Original Article

In-vitro Testing of Extracts and Fractions From two Cameroonian Medicinal Plants on Bacteria Gastroenteritis

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ABSTRACT

In vitro antibacterial activities of extracts and fractions from two Cameroonian pharmacopeia plants, *Carapa procera* (Meliaceae) and *Moringa oleifera* (Morigaceae) were evaluate on five gastroenteritis-causing bacteria. The crude extracts of leaves and barks of *Carapa procera* (Meliaceae) as well as the seeds and leaves of *Moringa oleifera* (Morigaceae) were obtained in methylene chloride/methanol (1/1) by maceration. Phytochemical screening was done on the crude extracts by colorimetric tests. The crude extracts and their fractions were tested against five (5) bacterial species (*Salmonella typhi, Salmonella paratyphi, E. coli, Campylobacter jejuni* and *Bacillus cereus*) using the diffusion method on wells. The inhibition parameters were determined by the macro-dilution method in liquid medium and by the dilution method incorporated in agar for the determination of the Minimal Inhibition Concentration (MIC). The Minimal Bactericidal Concentration (MBC) was determined after subculture. The phytochemical screening revealed that, all plant extracts contain not only phenolic compounds (phenols, tannins and flavonoids), but also alkaloids, triterpene, sterols and lipids. The crude extracts and methanolic fractions of the leaves and barks of *C. procera* and the extract of seeds of *M. oleifera* were active against four (4) bacterial species: *S. typhi, S. paratyphi, E coli* and *B. cereus*. Concisely, the diameter of inhibition of these active fractions ranges between 10 and 26 mm for extracts concentration of 80 mg/ml. The active extracts and fractions gave MIC range from 2.5 to 10 mg/ml. The results obtained indicate that the seeds of *Moringa oleifera* (Morigaceae) and the barks of *Carapa procera* (Meliaceae) extracts as well as its methanolic fraction showed a bactericidal activity on four (4) sensitive species. Thus, the antibacterial properties of the extracts and fraction of those plants confirm their use in traditional

medicine.

Keywords: Cameroon, Gastroenteritis, Antibacterial, *Moringa oleifera, Carapa procera*.

INTRODUCTION

Infectious diseases are the consequences of the invasion of an animal or human organism by pathogenic bacteria, viruses, fungi and unicellular parasites[1] among which gastroenteritis. Gastroenteritis can result from a microbial infection, often accompanied by damage to the stomach wall (gastric ulcer). Infectious bacterial gastroenteritis is more severe than those of viral origin[2]. About one person in ten suffers from infectious gastroenteritis disease during his life time[3]. These diseases are still today a major public health problem: 400 million cases per day worldwide, 2 million people die of gastroenteritis in the world with 70 % of cases in developing countries against 30 % in industrialized countries[4]. At least 20 % of deaths in children between 0 to 4 years are associated with gastroenteritis in developing countries[5]. In Cameroon, it is the second leading cause of death in children under 5 years after acute respiratory infections[6]. At least 31.62 % of Cameroon's population suffers from gastric ulcers[7]. This evil is a frequent reason for consultation, especially in industrializing countries where fecal and food hygiene is precarious[8]. The most effective mode of treatment is antibiotics. However, the inappropriate use of antimicrobials, inadequate management of infection and the emergence of resistant pathogenic bacteria to humans, making it more and more ineffective antibiotic therapy[9,10]. In addition, there are also the "side effects" and the high costs of antimicrobial treatments.

Faced with these problems, the search for alternative therapies for the treatment of infectious gastroenteritis is an urgent need. Therefore, in developing countries like Cameroon, 80% of the populations use herbal medicine[8] because the plant-based medicines are available and low cost for people in rural area. *Carapa procera* (Meliaceae) and *Moringa oleifera* (Moringaceae) which are used in traditional medicine to treat patients in case of ulcers, inflammation and diarrhea[11,12]. Therefore, they constitute a promising source of biomolecules antibacterial. To verify this property we assessed *in-vitro* the action of extracts and fractions from these plants on the gastroenteritis-causing bacteria.

MATERIAL AND METHODS

The plant material consisted of: *Carapa procera* (*Meliaceae*) leaves and bark were harvested in the Kribi, South Region-Cameroon (Mont Elephants) in the afternoon, on August 2012. The plant was identified at the National Herbarium of Cameroon under the specimen number 31947/SRFCam. The choice was made according to the ethnopharmacological and pharmacological studies. *Moringa oleifera* (Moringaceae): The seeds and leaves were harvested in Yaounde (Biyem-Assi), Cameroon, Center region in the afternoon in the month of December 2012. It identification was also done at the National Herbarium Cameroon under the specimen number 49178/CST. The choice was made according to the ethnobotanical, pharmacological and ethnopharmacological studies.

The bacterial material was made up of five bacterial species including four gram

- : *S. typhi, S. paratyphi, E. coli, C. jejuni* and one Gram +: *B. cereus*. They were provided by the Microbiology Laboratory of the University of Yaounde I, Cameroon. These species were from clinical isolates of patients collected in Medical Bacteriology Laboratory of Centre Pasteur of Cameroon. They were conserved at 4°C on nutrient agar slopes. Each bacterial species was activated on agar medium before carrying out antibacterial tests.

Sterile sheep blood was used to supplement the culture medium of *C. jejuni*. Non-biological material consisted module sheep blood to supplement the culture medium of *C. jejuni*. Mueller Hinton agar medium was used for the activation of bacterial species, achieving diffusion tests for the MIC determination by the solid medium dilution method (by incorporation into agar) and the determination of the MBC by subculture. Brain Heart Infusion Broth (Fortress) (ref: BXM0045 B) enriched with 5% agar supplemented with sheep blood and selective antibiotic were used for the growth of *C. jejuni*. Nutrient broth containing 0.05% phenol red and supplemented with 10% glucose (BNGP) was used for the determination of the inhibition parameters MIC by the macrodilution technique. Métroimidazole and gentamicin were used as reference antibiotics.

Preparation of Crude Extracts and Fractionation of Plants

Procedure

Bark and leaves of *C. procera* as well as seeds and leaves of *Moringa oleifera* were cut into pieces, dried and ground. The powders obtained bark (3kg) and leaves (599.5g) of *C. procera* and seeds (200g) and leaves (400g) of *M. oleifera* were macerated in a mixture of methanol/chloride methylene (1:1) for 48 hours. The crude extract was obtained after filtration and evaporation of the macerate in a rotary evaporator (461 BÜCHI Water Bath). The different fractions were obtained after exhaustion of 30g of the crude extracts of leaves and bark of *C. procera* in solvents of increasing polarity, namely: hexane, methylene chloride, ethyl acetate and the methanol. No fraction containing the leaves of *M. oleifera* was done because preliminary activity tests of the crude extract were not positive on bacterial species used.

The extraction yield was calculated using the formula:

R % = mass of extract obtained/mass of powder x 100

Phytochemical Screening of Each Extract

The phytochemical analysis was carried out to determine the class of phytochemicals in the plants powders. For this purpose, several types of tests described by Harborne[13] and Odebiyi Sofowora[14] were used:

In Vitro Evaluation of Antibacterial Activity

After several preliminary tests, the well diffusion method was carried out for sensitivity test[15]. Two techniques were used to measure inhibition parameters of the different extracts and active fractions:

The of liquid medium macrodilution method in tubes containing nutrient broth, 5 % phenol red and supplemented with 10 % glucose (BNGP) for the determination of the inhibition parameters of the seed extract of *M. oleifera*[16].

The solid medium dilution method (by incorporation into agar) for the determination of the inhibition parameters of the extracts and fractions from *C. procera*[16].

Diffusion Technique by Wells

Procedure

A young colony within 24 hours old

of each test bacterium on the sensitivity was suspended in 1 ml of sterile distilled water. To promote the achievement of a layer of semi-confluent colonies, the inoculum obtained above was diluted to 1/50th[17]. Wells were made using ferrules. Then 50 µl of different extracts, fractions, DMSO and the reference antibiotic solution (gentamicin or métroimidazole) prepared to a concentration of 100 mg/ml DMSO 10 % and 1 mg/ml were deposited respectively. Two controls were carried out: a negative control with 10 % DMSO and a positive control with the antibiotic (gentamicin or métroimidazole).

After a pre-diffusion of the test substances for 15 minutes at room temperature, the plates were incubated at 37°C for 24 hours. The diameters of the inhibition zones around the disks were measured using a caliper. Each test was performed three times and the values were expressed as mean ± standard deviation. The choice of extracts and fractions of different plants used for further work was directed at the conclusion of this preliminary test, which was used to select the active extracts and fractions against the target bacterial species.

Determination of Minimum Inhibitory Concentration (MIC)

Two techniques were used for this purpose for the MIC determination namely macrodilution technique in liquid medium and the solid medium dilution technique by incorporation into agar.

Liquid Medium Macrodilution Technique

Procedure

Two milliliters of extract of *M. oleifera* seeds at a concentration of 20 mg/ml were added to a sterile tube containing 2 ml of nutrient broth. Serial dilution in the cascade of 2 was carried out and the last volume eliminated. 1.87 ml of broth and 13 µl of bacterial inoculum density equivalent to 0.5 standard Mac Ferland (10^{-6} UFC ml^{-1}) were added to have a final volume of 4 ml in each tube. This gave a final concentration ranging between 10 and 0.078 mg/ml. Positive control tubes having only the 13 µl of the standardized inoculum and the nutrient broth and the negative control tube having only the extract and nutrient broth (containing the entire phenol red) without the microorganisms were performed. After incubating the tubes at 37°C for 24 hours, the MIC of the tested extract was deduced from the tube where no visible yellow color change was observed. Each experiment was repeated three times over three successive experiments.

Incorporation Technique in AGAR

Procedure

Two milliliters of an extract or fraction to be tested of concentration 80 mg/ml were added to a sterile tube containing 2ml medium Mueller Hilton agar. A double dilution series was made and the last volume discarded. Then, 1.95ml of medium was added to have a final volume of 3.95ml in each tube and to obtain a concentration range between 20 and 0.156mg/ml. The contents of each dilution were poured into Petri dishes. After solidification, 5µl of bacterial inoculums equivalent density standard 10^{-4} $UFCml^{-1}$ Mac Ferland were inoculated by spreading. The MIC was defined as the concentration of the extract or fraction leaving no or at most 1-3 colonies after 18 hours of incubation at 37°C. Each experiment was repeated three times[18,19,16].

Determination of Minimum Bactericidal Concentration (MBC)

MBC was determined by subculture. For this, 50µl of the contents of tubes at concentrations higher or equal to MIC were

inoculated onto agar in Petri dishes and incubated for 24 hours at 37°C. Minimum Bactericidal Concentration (MBC) was taken as the lowest concentration of extract that killed more than 99.9 % of the initial bacterial inoculum (less than 0.01 % of survivors). Each experiment was performed three times. Calculating the ratio MBC/MIC made it possible to determine the bactericidal, bacteriostatic and "tolerance" activity of the microbial strain. The results were discussed using those of Berche and Weil[5], and those of Fauchere and April[20] who showed that when the MBC of antibiotics on a given strain is close to the MIC (MBC/MIC = 1 or 2), the antibiotic is bactericidal; in contrast, if these values are relatively higher (4 < MBC/MIC > 16), the antibiotic is bacteriostatic. Finally, if MBC/MIC > 32, we talk about "tolerance" of the microbial strain.

Statistics Analysis

Statgraphic plus 5.0 solfware for windows was used for statistical analysis. The inhibition diameters of the growth of bacteria species by extracts during the Fisher test, P values < 0.05 were considered as significant. The results wer expressed as means ± standard deviations.

RESULTS

Physical Characteristics and Yields of Plant Extracts

Crude extracts of leaves and bark of *C. procera* as well as leaves and seeds of *M. oleifera* were obtained after maceration and evaporation. The physical characteristics and the extraction yields are given in Table 1.

Phytochemical Screening

The results of the phytochemical screening showed that the leaves of *Moringa oleifera* are rich in sterols, triterpenes (terpenoids), saponins, phenols, fats, flavonoids, tannins and alkaloids. Among all different parts of plants used for screening test, only the seeds of *M. oleifera* contain sugars and glucosides. These results are similar to that obtained on the leaves and seeds of *Moringa oleifera*[21-23]. Sterols and triterpenes (terpenoids), flavonoids, phenols, saponins, lipids, alkaloids and tannins are present in the leaves and bark of *C. procera*. Table 2 below shows the results of the phytochemical screening of different plant extracts.

Antibacterial Activities of Different Extracts and Fraction

Inhibition Diameters

The diameter of inhibition is a parameter to characterize the sensitivity of bacterial species to the antibacterial substances. The diameters of the zones of inhibition of bacterial species extracts and fractions are shown in Table 3 and illustrated by the histogram in Figure 1.

The observation of Table 3 shows that the diameters of inhibition of the extracts and fractions in general, vary from 10 mm to 26 mm. Indeed, the growth of four bacterial species (*S. typhi, S. paratyphi, B. cereus* and *E. coli*) on the 5 tested is inhibited by 3 out of 4 tested extracts (the crude extract of bark (E1) and leaves (F1) of *C. procera* and the crude extract of seeds (GM) and leaf (FM) of *M. oleifera*).

Regarding the activity of the extracts, the diameters of inhibition of the growth of different bacterial species vary from 11.33 to 22 mm for the crude extract of bark of *C. procera* (E1); from 12.66 to 18.33 mm for the crude extract of leaves of *C. procera* (F1) and from 12 to 26 mm for crude extract of seed *M. oleifera* (G_M). Against by crude extract of leaf (F_M) *M. oleifera* showed no activity vis-à-vis all targeted bacterial species.

The highest antibacterial activity is obtained with the extract of *M. oleifera* seeds.

This activity was observed against *E. coli* and *S. Paratyphi*. These are bacterial species highly susceptible to all samples with diameters of 22 mm and 26 mm respectively. Furthermore, *S. typhi* and *B. cereus* are bacterial species susceptible vis-à-vis the active extracts with diameters ranging from 10 mm to 12 mm. *C. jejuni* is the bacterial species not susceptible to extracts of both plants.

As for the fractions, the growth of four bacterial species (*S. typhi, S. paratyphi, B. cereus* and *E. coli*) on the 5 tested was inhibited by 2 out of 8 tested fractions (methanolic fractions of the leaves (F5) and bark (E5) of *C. procera*). The inhibition diameters varied from 11 mm to 22 mm for the methanolic fraction of bark of *C. procera* (E5) and 10.66 to 19.66 mm for the methanolic fraction of leaves of *C. procera* (F5). Contrarily, the fractions with hexane (E2 and F2), chloride of methylene (E3 and F3) and ethyl acetate (E4 and F4) of leaves and bark of *C. procera* showed no relation to the activity against all the bacterial species subject to the test.

In summary, the highest antibacterial activity was obtained with the methanolic fraction of bark of *C. procera* (E5). This activity was observed against *E. coli* and *S. Paratyphi*. These are still highly sensitive bacterial species to the methanolic fraction of bark of *C. procera* (E5) (diameters > 20 mm) and very sensitive to the methanolic fraction of leaves of *C. procera* (F5) (diameters between 15 and 19). Moreover, *S. typhi* and *B. cereus* bacterial species are sensitive to both methanolic fractions of *C. procera* (diameters from 10-12 mm). *C. jejuni* was not sensitive to crude extracts and fractions.

In general, such activities of extracts and fractions of both plant on target bacterial species is lower than that of gentamicin and métroimidazole considered as reference molecules in this work (see Table 3 and Figure 1).

Bacterial Inhibitions Parameters: CMI, cmb and CMB/CMI

The MIC and MBC are two parameters that help to calculate the ratio MBC/MIC. After determining the MIC, MBC and calculation of ratios, the results of these various parameters are shown in Table 4.

The analysis of this table shows that the MIC ranges from 2.5 mg/ml to 10 mg/ml in the whole. It is 5 mg/ml on the crude extract of bark of *C. procera* on all susceptible bacterial species. Calculating the ratio MBC/MIC shows bactericidal activity of this extract on all susceptible bacterial species (MBC/MIC of between 1 and 2).

The crude extracts of leaves of *C. procera*, have the MICs ranging from 5 mg/ml (*S. typhi* and *B. cereus*) to 10 mg/ml (*E. coli* and *S. paratyphi*). The calculation of ratios CMB/MIC shows a bactericidal activity only on *E. coli* and *S. Paratyphi* (MBC/MIC = 2) and a bacteriostatic activity on *S. typhi* and *B. cereus* (MBC/MIC = 4).

Finally, the extract of *M. oleifera* seeds had MICs ranging from 2.5 (*E. coli* and *S. paratyphi*) to 10 mg/ml (*S. typhi* and *B. cereus*). The lowest MIC is 2.5 mg / ml obtained on *E. coli* and *S. Paratyphi*. This confirms the results of preliminary tests where these bacterial species showed higher inhibition zones. Calculating the ratio MBC/MIC showed that the extract of *M. oleifera* seeds was bactericidal against all susceptible bacterial species (MBC/MIC of between 1 and 2).

As to the active fractions, the MIC ranged from 5 to 10 mg/ml in the whole. Regarding the methanolic fraction of bark of *C. procera*, MIC range from 5 (*S. paratyphi*) to 10 mg/ml (*E. coli, S. typhi* and *B. cereus*). Calculation MBC/MIC showed that the methanolic fraction of bark of *C. procera* was bactericidal against all susceptible bacterial species (MBC/MIC between 1 and 2). The methanolic fraction of leaves of *C. procera* had the MIC of 10 mg/ml for all susceptible

bacterial species. Bactericidal activity was obtained from *E. coli* and *S. Paratyphi* (MBC/MIC = 2). On the other hand, the MBC was not on *S. typhi* and *B. cereus*.

In summary, of all these extracts, the highest antibacterial activity was obtained with extracts of *M. oleifera* seeds and bark of *C. procera*. They were bactericidal on all susceptible bacterial species. Among the active fractions, the methanolic fraction of bark of *C. procera* was the most active with a bactericidal activity on all susceptible bacterial species. The bacterial species most sensitive to extracts and fractions were *S. paratyphi* and *E. coli* (MIC = 2.5 mg/ml) and the bacterial species less sensitive to extracts and fractions were *S. typhi* and *B. cereus* (MIC = 10 mg/ml).

Contrarily, although some extracts and fractions have shown nearby activities of the gentamicin (reference antibiotic), the latter showed an absolute bactericidal activity (MBC/MIC = 1) on all susceptible bacterial species (*B. cereus, S. typhi, S. paratyphi* and *E. coli*) (see Table 4).

DISCUSSION

The greater extraction yield value was obtained from the leaves of *Moringa oleifera* (37.7 %), this could be due to the extraction solvent system methanol/methylene chloride (1/1) which is capable of fragmenting and extracting the majority of compounds in the plant material. This result is higher than that of Millogo et al[23] which they obtained 5 % by using water as solvent for the extraction of components of the leaves of the same plant. This could be due to the extraction solvent that may influence the extraction yield.

In general, bacterial species (*B. cereus, S. typhi, S. paratyphi* and *E. coli*) were sensitive to crude extracts of both plants as well as methanolic fractions from leaves and barks of *C. procera*. Antibacterial activities obtained with different extracts and active fractions of the two plants could be explained by the presence of various classes of secondary metabolites revealed by the phytochemical screening such as terpenoids, flavonoids, tannins and alcaloïdes which are potentially endowed with antibacterial properties[24,25]. These antibacterial properties are consistent with those already revealed by Florian[26], who had obtained a high sensitivity of *E. coli* with respect to the extract of *M. oleifera* seeds. According to the classification of Aligiannis *et al*.[27], almost all extracts and active fractions inhibited the growth of Gram + and Gram - bacteria except *C. jejuni*. These extracts and fractions have a broad spectrum antibacterial action.

Overall, the crude extract of bark of *C. procera* (E1) has been active on *S. typhi, S. paratyphi B. cereus* and *E. coli*. This can be justified by the fact that it is rich in triterpenes: touloucounin and carapin. The work of Eugene[28] showed that these compounds have antibacterial activities.

In addition, the extract of *M. oleifera* seeds was more active than the extract of leaves and bark of *C. procera* on sensitive bacterial species. We can explain this activity that the extract of *M. oleifera* seeds is rich in natural compounds following: pterygospermin, benzyl isothiocyanate, 4- (α-L-rhamnopyra-nosyloxy) benzylglucosinolate having a strong antibacterial activity[29-31,12]. The work of Duke[32] also showed that the benzyl isothiocyanate has a strong antibacterial activity. These results also corroborate with those obtained by Florian[27] which isolated the polypeptide "Flo" from the seeds of *M. oleifera* having a strong antibacterial activity against *E. coli*.

In terms of fractions, only the methanolic fractions of leaves and bark of *C. procera* showed antibacterial activities. Sometimes activities similar to those of the crude extracts, which shows that the majority of natural antibacterial compounds of this plant are soluble in methanol.

In general, bacterial species are sensitive to the crude extract of *M. oleifera* seeds as well as crude extracts of bark and leaves of *C. procera* Except for *S. paratyphi* and *E. coli*, which are extremely sensitive to crude extracts of *M. oleifera* seeds and barks of *C. procera* as well as the methanolic fraction of bark of *C. procera* ($p < 0, 05$). These bacterial species distinct sensitivities to the two extracts in well during the test on solid medium and in the liquid medium would be due, firstly, to specific factors intrinsic to each microorganism and secondly to the phytochemical profile of the extract. Indeed, according to Takeo *et al*.[33], the mechanisms of action of the active ingredients may vary from one species to another and also from one strain to another. This observation also justifies the insensitivity of *C. jejuni* to the extracts and fractions of the two plants. Furthermore, this non-sensitivity could also be justified by the fact that the antibacterial molecules in the two extracts were not sufficiently concentrated to inhibit the growth of this bacterium. The possibility of phenotypic resistance would also be considered[34].

No targeted bacterial species were sensitive to methanol/methylene chloride leaves extract *of M. oleifera*. This is contrary to the work of Millogo *et al*.[24] who showed rather that the aqueous extract of *M. oleifera* leaves had antibacterial activity against *S. typhi, E. coli* and *B. cereus*. On this point, we can assume that the extraction solvent could have an influence on the extraction of antimicrobial compounds in the leaves of *M. oleifera* or antimicrobial compounds are complexed with other natural compounds in the last extract or microbial strains have developed resistance mechanisms to natural antimicrobials[34].

We obtained the MIC of various extracts and fractions active on susceptible bacterial species ranging from 2.5 to 10 mg/ml. In addition, bactericidal activity was obtained on the majority of susceptible bacterial species. This activity is comparable to that of gentamicin. From these facts, the plants have a set of molecules that justify the renewed interest in the exploitation of this natural resource, with the aim to develop new antibacterial substances in order to overcome the problem of the narrow spectrum of activity posed by references molecules used for the treatment of infectious gastroenteritis.

CONCLUSION AND PERSPECTIVES

At the end of this work, whose general objective was to make an *in vitro* assay on the extracts and fractions of *C. procera* leaves and bark and the leaves and seeds of *Moringa oleifera* on the gastroenteritis-causing bacteria, it appears that the highest value of extraction yield was obtained from the leaves of *Moringa oleifera* (37.7 %); phytochemical characterization of two extracts revealed the presence of several families of chemical compounds with important biological activities including terpenoids, tannins, alkaloids, phenols, flavonoids. Extracts of bark and leaves of *C. procera* and the extract from the seeds of *Moringa oleifera* inhibited the growth of four (4) bacterial species (*S. typhi, S. paratyphi, E. coli* and *B. cereus*) except *C. jejuni*. The extract of *M. oleifera* leaves presented no antibacterial activity against the target bacterial species. In general, seed extracts of *M. oleifera* and bark of *C. procera* showed strong activity against all sensitive bacterial species. Only the methanolic fractions of leaves and bark of *C. procera* are active on susceptible bacterial species. However, these activities are still low compared to those of the reference antibiotics (gentamicin and metroimidazol). In order to reinforce this approach to the production of Enhanced Traditional Medicines (MTA) and the discovery of new substances with antimicrobial activity, the accomplishment of

this work is a challenge. We will consider for future studies:

- Work on many microbial strains that are pathogenic to man on several preparations from this plant;
- Isolate pure molecules in each fraction and evaluate their antimicrobial activities;
- Evaluate *in vivo* toxicity of the crude extracts, their fractions and molecules that are isolated.

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Table 1. Yields and physical characteristics of crude extracts of various plants used

Extracts	Solvent	Extract Quantity	Color	Aspect	Yield (%)
Leaves of *C. procera* (599,5 g)	MeOH /$C_2H_2Cl_2$ (1/1)	76,9 g	Green	Pasty	12,8
Bark of *C. procera* (3 Kg)	MeOH /$C_2H_2Cl_2$ (1/1)	270,7 g	Reddish	Pasty	9,7
Seed of *M. moringa* (200 g)	MeOH/$C_2H_2Cl_2$ (1/1)	75,4 g	Three colors: purplish, white, yellow	Oily	20,2
Leaves of *M. moringa* (400 g)	MeOH /$C_2H_2Cl_2$ (1/1)	80,8 g	Green	Pasty	37,7

Table 2. Phytochemical screenings of +different extracts

Chemical compound	Extracts			
	Leaves of *C. procera*	Bark of *C. procera*	Seed of *M. oleifera*	Leaves of *M. oleifera*
Phenols	++	++	++	++
Tanins	+	++	+++	++
Saponines	+	+	++	+
Flavonoids	+	+	++	++
Alcaloids	++	++	++	+
Terpenes et sterols	+	++	+++	+
Lipids	+	+	+++	+
Sugars	-	-	+++	-
Glucosides	-	-	+++	-

+ = low ++ = Medium +++ = strong - = missing

Table 3. Diameters means zones of inhibition of the growth of bacterial species with gentamicin and the extracts and fractions of the plants used

Species Extraits	*B. cereus*	*S. paratyphi*	*S. typhi*	*E. coli*	*C. jejuni*
E_1	$11,33^a \pm 0,57$	$21,33^a \pm 0,57$	$11,33^a \pm 0,57$	$22^b \pm 0,00$	0
E_2	0	0	0	0	0
E_3	0	0	0	0	0
E_4	0	0	0	0	0
E_5	$11^a \pm 1,00$	$20,66^a \pm 0,57$	$11,33^a \pm 0,57$	$22^b \pm 1,00$	0
F_1	$12,66^b \pm 0,57$	$16,66^b \pm 0,57$	$13^b \pm 1,00$	$18,33^a \pm 0,57$	0
F_2	0	0	0	0	0
F_3	0	0	0	0	0
F_4	0	0	0	0	0
F_5	$10,66^a \pm 0,57$	$17^b \pm 0,57$	$11,66^a \pm 0,57$	$19,66^a \pm 0,57$	0
G_M	$12^a \pm 1,00$	$26^c \pm 0,00$	$12,66^b \pm 0,57$	$22^b \pm 0,57$	0
F_M	0	0	0	0	0
Genta	$27,66^c \pm 0,57$	$34,33^d \pm 0,57$	$26,66^c \pm 1,52$	$35,33^c \pm 0,57$	
DMSO	0	0	0	0	0
Métro.					13 ± 0,66

The values of the diameters carrying different letters are statistically different at probability level for the columns (P = 0.05%).

Legend : Genta: gentamicin, Metro: Métroimidazol, .G_M: extract of *M. oleifera* seeds, E1: crude extract of bark of *C. procera*, E5: methanolic fraction bark of *C. procera*, F1: crude extract of leaves of *C. procera,* F5: methanol fraction leaves of *C. procera.*

Table 4. Parameters of inhibition (MIC, MBC, MBC / MIC) of the extracts and fractions / gentamicin obtained after macrodilution technique and the incorporation agar technique

Extracts/Genta	Bacterial Parameters (mg/ml)	Sensitive Bactérial Species			
		S. typhi	*S. paratyphi*	*B. cereus*	*E. coli*
E_1	CMI	5	5	5	5
	CMB	10	5	10	5
	CMB/CMI	2	1	2	2
E_5	CMI	10	5	10	10
	CMB	10	5	20	10
	CMB/CMI	1	1	2	1
F_1	CMI	5	10	5	10
	CMB	20	20	20	20
	CMB/CMI	4	2	4	2
F_5	CMI	10	10	10	10
	CMB	ND	20	ND	20
	CMB/CMI	ND	2	ND	2
G_M	CMI	5	2,5	5	2,5
	CMB	5	2,5	10	2,5
	CMB/CMI	1	1	2	1
Genta (µg/ml)	CMI	0,25	0,125	0,25	0,125
	CMB	0,25	0,125	0,25	0,125
	CMB/CMI	1	1	1	1

Legend: Genta: gentamicin, G_M: extract of *M. oleifera* seeds, E1: crude extract of bark of *C. procera*, E5: methanolic fraction bark of *C. procera*, F1: crude extract of leaves of *C. procera*, F5: methanolic fraction of leaves of *C. procera*.

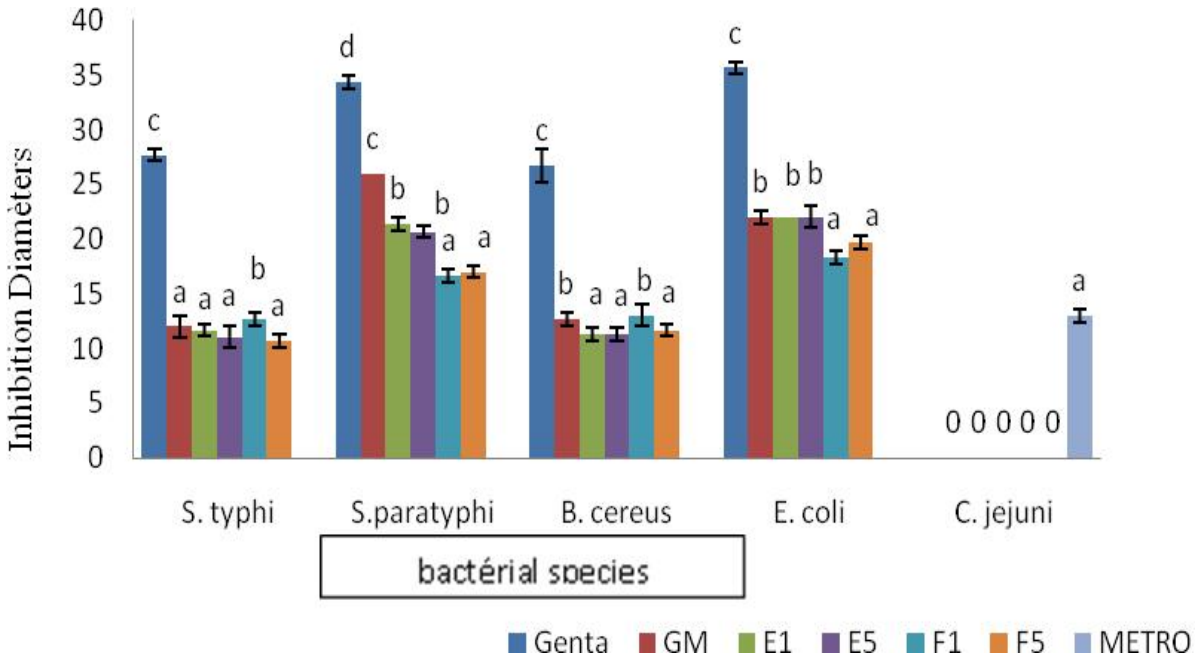

Fig. 1: Histogram representing the activity of the different extracts and fractions to each tested bacterial species