol	1189	1188	1683	0.5	<0.1	-
Sesquiterpenes				**83.9**	**73.8**	**80.8**
δ-elemene	1334	1338	1474	-	<0.1	-
α-copaene	1372	1376	1494	<0.1	<0.1	-
α-2-*epi*-funebrene	1392	1382	-	<0.1	-	-
β-elemene	1387	1390	1609	-	1.2	-
7-*epi*-sesquithujene	1389	1391	1716	<0.1	-	-
italicene	1394	1405	1541	<0.1	-	-
sesquithujene	1404	1405	-	<0.1	<0.1	-
α-cedrene	1413	1411	1572	0.8	<0.1	-
α-*cis*-Bergamotene	1415	1412	1568	<0.1	-	-
***(E)-β*-caryophyllene**	**1422**	**1419**	**1614**	**26.5**	**12,7**	**24.6**
β-Ionone	**1468**	**1430**				**4.7**
β-copaene	1430	1432	1542	<0.1	<0.1	-
sesquisabinene	1443	1459	1720	-	<0.1	-
α-*trans*-bergamotene	1446	1434	1599	0.6	-	-
β-*epi*-santalene	1452	1447	1643	<0.1	-	-
***α*-humulene**	**1454**	**1454**	**1674**	**3.9**	**4.6**	**-**
(E)-β-farnesene	1456	1456	1676	<0.1	<0.1	0.4
9-*epi*-*(E)*-caryophyllene	1458	1466	-	-	<0.1	-
α-acoradiene	1461	1466	1672	<0.1	-	-
β-acoradiene	1466	1470	1666	1.7	<0.1	-
β-10-*epi*-acoradiene	1465	1475	-	<0.1	-	-
γ-muurolene	1471	1479	1691	<0.1	<0.1	-
***γ*-curcumene**	**1473**	**1482**	**1682**	2	**3.8 ***	**-**
germacrene D	1473	1485	1696	-	0.5 *	**41.6**
ar-curcumene	1479	1480	1776	1.5	1.9	-
β-selinene	1480	1490	1708	<0.1	-	-
α-selinene	1486	1498	1713	<0.1	-	-
***α*-zingiberene**	**1488**	**1493**	**1728**	-	**5 ***	**-**
isodaucene	**1488**	**1500**	**-**	**3.7**	-	-
bicyclogermacrene	1488	1500	1734	-	2.3 *	-

α-muurolene	1493	1500	-	<0.1	-	-
(Z)-α-bisabolene	1496	1507	-	3.4	-	-
β-curcumene	1496	1515	1696	-	3.4	-
β-bisabolene	1506	1505	1724	2.9	-	2.9
Cubebol + γ-cadinene	1520	1515	-	-	<0.1	-
δ-cadinene	1502	1523	1755	-	2.2	1.3
γ-cadinene	1509	1513	1682	<0.1	-	-
β-curcumene	1509	1515	1731	1.1	-	-
(Z)-γ-bisabolene	1513	1515	-	0.7	-	1.4
β-sesquiphellandrene	**1521**	**1522**	**1756**	**4.3**	-	-
(E)- γ-bisabolene	1529	1531	1740	3.3	-	-
α-copaene-11-ol	1535	1541	2065	-	<0.1	-
italiceneether	1544	1537	1853	<0.1	-	-
(E)-α-bisabolene	1534	1540	-	0.8	-	-
germacrene B	1547	1561	1825	-	0.4	-
davanone B	1569	1566	1953	-	0.6	-
(E)-nerolidol	**1559**	**1563**	**2030**	**7.2**	**26.4**	**-**
davanone D (isomère 1)	1562	-	1950	-	0.2	-
caryolan-8-ol	1565	1572	-	<0.1	-	-
spathulenol	1576	1577	2113	-	0.6	-
caryophyllene oxide	1580	1583	1962	2.2	0.7	0.5
davanone D (isomère 2)	1585	1585	2030	-	0.6	-
Gleenol	1589	1587	2038	<0.1	-	-
Zingibérenol	1595	-	-	-	-	**3.4**
epoxyde d'humulene II	1596	1608	2034	<0.1	-	-
not identified 1	1600	-	-	-	0.6	-
2,(7Z)-bisaboladien-4-ol	1604	1619	-	<0.1	-	-
not identified 2	1605	-	-	-	0.7	-
epi-cubénol	1624	1628	2044	<0.1	-	-
eremoligenol	1626	1631	-	<0.1	-	-
α-acorenol	1630	1633	2166	0.7	-	-
not identified 3	1630	-	-	-	2.7	-
naphth-1-ol	1635	1641 **	-	-	1	-
caryophylla-4(12),8(13)-dien-5-α-ol	1637	1640	-	1.4	-	-
α-epi-cadinol	1640	1640	2163	<0.1	-	-
α-muurolol	1642	1646	2176	0.5	-	-
not identified 4	1647	-	2419	-	0.9	-
neo-intermedeol	1655	1660	2125	3.1	-	-
not identified 5	1663	-	-	-	0.1	-
β-bisabolol	**1665**	**1675**	**2142**	**8.9**	-	-
not identified 6	1667	-	-	-	0.8	-
α-epi-bisabolol	1674	1684	-	<0.1	-	-
α-bisabolol	1676	1685	2187	2.2	-	-

(2Z,6E)-farnesol	1699	1723	2355	0.5	-	-
Diterpenes					**1.6**	**-**
Phytol	2068	1943	2591	-	1.6	-
Total identified				**99**	**99.4**	**88.7**

**=relatives proportions obtained on polar column; **=retention indices obtained from FFNSC2.L*

Table 4: Phytochemical analysis of plant extracts using qualitative tests

	A. conyzoïdes			*L. camara*			*P. guajava*			*S. acuta*		
Phytochemical compounds	AE	EE	HEE	AE	EE	HEE	AE	EE	HEE	AE	EE	HEE
Alcaloïds	+	+	+	+	-	+	+	-	-	+	+	+
Athocyanins	+	+	+	+	-	+	+	+	+	+	+	+
Flavonoïds	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	-	+	-	+	+	+	-	+	-	-	+	-
Polyphenols	+	-	+	-	-	-	+	-	+	+	-	+
Saponins	-	+	+	-	+	+	+	+	+	-	+	+
Stéroids	+	+	-	-	+	-	-	+	-	+	+	-
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Triterpenes	-	-	+	+	-	+	-	-	+	-	-	+

+ = present; - = absent ; AE = Aqueous extract; EE = Ethanolic extract; HEE = Hydro ethanolic extract

Table 5: Inhibition zone diameters of the essential oils and Gentamicin against the tested bacteria using the disk diffusion assay.

	Bacterial species Inhibition zone diameters (mm)				
Essential oils	*B. cereus*	*E. coli*	*S. enteritidis*	*Shigella spp*	*S. aureus*
A. conyzoides	7.0 ± 0	8.5 ± 0.5	7.3 ± 0.6	7.6 ± 0.5	9.7 ± 0.6
L. camara	-	9.0 ± 1.0	13.0 ± 2.0	11.0 ± 0.5	-
P. guajava	10.6 ± 0.5	11.6 ± 0.5	13.6 ± 2.5	10.6 ± 0.5	12.6 ± 0.5
Gentamicin	33.0 ± 1.0	30.6 ± 0.5	31.6 ± 7.0	37.6 ± 2.5	27.6 ± 5.5

Table 6: Inhibition zone diameters of the crude extracts against the tested bacteria using the disk diffusion assay

	Bacterial species Inhibition zone diameters (mm)				
Extract	*B. cereus*	*E. coli*	*S. enteritidis*	*Shigella spp*	*S. aureus*
AE_{Ac}	12.0 ± 1.0	11.0 ± 1.0	11.3 ± 0.6	11.3 ± 0.6	12.7 ± 2,1
EE_{Ac}	-	-	-	-	-
HEE_{Ac}	-	-	9.3 ± 0.6	-	-
AE_{Lc}	-	-	-	-	-
EE_{Lc}	13.0 ± 2.0	14.3 ± 2.1	12.8 ± 1.6	9.7 ± 0.6	-
HEE_{Lc}	11.0 ± 1.0	12.3 ± 0.6	15.0 ± 3.5	12.3 ± 1.5	11.7 ± 1.5
AE_{Pg}	-	-	14.0 ± 5.3	-	-
EE_{Pg}	11.3±1.1	11.7 ± 0.6	11.7 ± 0.6	10.3 ± 0,58	10.3 ± 0.6
HEE_{Pg}	-	12.0 ± 1.0	16.0 ± 3.6	12.0 ± 1.0	11.3 ± 2.3
AE_{Sa}	-	-	-	-	-
EE_{Sa}	-	9.3 ± 1.5	-	-	-
HEE_{Sa}	10.0 ± 1.0	9.3 ± 0.6	13.0 ± 2.0	10.0 ± 0.0	8.7 ± 1.5

AEAc = Aqueous extract from *A. conyzoïdes*; EEAc = Ethanolic extract from *A. conyzoïdes*; HEEAc = Hydro ethanolic extract *from A.conyzoïdes* ; AELc = Aqueous extract *from L. camara*; EELc = Ethanolic extract from *L. camara*; HEELc = Hydro ethanolic extract from *L. camara* ; AEPg = Aqueous extract from *P. guajava;* EEPg = Ethanolic extract from P. *guajava* ; HEEPg = Hydro ethanolic extract from *P. guajava* ; AESa = Aqueous extract from *S. acuta*; EESa = Ethanolic extract from *S. acuta* ; HEESa = Hydro ethanolic extract from *S. acuta*.

Table 7: Growth Inhibition parameters (MIC, MBC and MBC/MIC) of the EO and Gentamicin on the tested bacteria (mg/mL)

	B. cereus			*E. coli*			*S. enteritidis*			*Shigellaspp*			*S. aureus*		
	MIC	MBC	MBC/ MIC	MIC	MBC	MB C/M IC	MIC	MBC	MB C/M IC	MIC	MBC	MB C/M IC	MIC	MBC	MBC /MIC
A. conyzoïdes	25	25	1	50	100	2	100	100	1	50	100	2	100	100	1
L. camara	25	100	4	25	25	1	25	25	1	12.5	25	2	25	25	1
P. guajava	25	25	1	25	25	1	25	25	1	6.25	25	4	12.5	12.5	1
Gentamicin	0.01	0.03	1	0.03	0.03	1	0.15	0.39	1	0.07	0.31	1	0.15	0.39	4

MIC =Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration

Table 8: Growth Inhibition parameters (MIC, MBC and MBC/MIC) of the non volatile extracts on the tested bacteria (mg/mL).

		B. cereus			*E. coli*			*S. enteritidis*			*Shigella spp.*			*S. aureus*		
Plants	Extracts	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC	MIC	MBC	MBC /MIC	MIC	MBC	MBC/ MIC	MIC	MBC	MBC/ MIC
A. conizoïdes	AE	100	100	1	50	100	2	25	25	1	50	100	2	25	25	1
	EE	6.25	6.25	1	12.5	12.5	1	12.5	12.5	1	12.5	25	2	25	25	1
	HEE	6.25	6.25	1	6.25	6.25	1	6.25	6.25	1	12.5	25	2	6.25	6.25	1
L. camara	AE	25	100	4	6.25	12.5	2	6.25	6.25	1	6.25	6.25	1	1.56	3.12	2
	EE	3.12	3.12	1	3.12	3.12	1	1.56	1.56	1	1.56	3.125	2	1.56	1.56	1
	HEE	0.78	0.78	1	0.78	0.78	1	0.78	1.56	2	0.78	3.125	4	0.39	1.56	4
P. guajava	AE	0.19	3.12	16	3.12	6.25	2	6.25	6.25	1	1.56	1.5625	1	0.78	3.12	4
	EE	0.78	12.5	16	3.12	6.25	2	6.25	6.25	1	3.12	12.5	4	0.78	12.5	16
	HEE	3.12	12.5	4	3.12	6.25	2	6.25	6.25	1	3.12	3.125	1	3.12	12.5	4
S. acuta	AE	6.25	12.5	2	50	50	1	12.5	12.5	1	6.25	6.25	1	12.5	100	8
	EE	0.78	1.56	2	3.12	3.12	1	3.12	6.25	2	1.56	6.25	4	1.56	6.25	4
	HEE	1.56	1.56	1	3.12	6.25	2	0.78	3.125	4	3.12	3.12	1	3.12	3.12	1

AE = Aqueous extract; EE= Ethanolic extract; HEE= Hydro ethanolic extract;

MIC =Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration