

Ethnobotanical Survey and Antibacterial Activity of African Plants used Against Diarrhea

Etoa François-Xavier

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Abstract

Context: In Africa, medicinal plants are usually used to treat diarrhea but few data are available concerning their identification and their antidiarrheal activities. **Aims:** This study aims to record some antidiarrhoea plants in the Medoneu locality (Gabon), describe their modalities of use and assess the chemical composition and antibacterial activities of four most commonly used plants. **Methods and Material:** Plants and their modalities of use were recorded through ethnobotanical survey. Four commonly used plants were harvested; their extracts and the essential oils (EO) were prepared from the leaves by maceration and hydro-distillation. The chemical compositions of EO were determined by Gas Chromatography while, the antibacterial activities of extract and EO were assessed using both agar diffusion and micro-dilution methods.

Index terms— diarrheal infections; african medicine, plants extracts; essential oils, antibacterial activity.

1 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 conyzoides, *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, ??0]. 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 conyzoides*, *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.

2 II.

3 Subjects and Methods

4 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 conyzoides*, *Lantana camara*, *Psidium guajava* and *Sida acuta* 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.

5 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 enteroinvasive diarrhea. These bacterial strains were kept at the Laboratory of Microbiology of the University of Yaounde I where antibacterial tests were carried out.

6 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 $\text{Yield (\%)} = \frac{\text{Mass of essential oil}}{\text{Mass of raw plant material}} \times 100$.

7 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 ethanolwater 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: $\text{Yield (\%)} = \frac{\text{Mass of extract}}{\text{Mass of raw plant material}} \times 100$ [13].

8 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 N₂ 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 quadrupole 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 H₂SO₄ 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 FeCl₃ 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 of triterpenes 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 [?].

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 μ L 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 μ L 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 μ L of Muller Hinton broth (supplemented with Tween 40 at 5% for the EO). Subsequently, 100 μ L 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 μ L 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 μ L 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 μ L of inoculum (with concentrations greater than or equal to the MIC) in 150 μ L 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].

12 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.

13 III.

14 Results

15 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 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*.

16 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. conyzoides* leaves showed the highest EO yield of $0.21 \pm 0.02\%$, while *S. acuta* did not have any essential oils. The highest yields were obtained from hydro ethanolic extracts of *A. conyzoides* (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.

17 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. conyzoides* 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%), β -humulene (3.9%) and 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 contains 7 main components namely (E)-nerolidol (26.4%), (E)- β -caryophyllene (12.7%), sabinene (8.4%), 1,8-cineole (6.9%), β -zingiberene (5%), β -humulene (4.6%) and β -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. conyzoides* 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.

18 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 hydroethanolic 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*.

19 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 nonvolatile 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. conyzoides* 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. conyzoides*, 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 ethanolic 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*).

20 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. conyzoides* 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

21 C

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*; hydroethanolic extract of *S. acuta* on *B. cereus* and ethanolic extract 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*, *Shigella* 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.

22 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 diversified 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 conyzoides*, *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. conyzoides*) is sesquiterpene with broad-spectrum antibacterial effects due to its hydrophobicity and ability. 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.

23 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 conyzoides*, *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

identified main plant families namely Anacardiaceae, Annonaceae, Apocynaceae, Aspleniaceae, Asteraceae, Bombacaceae, Chenopodiaceae, 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 reticulata*, *Alchornea cordifolia*, *Bridelia atroviridis*, *Hymenocardia acida*, *Jatropha curcas* and *Uapaca densifolia*). The second most encountered family was the Asteraceae with 6 species (*Acanthospermum hispidum*, *Ageratum conyzoides*, *Apodocephala pauciflora*, *Biden spilosa*, *amygdalina*)

Euphorbia hiriMacaranga spp.,

Vernoniahalophora and Vernonia

Figure 1:

Figure 2:

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Plants	Extraction yields (%)			
	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

Figure 4: Table 2 :

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Figure 5: Table 3 :

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Phytochemical compounds	A. conyzoides			L. ca- mara			P. gua- java			S. acuta	
	AE	EE	HEE	AE	EE	HEE	AE	EE	HEE	AE	HEE
Alcaloids	+	+	+	+	-	+	+	-	-	+	
Athocyanins	+	+	+	+	-	+	+	+	+	+	
Flavonoïds	+	+	+	+	+	+	+	+	+	+	
Phenols	-	+	-	+	+	+	-	+	-	-	
Polyphenols	+	-	+	-	-	-	+	-	+	+	
Saponins	-	+	+	-	+	+	+	+	+	-	
Stéroïds	+	+	-	-	+	-	-	+	-	+	
Tannins	+	+	+	+	+	+	+	+	+	+	
Triterpenes	-	-	+	+	-	+	-	-	+	-	

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

Figure 6: Table 4 :

5

Bacterial species
Inhibition zone diameters (mm)

Figure 7: Table 5 :

6

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

Extract	AE	B.	Bacterial species	Inhibition zone diameters (mm)	E. coli	S. enteritidis	Shigella sp
Ac	EE	Ac	cereus				
HEE	Ac	AE	12.0				
Lc	EE	Lc	± 1.0				
HEE	Lc	AE	$13.0 \pm$				
Pg			$2.0 \pm$				
			$11.0 \pm$				
			$1.0 -$				
EE	Pg		11.3 ± 1.1	11.7 ± 0.6	$11.7 \pm$	$10.3 \pm$	
					$0.6 \pm$	$0.58 \pm$	
HEE	Pg	-	12.0 ± 1.0		$16.0 \pm$	$12.0 \pm$	
					$3.6 \pm$	$1.0 \pm$	
AE	Sa	-	-		-	-	
EE	Sa						

Figure 8: Table 6 :

7

	B. cereus			E. coli			S. enteritidis			Shigellaspp		
	MIC	MBC	MBC/ MIC	MIC	MBC	MB C/M IC	MIC	MBC	MB C/M IC	MIC	MBC	M C IC
A. conyzoides	25	25	1	50	100	2	100	100	1	50	100	2
L. camara	25	100	4	25	25	1	25	25	1	12.5	25	2
P. guajava	25	25	1	25	25	1	25	25	1	6.25	25	4
Gentamicin 0.01 0.03			1	0.03	0.03	1	0.15	0.39	1	0.07	0.31	1
MIC =Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration												
Year 2020 (mg/mL).												

2

[Note: AE = Aqueous extract; EE= Ethanollic extract; HEE= Hydro ethanollic extract; MIC =Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration]

Figure 9: Table 7 :

8

Figure 10: Table 8 :

329 results explain the use of those plants to treat diarrheal infections and show a potential source of antimicrobial
 330 compounds. ¹

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- [Kotloff et al. ()] ‘Burden and etiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, casecontrol study’. K L Kotloff , J P Nataro , W C Blackwelder , D Nasrin , T H Farag , S Panchalingam . *Lancet* 2013. 382 p. .
- [Boukri ()] *Contribution à l’étude phytochimique des extraits bruts des épices contenus dans le mélange Ras-el-hanout*, Neh Boukri . 2014. p. 99. Mémoire de fin de cycle en Biologie, Université KASDI MERBAH Ouargla
- [Mindiédiba] ‘Etude phytochimique et activités biologiques des tiges feuillées de *Lantana camara* L. et de *Lippia chevalieri* Moldenke: deux Verbenaceae du Burkina Faso” Thèse d’Etudes approfondies pour obtenir le grade de Docteur de l’Université de Ouagadougou Option: Sciences appliquée (plantes médicinales et phytothérapie). J B Mindiédiba . *Spécialité: Biochimie et chimie des substances naturelles*, (Université d’Ouagadougou. 2012 : pp 113)
- [Amégninou et al.] *Evaluation des activités*, A Amégninou , A G Koffi , K A Eyana , T Kokou , B Komlan , K Kossi .
- [Nguendo ()] ‘Landforms and diarrheal diseases in Yaounde (Cameroon): A medical geomorphology approach in a tropical urban setting’. Yhb Nguendo . *Canadian Journal of Tropical Geography* 2014. 1 p. .
- [Atokare ()] *Mémoire de fin d’études d’obtention du Diplôme d’Etudes Supérieures Spécialisées en Démographie, Institut de formation et de recherche démographique*, A Atokare . 2008. p. . (?Facteurs explicatifs de la morbidité diarrhéique chez les enfants de moins de cinq ans au Tchad)
- [Biyiti et al. ()] ‘Recherche de l’activité antibactérienne de quatre plantes médicinales Camerounaises’. L F Biyiti , ’ Meko , Djl , V Tamze , Amvam Zollo , PH . *Pharmacopée et Médecine Traditionnelle Africaine* 2004. 13 p. .
- [WH O (2009)] *World health statistics. Cause-specific mortality and morbidity*, WH O . http://www.who.int/gho/publications/world_health_statistics/FR_WHS09_Full.pdf 2009. 14 August 2018.
- [Zerbo et al. ()] P Zerbo , J Millogo-Rasolo , O G Nacoulma-Ouedraogo , P Van Damme . *Contribution à la connaissance des plantes médicinales utilisées dans les soins infantiles en pays*, (San, au Burkina Faso) 2007. 1 p. .