Bactericidal and bacteriostatic effects of four different parts of 2 plants*Moringa*extracts against the bacteria *Vibrio cholerae*and *Salmonella typhi*, and some phytochemical properties

ABSTRACT

Background and Objectives: Typhoid fever and cholera are two major health challenges in many developing countries. The use of extracts of the plants *Moringa*for their treatment is often done without perfect knowledge of the antimicrobial properties of each part of plant. This study aims to evaluate the bactericidal and bacteriostatic effects of extracts of *M. oleifera* and *M. stenopetala* against the bacteria *S. typhi* and *V. cholera* which cause typhoid fever and cholera respectively.

Materials and Methods:Four different parts (leaves, seeds, stem, and roots) of each plant species were dried and then ground. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the extracts of each part of the plantsagainstthe considered bacteriawere determined by dilution method. Their phytochemical profiles were determined by chromatographic and mass spectral analyses.

Results: It has been noted that MICs and MBCs varied from one part to another of the same *Moringa* species, and from one bacterium to another for the same extract. With *M. oleifera*, the lowest MBC (0.078 mg/mL) against *S. typhi* and *V. cholerae* came from seeds and roots extracts, respectively. With *M. stenopetala*, the lowest MBCs against *S. typhi* (0.156 mg/mL) and *V. cholerae* (0.312 mg/mL) were from seeds extracts. Extracts of different parts of *M. oleifera* have bactericidal effects against *S. typhi*, and bacteriostatic effects against *V. cholerae*. Extracts from all considered parts of *M. stenopetala* have bacteriostatic effects against both bacteria. Although some phytochemicals were found in both *Moringa*, others were specific to one species or to a given part.

Conclusion: In case of co-infection by both bacteria,*M. oleifera*roots extracts could be the most recommended in the treatment because of their lowest MIC and MBC. With *M. stenopetala*, roots and seeds extracts could be the most recommended because of their relatively lower MIC and MBC, respectively.The next studies will determine for each microorganism, the activity of each identified and purified chemical compound

Key words:plants *M. oleifera*and *M. stenopetala*, plant part's extract, antimicrobial activities,*S. typhi* and *V. cholerae,* MICs and MBCs, phytochemicals

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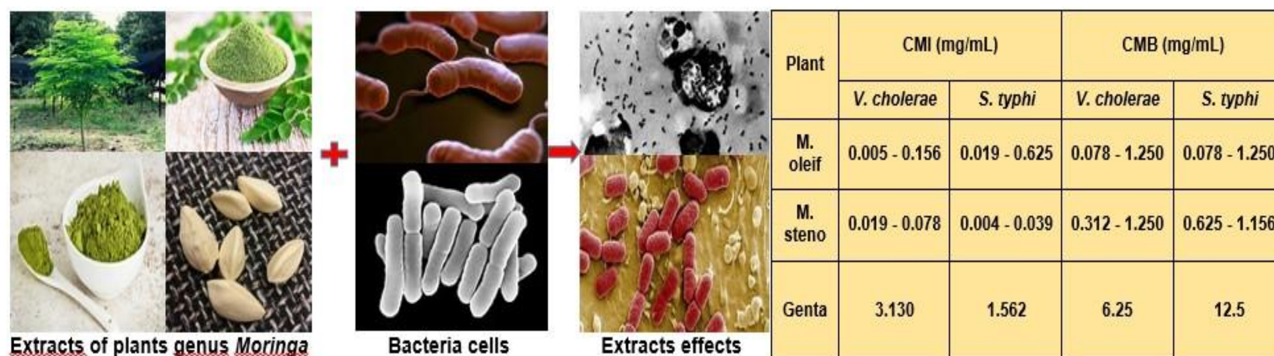
Graphical Abstract



Plant	CMI (mg/mL)		CMB (mg/mL)	
	V. cholerae	*S. typhi*	*V. cholerae*	*S. typhi*
M. oleif	0.005 - 0.156	0.019 - 0.625	0.078 - 1.250	0.078 - 1.250
M. steno	0.019 - 0.078	0.004 - 0.039	0.312 - 1.250	0.625 - 1.156
Genta	3.130	1.562	6.25	12.5

1. INTRODUCTION

Many people on earth suffer from water-borne diseases due to the lack of appropriate drinking water.Among these diseases, typhoid and cholera are two major health challenges in many African and Asian countries [1, 2]. Typhoid is caused by the bacteria named *Salmonella typhi*and *S. paratyphi*, while cholerais caused by *Vibrio cholera*[2, 3].The problem is that there is an increasing resistance to antibiotics by the 3 bacteria species above. The 3 bacteria *S. typhi, S. paratyphi* and *V. cholerae* essentially live in water and humans often become contaminated by drinking polluted water.In modern medicine, both diseases are treated by the consumption of the appropriate antibiotics. Due to the low socio-economic level and the often high cost of antibiotics, people in many developing countries use several local plants to treat these diseases.

These plants are supposed to contain certain substances or chemical elements which would act against the microorganisms at the origin of these diseases. The synthesis of these substances or chemical elements would depend not only on the genetic properties of the plant, but also on the mineralogical and petrographic properties of the crop soils, as well as meteorological parameters such as temperature, precipitation, insolation, light intensity and degree of humidity[4]. The nature, properties and concentrations of these chemical elements would vary depending on the part of the plant [4, 5].

Medicinal plants include among others *Moringa oleifera*Lam. and *M. stenopetala* (Baker f.) Cufod. Both species belong to the family ofMoringaceae, order of Capparidales, class of Dicotyledonae, subphylum of Angiospermae, phylum of Spermatophyta, kingdom of Plantae, domain of Eukaryota[6-8]. The parts of each plants above used to treat each diseasevaries from one region of the world to another, as well as from one traditional healer (doctor) to another. Their leaves, flowers, seeds, stem barks or roots are often used to treat typhoid fever and cholera, and the

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set-up include decoction, infusion,maceration[7, 9]. A variety of liquid is often used by traditional healers to prepare plant extract.Water and alcohol are regularly used [10].

Effective treatment of an infectious disease depends on the mode of action of the antimicrobial agent against the infectious agent. The antimicrobial agent can use bacteriostatic or bactericidal mechanism to fight against the pathogen. Bacteriostatic antimicrobial agent limit just the growth of bacteria.Its actions are reversible.In contrast, bactericidal antimicrobials kill their target bacteria [11, 12].

In modern medicine, the decision to use a bacteriostatic or bactericidal drug depends on the type of infection and the immune status of the patient.In traditional herbal medicine, there is in most cases insufficient knowledge of the mode of action of the extract from each part of the plant concerned.The perfect knowledge of the mechanism of action of the extract of a precise part of a medicinal plant would not only quickly eradicate the microorganism responsible of the disease, but would also allow the patient to save money while shorteningthe duration of treatment.It would also make it possible to avoid sensitization of the microorganism which often leads to resistance of the germ concerned to antimicrobial agents.

The use of *Moringa* extracts to fight against *Salmonella* and *Vibrio* bacteria is often done without perfect knowledge of the antimicrobial properties of each plant part. Little is known about the differences in extract activities from one part of the plant to another to fight against the same bacterium, as well as from one bacterium to another for the extract of the same plant part. The present study aims to evaluate the bactericidal or bacteriostatic effect of extracts of several parts of *M. oleifera* and *M. stenopetala* to fight against the bacteria *S. typhi* and *V. cholera*which respectively cause typhoid fever and cholera in humans.

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2. MATERIALS AND METHODS

The equipments and softwareused for this study include the membrane filter apparatus, incubator, porcelain mortar, Bunsenbeak, the mass spectrometer, the HPLC (High Performance Liquid Chromatography),Xcalibur software and wells plate.

2.1 Harvesting the parts of the plant and the microorganisms used

The plant species *Moringa oleifera*Lam. and *M. stenopetala* (Baker f.) Cufodare cultivated in Yaoundé (Cameroon, Central Africa). The plant names have been checked with http://www.theplantlist.org/tpl1.1/search?q=Moringa web site (accessed on 28th May 2023). They

were identified at the National Herbarium at Yaounde (Cameroon) and registered at No. 8241/SRF/Cam for *M. oleifera* and 67475/HNC for *M. stenopetala*. The parts of plant considered were the seeds, leaves, roots, and stem.Each of them was dried and then ground.

"The microorganisms *V. cholerae* and *S. typhi*were isolated from the wells of Yaoundé (Cameroon, Central Africa) by the membrane filter method (cellulose ester membranes, porosity 0.45 µm). The agar culture media used were TCBS (Bio-Rad) and Wilson-Blair media (Oxoid) for isolation of *V. cholerae* and *S. typhi* respectively. Incubations were done at 37 °C for 24hrs. Each bacterial specie was subsequently identified using conventional biochemical tests"[13, 14].

2.2Preparation of *M. oleifera* and *M. stenopetala*powders

The leaves, the bark of the roots and stems were cut up and dried in the shade, at laboratory temperature (25 ± 2 °C) for 30 days.The dried seeds were pulped, and the almond also dried at laboratory temperature (25 ± 2 °C) for 30 days.The different parts of the 2 chosen plants have been dried, crushed and then ground. Their crushing was done in an autoclave-sterilized porcelain mortar around the flame of the Bunsenbeak. The sterility of the powderwas then checked by the negative search for the 2 bacteria considered.

"To check the absence of *S. typhi*, 5 g of powder from each part of the plant were dissolved in 5 ml of sterile physiological water. After homogenization, 1 ml of this suspension was taken and diluted in 10 ml of sterile physiological water, and the whole was then filtered on a Millipore membrane (porosity 0.45 µm). This membrane was then deposited on the Wilson-Blaird agar culture medium contained in a Petri dish. Then the whole was incubated at 37° C for 24 hours. The absence of colony formed unit reflected the absence of contamination of the powder by this bacterial species. For the control of the absence of *V. cholerae*, the same protocol was used. The culture medium used was TCBS agar. The absence of colony formed unit reflected the absence of contamination of the powder by this bacterium" [52].

2.3Preparation of the methanoic plant extract

The same protocol was used for each plant material. Thirty grams (30 g) of plant material, was extracted with methanol at room temperature for 24 hours (1 g of plant in 10 mL of methanol), repeated three times. After filtration, the solvent was removed under vacuum to furnish a crude extract [15].

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2.4Preparation of plant extracts for chromatographic analyses and mass spectral determination

The method used was that described by other authors [16, 17]. From the crude methanoic extract prepared above of the different parts of the plant *M. oleifera* on one hand and *M. stenopetala* on the other hand, 5 mg of material were diluted in 5 mL of Methanol. Then the solution was filtered first through a 0.45 µm porosity membrane, and secondly through a 0.22 µm porosity membrane. Part of the filtrate was transferred to an HPLC vial for analysis.

Data was processed using Xcalibur software (Thermo Fisher Scientific Inc., MA, USA). The identification of all compounds described was carried out using the negative ionization mode.

Mass spectrum data were registered in a standard Excel format. These data included the exact mass of each compound. Each compound detected was characterized. Its potential raw formula was then calculated using Xcalibur software, which takes into account its relative abundance, its molar masses and its retention time. The names of chemical compounds were determined by comparison with analytical standard or according to literature data [18-21].

2.5Preparation of plant extracts for antimicrobialactivities

The protocolusedwasthatdescribed by Ashraf et al [22].A stock solution of extract of 2.5 mg/mL concentration wasprepared in a volume of 5 mL of 8.5 g/L NaClsterile solution. The mixture obtainedwashomogenized in a vortex, and thenfilteredthroughafilter membrane (porosity 0.2 µm).

For antimicrobialactivity, the standard antibioticchosenwasGentamicin.The microbial inoculum waspreparedfrom a pure 18-24 hours' culture on standard agar, and thenadjustedat standard solution using a densitometer (density 0.5 on the Mc Farlandscale) containingapproximately 1.5 x 10^8 CFU/mL, and diluted to 1/100for the saline dilution test [23]. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) werethendetermined.

2.6Determination of the minimum inhibitory concentrations (MICs)

The method of 96 wells plate was used and this has been described by others [12, 24]. A sterile Muller Hinton nutrient broth was used. From the bacterial suspension indicated above, bacterial inoculum containing approximately 10^6 CFU/mL was prepared by dilution. From this suspension, 100 µL was inoculated into each well. The serial dilutions of extract ranging from 1.25

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to 0.0045 mg/mL were then introduced in 96-well microplates. A sterility control well and a growth control well were also done. Plates were then incubated at 37 ° C for 18 to 24 hours. After incubation, 40 µL of the colorlessiodonitrotetrazolium chloride (INTC) solution were introduced into each of the wells of the plate. INTC reagent is an indicator of microbial growth. About 30 minutes after its addition, the appearance of a red color indicates the cells growth. The unstained well and which contained the lowest concentration of the extract corresponds to the MIC. This was done in triplicate. The antibiotic Gentamicin was used for positive control, and the negative control was at the absence of plants extract.

2.7Determination of the Minimum Bactericidal Concentrations (MBCs)

The protocolusedwasthatindicated by otherauthors[24-26]. 50 µL of extracttakenfrom the wellscorresponding to the MICsobtainedwereadded. Also, 50µL of extractwastakenfrom the wellsthatprecededthosewith MIC. The sterilitychecksconsisted of 200 µL of Mueller Hinton broth. The positive control consisted of Gentamicin, treatedlike the extracts. The negative control was the medium withoutextract. Incubation wasat 37°C for 18 - 24hrs. The CMB was the lowest concentration of extractwith no visible microbialgrowth (around 99,9% of cells have been killed). The tests weredone in triplicate.

2.8 Determination of the MBC/MIC ratios

The ratio obtainedaimed to concludewhetherextractstestedwerebacteriostatic or bactericidal.Whenthis ratio was> 4, extractwasconsideredbacteriostatic.Whenitwas ≤ 4, thenextractwasconsideredbactericidal[24, 26].

3. RESULTS

3.1Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of each part of plants extractsagainst*S. typhi*and *V. cholerae*

For each of the 2 bacterial species; *S. typhi*and *V. cholerae*, the mean values of MICs and MBCs obtained with the extracts of each part of *M. oleifera* and *M. stenopetala* were calculated. They are presented in Fig. 1. For both bacterial species Gentamicin has been chosen as the reference antibiotic. It has a MIC of 1.562 mg/mL to fight against *S. typhi* and 3.130 mg/mL to fight against *V. cholerae*. With plants, it was noted that the MICs values of extracts parts of *M. oleifera* against *S. typhi* varied from 0.625 mg/mL to 0.019 mg/mL. The highest value was registered with leaves and

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stem extracts, while the lowest was recorded with seeds extract. The MIC value with roots extract was 0.078 mg/mL. Against *V. cholerae*, the MICs with extract from this plant species varied from 0.156 mg/mL to 0.005 mg/mL. The highest value was registered with leaves and stem extracts while the lowest value was recorded with roots extract. With the seeds extract the MIC against *V. cholerae* was 0.009 mg/mL (Fig. 1).

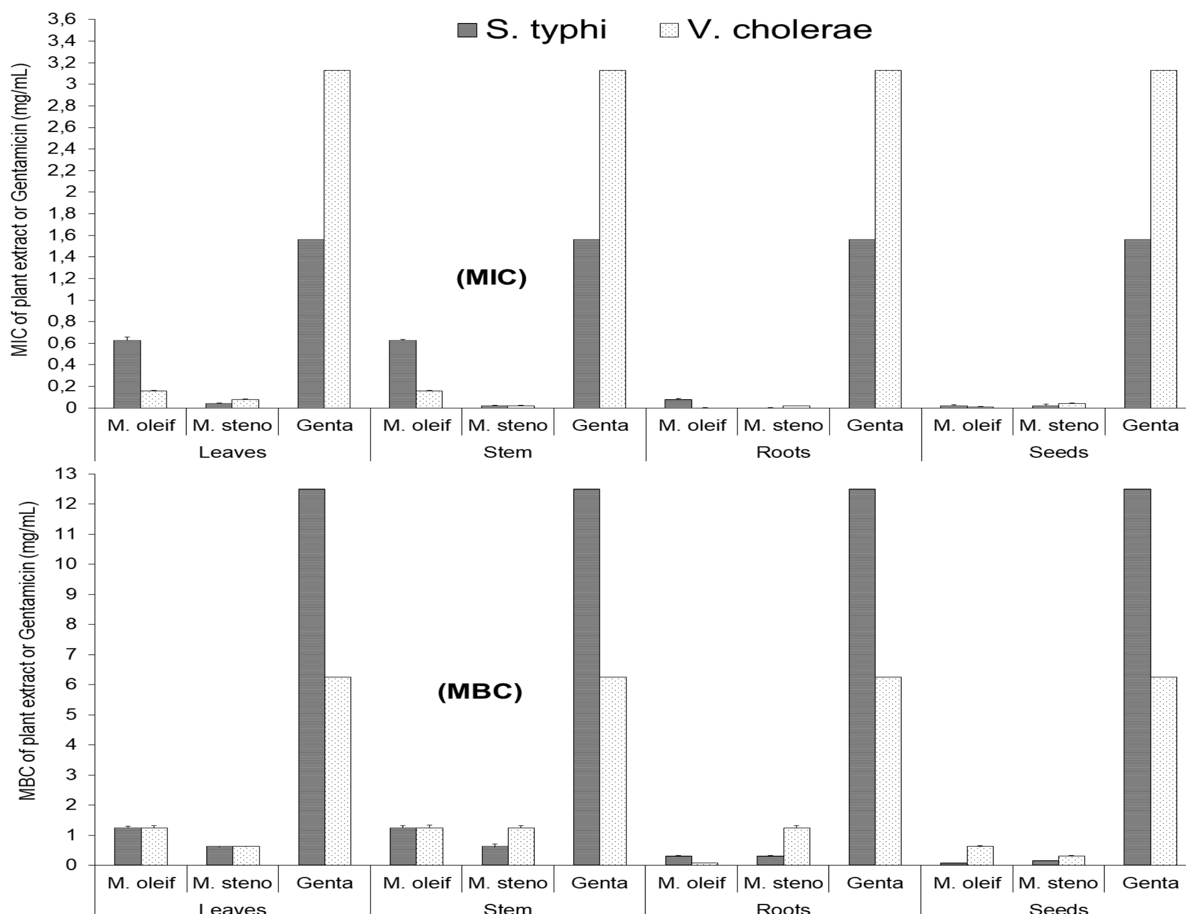

Fig. 1. Variation in MICs and MBCs of leaves, seeds, stem, and roots extracts of *M. oleifera* (**M. oleif**) and *M. stenopetala* (**M. steno**) as well as Gentamicin (**Genta**) against *S. typhi* and *V. cholerae*

When using *M. stenopetala* extracts, the MICs against *S. typhi* varied from 0.039 mg/mL to 0.004 mg/mL. The highest value was registered with leaves extract while the lowest value was recorded with roots extract. The MICs of stem and seeds extracts was 0.019 mg/mL. Against *V. cholerae*, MICs with *M. stenopetala* extracts varied from 0.078 mg/mL to 0.019 mg/mL. The highest MIC value was recorded with leaves extracts while the lowest value was obtained with stems and roots extracts. With seeds extracts, the MIC value was 0.039 mg/mL (Fig. 1).

For both bacterial species, Gentamicin chosen as the reference antibiotic has an MBC of 12.5 mg/mL against *S. typhi* and 6.25 mg/mL against *V. cholerae*. The MBCs when using *M.*

*oleifera*extracts against *S. typhi* varied from 1.250 mg/mL to 0.078 mg/mL. The highest value was obtained with leaves and stem extracts and the lowest value with seeds extracts. With roots extracts, it was 0.312 mg/mL. In the presence of *V. cholerae*, the MBCs with *M. oleifera* extracts also varied from from 1.250 mg/mL to 0.078 mg/mL. The highest was registered with leaves and stem extracts while the lowest was obtained with roots extracts. With seeds extracts the MBC was 0.625 mg/mL (Fig. 1).

The MBCs values when using *M. stenopetala* extracts, to fight against *S. typhi* varied from 0.625 mg/mL to 1.156 mg/mL. The highest was obtained with leaves and stems extracts while the lowest was recorded with seeds extracts. With roots extracts, the MBC against *S. typhi* was 0.312 mg/mL (Fig. 1). When using *M. stenopetala* extracts, the MBCs in the presence of *V. cholerae* varied from 1.250 mg/mL to 0.312 mg/mL. The highest value was recorded with stems and roots extracts while the lowest value was registered with seeds extracts. With leaves extracts, the MBC obtained was 0.625 mg/mL (Fig. 1).

3.2 Effect of the extract of each part of *M. oleifera* and *M. stenopetala* against *S. typhi* and *V. cholerae*

From the values of the MBC/MIC ratios, the antibacterial properties of each extract from each *Moringa* species and on each microorganism considered were determined. These different properties are presented in Table 1. It was noted that extracts of each part from the two *Moringa* species are bacteriostatic against *V. cholerae*. Whereas against *S. typhi*, extract of each considered part *of M. oleifera* is bactericidal, and extracts from each part of *M. stenopetala* are bacteriostatic (Table 1). It should be noted that Gentamicin as our control is bactericidal against *V. cholerae* but bacteriostatic *against S. typhi* (Table 1).

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Table 1. Effect of the extract of each part (leaves, seeds, stem and roots) of *M. oleifera* and *M. stenopetala* against *S. typhi* and *V. cholerae*

Part of the plant species and standard antibiotic used		Bacteria species and extract's effect	
Plant part	**Plant species**	***S. typhi***	***V. cholerae***
Leaves	*M. oleifera*	Bactericidal	Bacteriostatic
	M. stenopetala	Bacteriostatic	Bacteriostatic
Stem	*M. oleifera*	Bactericidal	Bacteriostatic
	M. stenopetala	Bacteriostatic	Bacteriostatic
Roots	*M. oleifera*	Bactericidal	Bacteriostatic
	M. stenopetala	Bacteriostatic	Bacteriostatic
Seeds	*M. oleifera*	Bactericidal	Bacteriostatic
	M. stenopetala	Bacteriostatic	Bacteriostatic
Gentamicin ($C_{21}H_{43}N_5O_7$)		Bacteriostatic	Bactericidal

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3.3 Comparison amongst the MICs and MBCs values for each bacteria specie considered for the 4 parts of *M. oleifera* and *M. stenopetala*

A comparison of the MICs and MBCs among the extract from the 4 parts of each the *Moringa* species against each bacterial species was made. The result is presented in Table 2. It can be noted that MICs of extract from different parts of *M. oleifera* significantly differed (P<0.05) against *V. cholerae* and *S. typhi*. However, MICs of extract from different parts of *M. stenopetala*do not

significantly differ from one part of the plant to another (P>0.05) against both bacteria species. MBCs of extract from different parts the *Moringa* species do not also significantly differ against the 2 bacteria species (Table 2).

Table 2. "*P*" value of the comparison for each considered bacteria species, of the MICs and MBCs values of the 4 parts of the plant

Plant species and cells species		Extracts' concentrations	
Plant species	**Bacterial species**	**MIC**	**MBC**
M. oleifera	*V. cholerae*	**P= 0.025***	P=0.392
	S. typhi	**P= 0.013***	P= 0.392
M. stenopetala	*V. cholerae*	P= 0.435	P= 0.392
	S. typhi	P= 0.835	P=0.392

***** : Significantdifference (P < 0.05) ; ddl=3

3.4 Phytochemical profiles of extracts

The chemical profiles of each extract have been established by mass spectra in negative ionization mode. The chemical compounds identified from extracts of leaves, seeds, roots and stems of *M. oleifera* and *M. stenopetala*are presented in Table 3.

Concerning *M. oleifera*, some compounds have been identified in several parts of the plant. Saccharose (glycoside) and Glucomoringinin (Glucosinolate) for example, were identified in leaves, seeds, stems and roots extracts. The O-acetylshanzhiside methyl ester was found in leaves, stems and roots extracts. The Glucotropaeolin was identified in stems and roots extracts. On the other hand, some compounds have only been found in certain specific parts of the plant. This is for example Neochlorogenic acid, Isoquercetin, Kaempferol-3-O-glucoside and Kaempferol acetyl glucoside which were found only in leaves extracts. It is the same for Quinic acid and Catechin which were found only in stems extracts (Table 3).

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Compounds identified are representative of 7 different classes (Table 3): heteroside, glucosinolate, phenolic acid, iridoid, flavonoid, flavonoid heteroside and carboxylic acid. The heteroside and glucosinolate identified were found in the 4 parts of the plants considered. The phenolic acid identified was found only in leaves. The iridoid identified was found in leaves, stems and roots extracts. The flavonoids identified were found in leaves and stems. The flavonoids heterosides identified were found only in leaves. The carboxylic acid identified was found only in stems (Table 3).

As in the case of *M. oleifera*, the extracts of *M. stenopetala* also show the presence of same compounds in different part of the plant. This is the case for Sucrose and Glucomoringin.Others are present only in certain parts of the plant, like Quercetin-3-O-rutinoside and Neochlorogenic acid which are only present in the leaves. Glucoconringiin-2-hydroxy-2-méthylpropylglucosinolate is only found in the seeds.Acetyl-4-(L-rhamnopyranosyloxy)benzylglucosinolate and 1,2-Di-O-galloyl-6-O-cinnamoyl-β-D-glucose are only in the stem. Glucose is present only in the roots. Glucotropaeolin is present only in the stem and roots.Citric acid and O-acetylshanzhiside methyl ester are present only in the leaves, stem and roots. All compounds identified are of 6 different classes: heteroside, glucosinolate, phenolic acid, iridoid, flavonoid and flavonoid-heteroside(Table 3).

Some main differences in the chemical profiles of the extracts of the 2 *Moringa* species have been highlighted. In leaves it is the presence of Isoquercetin(Flavonoid), Kaempferol-3-O-glucosideand Kaempferol-acetyl glucoside(Flavonoid heteroside) only in *M. oleifera*, and the presence of Quercetin-3-O-rutinoside (Flavonoid heteroside), only in*M. stenopetala*. In stems extracts, it is the presence of Quinic acid (Carboxylic acid) and Catechim (Flavonoid) only in *M. oleifera* and that of Acetyl-4-(α-L-rhamnopyranosyloxy)benzyl glucosinolate (Glucosinolate), 1,2-Di-O-galloyl-6-O-cinnamoyl-β-D-glucoseand Citric acid (organic acid)only in *M. stenopetala*. In roots, it is the presence of Glucose (heteroside) and Citric acid (organic acid) only in *M.stenopetala*. In seeds Glucoconringiin-2-hydroxy-2-méthylpropylglucosinolate has been found in *M. stenopetala* and not in *M. oleifera* (Table 3).

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Table 3. Phytochemical profile of MeOH extracts of each part considered of *M. oleifera*and *M. stenopetala*(comparison with analytical standard and according literature data [18-21])

Part of the plant considered	Plant species and chemical compound identified									
	M. oleifera					*M. stenopetala*				
	Rt (min)	M-H$_{exp}$ (m/z)	Compound formula	Compound name	Class of molecule	Rt (min)	M-H$_{exp}$ (m/z)	Compound formula	Compound name	Class of molecule
Leaves	3.99	341.1086	$C_{12}H_{22}O_{11}$	Saccharose	Heteroside	3.96	341.1086	$C_{12}H_{22}O_{11}$	Saccharose	Heteroside
	8.99	570.0956	$C_{20}H_{29}NO_{14}S_2$	Glucomoringinin	Glucosinolate	6.84	191.0189	$C_6H_8O_7$	Citric acid	Organic acid
	11.34	353.0875	$C_{16}H_{18}O_9$	Neochlorogenic acid	Phenolic acid	9.01	570.0956	$C_{20}H_{29}NO_{14}S_2$	Glucomoringinin	Glucosinolate
	14.13	447.1508	$C_{19}H_{28}O_{12}$	O-acetylshanzhiside methyl ester	Iridoid	11.32	353.0875	$C_{16}H_{18}O_9$	Neochlorogenic acid	Phenolic acid
	18.55	463.0883	$C_{21}H_{20}O_{12}$	Isoquercetin	Flavonoid	14.11	447.1508	$C_{19}H_{28}O_{12}$	O-acetylshanzhiside methyl ester	Iridoid
	22.12	447.0931	$C_{21}H_{20}O_{11}$	Kaempferol-3-O-glucoside	Flavonoid heteroside	16.98	609.1465	$C_{27}H_{30}O_{16}$	Quercetin-3-O-rutinoside	Flavonoid heteroside
	26.65	489.1037	$C_{23}H_{22}O_{12}$	Kaempferol acetyl glucoside	Flavonoid heteroside	/	/	/	/	/
Seeds	4.04	341.1093	$C_{12}H_{22}O_{11}$	Saccharose	Heteroside	3.98	341.1086	$C_{12}H_{22}O_{11}$	Saccharose	Heteroside
	8.78	570.0966	$C_{20}H_{29}NO_{14}S_2$	Glucomoringinin	Glucosinolate	7.29	390.0533	$C_{11}H_{21}NO_{10}S_2$	Glucoconringiin-2-hydroxy-2-méthylpropyl-glucosinolate	Glucosinolate
	/	/	/	/	/	8.91	570.0957	$C_{20}H_{29}NO_{14}S_2$	Glucomoringinin	Glucosinolate
	3.85	191.0547	$C_7H_{12}O_6$	Quinic acid	Carboxylic acid	3.98	341.1087	$C_{12}H_{22}O_{11}$	Saccharose	Heteroside

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Stems	4.00	341.1046	$C_{12}H_{22}O_{11}$	Saccharose	Heteroside	6.86	191.0185	$C_6H_8O_7$	Citric acid	Organic acid
	9.03	570.0955	$C_{20}H_{29}NO_{14}S_2$	Glucomoringinin	Glucosinolate	8.98	570.0959	$C_{20}H_{29}NO_{14}S_2$	Glucomoringinin	Glucosinolate
	12.89	408.0426	$C_{14}H_{28}NO_9S_2$	Glucotropaeolin	Glucosinolate	11.66	612.1067	$C_{22}H_{31}NO_{15}S_2$	Acetyl-4-(α-L-rhamnopyranosylox) benzyl glucosinolate	Glucosinolate
	13.09	289.0718	$C_{15}H_{14}O_6$	Catechin	Flavonoid	12.26	612.1066	$C_{29}H_{25}O_{15}$	1,2-Di-O-galloyl-6-O-cinnamoyl-β-D-glucose	Acyl glucoside
	14.11	447.1506	$C_{19}H_{28}O_{12}$	O-acetylshanzhiside methyl ester	Iridoid	12.80	408.0428	$C_{14}H_{28}NO_9S_2$	Glucotropaeolin	Glucosinolate
	/	/	/	/	/	14.12	447.1509	$C_{19}H_{28}O_{12}$	O-acetylshanzhiside methyl ester	Iridoid
Roots	3.99	341.1087	$C_{12}H_{22}O_{11}$	Saccharose	Heteroside	3.23	179.0554	$C6H_{12}O_6$	Glucose	Heteroside
	9.04	570.0956	$C_{20}H_{29}NO_{14}S_2$	Glucomoringinin	Glucosinolate	3.98	341.1087	$C_{12}H_{22}O_{11}$	Saccharose	Heteroside
	12.85	408.0426	$C_{14}H_{28}NO_9S_2$	Glucotropaeolin	Glucosinolate	6.85	191.0186	$C_6H_8O_7$	Citric acid	Organic acid
	14.12	447.1507	$C_{19}H_{19}O_{12}$	O-acetylshanzhiside methyl ester	Iridoid	9.02	570.0959	$C_{20}H_{29}NO_{14}S_2$	Glucomoringinin	Glucosinolate
	/	/	/	/	/	12.77	408.0428	$C_{14}H_{28}NO_9S_2$	Glucotropaeolin	Glucosinolate
	/	/		/	/	14.11	447.1509	$C_{19}H_{28}O_{12}$	O-acetylshanzhiside methyl ester	Iridoid

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4. DISCUSSION

This study shows that some compounds in the extracts of *Moringa* species, are found in all parts of the plant, while others are only localized in some specific parts (Tables 3). These qualitative and probably quantitative variations (because of the variations in retention times), would be at the origin of the apparent variations of the MICs and MBCs from one part of the plant species to another.

Four compounds identified in *M. stenopetala* extracts were absent in *M. oleifera*. They are Quercetin-3-O-rutinoside, Citric acid, Acetyl-4-(α-L-rhamnopyranosyloxy)benzylglucosinolate, and glucose. Five compounds identified in *M. oleifera* extracts, were absent in the *M. stenopetala* extracts. They are Isoquercetin, Kaempferol-3-O glucoside, Kaempferol acetyl glucoside, Quinic acid and Catechim. Each of the chemical compound has its own properties towards microorganisms.

Quinic acid which is a carboxylic acidwas found in *M. oleifera* extract, unlike extracts from *M. stenopetala* (Tables 3). Carboxylic acids are known to facilitate the destruction of microbial membranes and cell walls. They act through the inhibition of peptidoglycan synthesis [27]. They can also inhibit bacterial growth through the inhibition of DNA/RNA replication, for example the inhibitory activity against DNA gyrase (an essential enzyme that controls the topological state of DNA replication) [28, 29]. Carboxylic acids also target horizontal gene transfer inhibition, quorum sensing inhibition, inhibition of efflux pumps and inhibitors of metabolic routes [29].

Isoquercetin was isolated only in *M. oleifera* and not in *M. stenopetala* extracts (Table 3). This flavonoid is a naturally occurring polyphenol that has antioxidant, anti-proliferative, anti-carcinogenic, neuroprotection, anti-inflammatory, antibacterial and antiviral properties [30].

The flavonoids Kaempferol-3-O glucoside, Kaempferol acetyl glucoside and Catechim have been found only in *M. oleifera* and the phenolic acidNeochlorogenic acid has been found in leaves extracts of both *Moringa* species (Table 3). Phenolic products can also inhibit the synthesis of nucleic acids of both gram negative and gram-positive bacteria although this antibacterial effect varies with chain length of the molecules [31].In other circumstances, they can form a complex with bacterial cell wall thus inhibiting the microbial growth [32]. The anti-microbial mechanisms of flavonoids and phenolic compounds include the inhibition of cell envelop synthesis, nucleic acid synthesis, bacterial motility, electron transport chain and ATP synthesis, bacterial toxins, bacterial quorum sensing, biofilm formation, bacterial enzyme-dependent virulence, bacterial efflux pumps, cytoplasmic membrane function causing marked increase in potassium loss from cells, the membrane disruption, among others [33-35].

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Quercetin-3-O-rutinoside and Citric acid were found only in *M. stenopetala* extracts. They respectively destroy the normal functioning of the endoplasmic reticulum and increase the intracellular acidity leading to the destruction of the bacterial cell [36, 37].

Traditionally, non-ionized citric acid is considered more antimicrobial, presumably due to the combined effects of the molecule and the acidic environment in which it occurs [37]. It is believed that at low pH, citric acid in its un-charged and un-dissociated state can freely cross the microbial membrane. Once inside the cytoplasm, it dissociates into citric acid anions and protons leading to the acidification of the intracellular media, while causing functional and structural damage to the cell [38-40].

Somecompounds asGlucomoringin, Neochlorogenic acid and O-acetylshanzhiside methyl ester have been found in the extracts of both *Moringa* species. Glucomoringin has been found in most parts of the plant studied. The antibiotic activity of this glucosinolate on *S. aureus* has been reported [21, 41]. Its bioactivation with myrosinate enzyme leads in the increasing power of the compound [41]. O-acetylshanzhiside methyl ester which is an iridoid has been detected in leaves, stems and roots extracts. Cytotoxic activities of iridoids have been indicated by many authors [42].

The differences in the resistance mechanisms to antimicrobial agents between *S. typhi* and *V. cholerae* could also be at the origin of the relative differences observed in the MICs and MBCs against the two bacteria species (Fig. 1), as well as the effect of the extract of each part of each *M. oleifera* and *M. stenopetala* against each bacteria species (Table 1).

It is indicated that *S. typhi*resists to antimicrobials by the following ways: inactivation of the antimicrobial agent and efflux or transport of the antimicrobial, modification of the antimicrobial target site and reduction of the permeability of the antimicrobial agent [43].These resistance mechanisms can be plasmid mediated or chromosome mediated[44, 45].

Antimicrobial resistance in *V. cholerae* is mostly due to acquisition of resistance genes from closely or distantly related microbial species through horizontal gene transfer [46]. *V. cholerae* can also acquire resistance to antimicrobial compounds by any of the following seven mechanisms :i) modification of the antibiotic target site, ii) antibiotic target replacement, iii) antibiotic target protection, iv) inactivation of the antibiotic by hydrolysis or chemical modification, v) preventing access to the target site by changing membrane permeability, vi) actively exporting antibiotics from bacterial cell, and vii) resistance by absence of antibiotic target [47, 48]. Both, spontaneous mutation and horizontal gene transfer could contribute to all the resistance mechanisms [48].

In the present study, the difference in behavior between the 2 pathogens would also vary according to the concentration of each antimicrobial agent identified, although this concentration

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was not determined in the extract of each part of the plant.The concentration of the antimicrobial agent is often said to be very closely related to the kinetics of its activity on the target bacterium [49, 50]. This could also partly explain the variations in the recorded MICs and MBCs, as well as the variation in the extract activity from one microorganism to another.

Gentamicin was considered as a control antimicrobial agent in this study. His chemical formula is $C_{21}H_{43}N_{5}0_{7}$. It is noted that it has a bacteriostatic effect against *S typhi* and bactericidal against *V cholerae*.This molecule contains a high number of nitrogen atoms, compared to the chemical compounds identified in the 2 plants used. According to Yoshizawa*et al*[51], its bactericidal action is by binding the 30S subunit of the bacterial ribosome, negatively impacting protein synthesis.

5. CONCLUSION

The MICs and MBCs of the *M. oleifera* and *M. stenopetala* extracts against *S. typhi* and *V. cholerae* relatively vary on one hand from one part of the plant to another for the same *Moringa* species, then from one bacterium species to the other for the same extract on the other hand. The leaves, seeds, stem, and roots extracts of *M. oleifera* have bactericidal effect against *S. typhi*, and bacteriostatic effect against *V. cholerae*. Extracts from all parts of *M. stenopetala* have bacteriostatic effects against each of the 2 bacterial species. These differences would be linked on one hand to the relative phytochemical differences amongst the parts of the 2 plant species, and on the other hand to the relative differences between the antimicrobial resistance mechanisms of the 2 bacteria. Although some phytochemicals were found in both *Moringa* species, others were specific to one species or to a given plants part. In case of co-infection by the 2 bacterial species,*M. oleifera* extracts could be the most recommended in the treatment, especially root extracts, because of their lowest MIC and MBC. With *M. stenopetala*, root and seed extracts could be the most recommended because of their relatively lower MIC and MBC, respectively.The next studies will make it possible to isolate and purify each chemical compound of the extract, then to determine its activity vis-à-vis each of the 2 microorganisms concerned.It seems important to explore in each region of the world, the antimicrobial properties of the extracts of different parts of each medicinal plant with respect to each microorganism or group of microorganisms which cause the diseases whose said extracts are used to treat the symptoms inHumans.This would increase the effectiveness of herbal medicine against the germs concerned and would also contribute to the fight against their resistance to antimicrobial agents.

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