

## MINIMUM INHIBITION AND BACTERICIDAL CONCENTRATIONS OF THE PLANT *MORINGA OLEIFERA* EXTRACTS AGAINST THE BACTERIA *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

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### ABSTRACT

This study aimed at assessing the MICs (Minimum Inhibitory Concentrations) and the MBCs (Minimum Bactericidal Concentrations) of the methanolic extracts from 4 different parts (leaves, seeds, stem, roots) of the plant *Moringa oleifera* against the bacteria *E. coli* and *S. aureus*. Phytochemical profile of each extract has been determined using LC/MS analyses. Microbial analyses were done using 96-wells plate method. Results showed that the four parts on the plants contain heterosides and glucosinolates. Iridoids were found in leaves, stem and roots. Flavonoids were found in leaves and stem. Phenolic acids were found only in leaves and carboxylic acids found only in stem. The abundance and the number of identified compounds of a given class varied from one part of plant to another. The MICs and MBCs varied with respect to the microorganism and the part of the plant. With *E. coli*, the MICs varied from 0.009 (leaves) to 1.25 mg/mL (seeds), and the MBCs varied from 0.156 (leaves) to 2.5 mg/mL (seeds and stem). With *S. aureus*, the MICs varied from 0.009 (stem and roots) to 0.039 mg/mL (leaves and roots), and the MBCs varied from 0.078 (leaves) to 4 mg/mL (stem). The bactericidal or bacteriostatic properties of the extracts varied depending on the part of the plant and the microorganism considered. Against *E. coli*, the leaves, stem and roots extracts were bacteriostatic whereas seeds extract was bactericidal. Against *S. aureus*, the seeds, stem and roots extracts were bacteriostatic whereas leaves extract was bactericidal.

**Keywords:** *Moringa oleifera* extracts, MIC, MBC, *E. coli*, *S. aureus*, phytochemistry, plant parts

### INTRODUCTION

A lot of microorganisms in nature can be found in various biotopes. For humans, some are pathogens or opportunistic pathogens while others are simply commensal. The commensal character of a germ, however, depends on several factors in its environment (Van Baarlen *et al.*, 2007; Hamilton *et al.*, 2019). Increased consumption of antimicrobial agents and their inappropriate use are among factors which further accelerated the increase of antibiotic resistant microorganisms. Furthermore, the continuous migration of people between countries as well as international tourism and business travel play an important role in the acquisition and spread of multidrug resistant strains (Van der Bij and Pitout, 2012; Allocati *et al.*, 2013; Galindo-Méndez, 2020).

The phytotherapy (herbal treatment) has become more and more common and around 14-28% of the plants listed in the world have medicinal use, and it is often empirical (Padulosi *et al.*, 2002). Plants used are of various families. In African and Asian plant medicine, various extracts are commonly used to treat various enteric infections. Antimicrobial resistance has seriously threatened human public health, and new antimicrobial agents are desperately needed (Laxminarayan *et al.*, 2016; Yuan *et al.*, 2021). Many plant ingredients present weak antimicrobial activities, while some of them can in reverse promote the resistance against antimicrobial agents. Most of them are considered nontoxic to human body because of their ubiquity in all sorts of plant derived foods and beverages (Tsagou Manfo *et al.*, 2014; Gorniak *et al.*, 2019; Yuan *et al.*, 2021). The extracts have sometimes been indicated to contain a variety of families of chemical compounds (Fuglie, 2001; Ani *et al.*, 2018).

The extracts of the plant *M. oleifera* have sometimes been indicated to contain a variety of families of chemical compounds including essential amino acids, tannins, alkaloids, phenols,  $\beta$ -carotenes, flavonoids, calcium, potassium, phosphorus, vitamin A, C and D among others (Kuate, 2010, 2017; Arora *et al.*, 2013; Metsopkeng *et al.*, 2019, 2020). Their impact on the growth of bacteria on the blood has also been indicated (Latifa and Muneera, 2016). Their role in prevention and treatment of chronic diseases as a result of possible cross-kingdom regulation by exogenous vegetal microRNAs and synergistic action of plant bioactive components on endogenous human microRNA regulation has been reported (Matic *et al.*, 2018). In several regions of the world, these extracts are

also used in traditional medicine to fight against fever, tuberculosis, anemia, skin infections and respiratory disorders (Fuglie, 2001). They are sometimes used to fight against some microorganisms of health importance including *Escherichia*, *Salmonella*, *Pseudomonas*, *Staphylococcus*, *Aeromonas*, *Bacillus*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Candida* cells species (Elgamily *et al.*, 2016; Metsopkeng *et al.*, 2019, 2020). Most of the above microorganisms are waterborne and the strains of some species are commensal, whereas other can be opportunistic pathogens and can thus cause harm to immunocompromised individuals (Kurosu *et al.*, 2013; Mecherghi *et al.*, 2019). The popular use of *M. oleifera* extracts against AIDS and related secondary infections associated with HIV has been indicated in human. They showed significant activities against viruses of human health importance like HIV, HSV, HBV, EBV, FMDV and NDV (Biswas *et al.*, 2020 ; Xiong *et al.*, 2022). The use of the leaves extract to improve the immune system in vaccinated and non-vaccinated broiler Hubbard chickens against some virus has also been indicated (Hala *et al.*, 2022).

Most of the antimicrobial effects indicated above were achieved by introducing the crude plant extracts into water contaminated with microorganisms or in agar culture medium containing microorganisms. Few data are available on the minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) of the most medicinal plants extracts with respect to the particular pathogenic microorganisms or opportunistic pathogens found in the environment. In addition, little is known about the variation in the activity of the extracts from different parts of the plant on the same microorganism, and extract from one part of the plant on one bacterial species to another. This study aimed to assess the activity properties of four different parts of the plant *M. oleifera* extracts against 2 bacteria *E. coli* (Gram negative) and *S. aureus* (Gram positive), by determination of the MICs and MBCs of the extract of each part of the plant and identification of the major chemical compounds present. The hypothesis is that the four parts of the plant are of different chemical properties and would have different activity density first towards a given microorganism, then from one microorganism to another for the extract of the same part of plant.

## MATERIAL AND METHODS

### Harvesting the parts of the plant and the microorganisms used

The plant species *Moringa oleifera* belongs to the superkingdom of Tracheobionta, superdivision of Spermatophyta, division of Magnoliophyta, class of Magnoliopsida, subclass of Dilleniidae, order of Capparales, family of Moringaceae (Mallenakuppe et al., 2019). Its common name varies according to the part of the world and it is considered to have its origin in the northwest region of India and south of the Himalayan Mountains. It is now cultivated throughout the Middle East, almost the whole tropical belt and it was introduced in Africa from India at the beginning of 20th century (Mallenakuppe et al., 2019).

It is cultivated in Yaounde (Cameroon, Central Africa). This region has an equatorial climate. This town is located at 3°52' latitude North, 11°32' longitude East and at 759 m altitude. The soil is fero-lateritic (Naah, 1990). The plant *M. oleifera* was identified at the National Herbarium of Cameroon (Yaoundé) and registered under No. 8241/SRF/Cam. The parts of plant considered were the seeds, leaves, roots and stem. Each of these parts was dried and then ground.

The microorganisms considered were the bacteria *E. coli* and *S. aureus*. These bacterial species were chosen because of their importance in hygiene and public health, as well as in the bio-indication of the microbiological quality of the water (Al-Mayah et al., 2017; Hamilton et al., 2019). They were isolated from the surface waters of Yaoundé (Cameroon, Central Africa) by the membrane filter method (cellulose ester membranes, porosity 0.45 µm). The agar culture media contained in Petri dish used were Endo (Difco) and salt-mannitol agar (BioRad) for the isolation of *E. coli* and *S. aureus* respectively. Incubation was done at 37 °C for 24h. Each bacterial species was subsequently identified by using conventional biochemical tests (Holt et al., 2000; Bautista-Trujillo et al., 2013; Prasad Sha et al., 2018).

### Preparation of *M. oleifera* powders

The leaves, the bark of the roots and stems were cut up and dried in the shade, at laboratory temperature ( $25 \pm 2$  °C) for 30 days. The dried seeds were pulped and the almond also dried at laboratory temperature ( $25 \pm 2$  °C) for 30 days. These different parts of the plant after drying were crushed and ground. Each part of the plant was crushed in an autoclave-sterilized porcelain mortar, within a radius of 15 cm around the flame of the Bunsen beak. The sterility of the powder was then checked by the negative search for the 2 bacteria considered.

To check the absence of *E. coli*, 5g of powder from each part of the plant was dissolved in 5ml 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 Endo agar culture medium contained in a Petri dish. Then the whole was incubated at 37° C for 24 hours. The absence of culture reflected the absence of contamination of the powder by this bacterial species. For the control of the absence of *S. aureus*, the same protocol was used. The culture medium used was Chapman Mannitol agar. The absence of culture reflected the absence of contamination of the powder by this bacterium.

### Preparation of the methanol extract

Thirty grams (30g) of plant material, for each part of the plant, were reduced to powder, then extracted three times with methanol for 24 hours at room temperature so that all the molecules could come into contact with the solvent and the compounds of interest bind well to methanol molecules. We used a plant/solvent ratio of 1:10 (mass:volume). The whole left on a magnetic stirrer at a speed of 500rpm. Subsequently, the mixture is filtered on whatmann N/4 paper, in order to remove impurities, and the rest of the ground material in the beaker is macerated again in methanol. The filtrate thus obtained is evaporated under vacuum with a rotavapor for 1 to 2 hours, in order to eliminate the methanol at a pressure of 337 mmHg (Millimercury), the boiling temperature of the methanol is found lowered causing the evaporation of the latter to 40° C in a water bath, leaving the compounds of interest in the flask. This method was according to Mellon et al. (2002).

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

From the crude methanolic extract prepared above of the different parts of the plant *M. oleifera*, 5 mg of material was diluted in 5 mL of MeOH HPLC Grade. The solution was then homogenized in an ultrasonic tank. Using a 2 ml syringe, the

solution was withdrawn and filtered first through a 0.45 µm porosity membrane, and then through a 0.22 µm porosity membrane. Part of the filtrate was transferred to an HPLC vial.

HPLC analyses were performed on an Agilent 1260 Infinity apparatus, with DAD detector equipped with an Uptisphere C18-3 (250 x 4.6 mm, 5µm) column from Interchim (Montluçon, France). LC-MS analyses were carried out on an UHPLC Ultimate 3000 RSLC chain and an Orbitrap Q-Exactive (Thermo Scientific, Waltham, USA) with the column mentioned above. Source operating conditions were: 3 kV spray voltage; 320 °C heated capillary temperature; 400 °C auxiliary gas temperature; sheath, sweep and auxiliary gas (nitrogen) flow rates were at 50, 10 and 2 arbitrary units, respectively; collision cell was used in stepped nCE mode with ionisation voltage between 10 and 50 arbitrary units. Full scan data were obtained at a resolution of 70 000. Data were processed using Xcalibur software (Thermo Fisher Scientific Inc., MA, USA). The identification of all compounds described was carried out using the negative ionisation mode.

For both analyses, the mobile phase was a mixture of 0.1% (v:v) formic acid in water (phase A) and 0.1% (v:v) formic acid in acetonitrile (phase B). The gradient of phase A was 100% (0 min), 80% (10 min), 73% (35 min), 0% (40–50 min) and 100% (51–60 min). The flow rate was 0.8 mL/min, and the injection volume was 5 µL.

Mass spectrum data were registered in a standard Excel format. These data included the exact mass of each compound, the mass M+H, the mass M-H, the mass in M+NH<sub>4</sub>, the mass in M-COOH, the mass M+Na and the experimental mass (Aubert et al., 2019; Gainche et al., 2021). Each compound detected was characterized. Its potential raw formula was then calculated using Xcalibur software (Thermo Fisher Scientific, Inc.), which takes into account its relative abundance, its molar masses and its retention time (Aubert et al., 2019; Gainche et al., 2021). Literature data and the website <https://pubchem/> were then used to determine the name of the compound (Shakeri et al., 2016).

### Preparation of plant extracts for antimicrobial activities

The protocol used is that recommended by Zirihi et al. (2003). A stock solution of extract of 2.5 mg/mL concentration was prepared in a volume of 5 mL of 8.5 g/L NaCl solution. The mixture obtained was homogenized in a vortex, then filtered through a filter membrane with a porosity of 0.2 µm (CHU-PS, 2003).

For antimicrobial activity, the standard antibiotics chosen was Gentamicin. The microbial inoculum was prepared from a pure 18-24 hours' culture on standard agar. This microbial suspension was adjusted to that of a standard solution using a densitometer (density 0.5 on the Mc Farland scale) containing  $1.5$  to  $2 \times 10^8$  CFU/ml and diluted to 1/100 for the saline dilution test (Andrews, 2001). The MICs and MBCs were then determined. The MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, the MBC as the lowest concentration of an antimicrobial that will prevent the growth of an organism after subculture onto antibiotic-free media (Andrews, 2001).

### Determination of the minimum inhibitory concentrations (MICs)

The method used is that of the plate described by Newton et al. (2002). A volume of 100µL of Mueller Hinton broth was introduced into the wells of a 96-well plate as indicated by other authors (Andrews, 2001; Newton et al., 2002; Naïtali and Brissonnet, 2017). In the wells of columns 1, 2 and 3 corresponding to the replications, a series of 100 µl of stock solution was introduced. These were diluted extract solutions, ratio 2, to obtain a range of nine concentrations from 1.25 to 0.0045 mg/mL.

From a 24-hours young culture on sloped agar medium, a microbial suspension of each germ was prepared in sterile physiological water (8.5% NaCl) by adjusting the turbidity to a 0.5 Mac Farland solution (0.5 mL of 0.0048M BaCl<sub>2</sub> (1.17% w/v BaCl<sub>2</sub> · 2H<sub>2</sub>O) added to 99.5mL of 0.18M H<sub>2</sub>SO<sub>4</sub> (1% v/v)), i.e.  $1.5 \times 10^8$  CFU/mL for bacteria and  $2 \times 10^8$  CFU/mL for yeast (Andrew, 2001; Kuete, 2010). A first dilution was then made to obtain intermediate solutions respectively at  $10^7$  CFU/mL and  $10^6$  CFU/mL. Then a second dilution to obtain inocula at  $10^6$  CFU/mL and  $10^5$  CFU/mL (CA-SFM/EUCAST, 2021). 100 µl of each inoculum were subsequently introduced into each well, for a final volume of 200 µl per well. The positive control was placed in columns 11 and 12. This positive control, consisting of Gentamicin was prepared under the same conditions as the extract and tested at a concentration of 08 successive dilutions between 50 and 0.39 mg/mL (Houssou et al., 2010). The negative control was made up of a mixture (culture medium + bacterial inoculum in the proportions (1/1; V/V). The wells containing only the culture medium were reserved for sterility control.

Plates were then incubated at 37 °C for 18 to 24 hours. After incubation, microbial growth was revealed using iodinitrotetrazolium chloride (INT). Its principle is based on the capture of protons emitted by dehydrogenase enzymes present on the membrane of living microorganisms (Houssou et al., 2018). 40 µl of the INT solution were thus introduced into each of the wells of the plate. After metabolism of glucose, a change from the medium to pink-red, after 30 min of re-incubation reveals an insufficient concentration of the metabolite to inhibit bacterial growth. In the unstained well and containing the lowest amount of secondary metabolite, the concentration of the extract corresponds to the MIC (Houssou et al., 2018). The MIC is the concentration of the extract from the well not colored in red. The tests were done in triplicate.

#### Determination of the Minimum Bactericidal Concentrations (MBCs)

Tests were carried out just after the determination of the MICs. The protocol used was that recommended by (Naïtali and Brissonnet, 2017; Okou et al., 2018). A volume of 150 µL of sterile medium (Mueller Hinton Broth) was introduced into the wells of another plate, also sterile and different from that of the MICs. Then 50 µl of extract taken from the wells corresponding to the MICs obtained were added. Also 50µL of extract was taken from the wells that preceded those with MIC (ISO standard 20766-1:2006). The sterility checks consisted of 200 µL of Mueller Hinton broth. The positive control consisted of Gentamicin, treated like the extracts. The negative control was the medium without extract. The CMB was represented by the well corresponding to the lowest concentration of extract with no visible microbial growth (Okou et al., 2015).

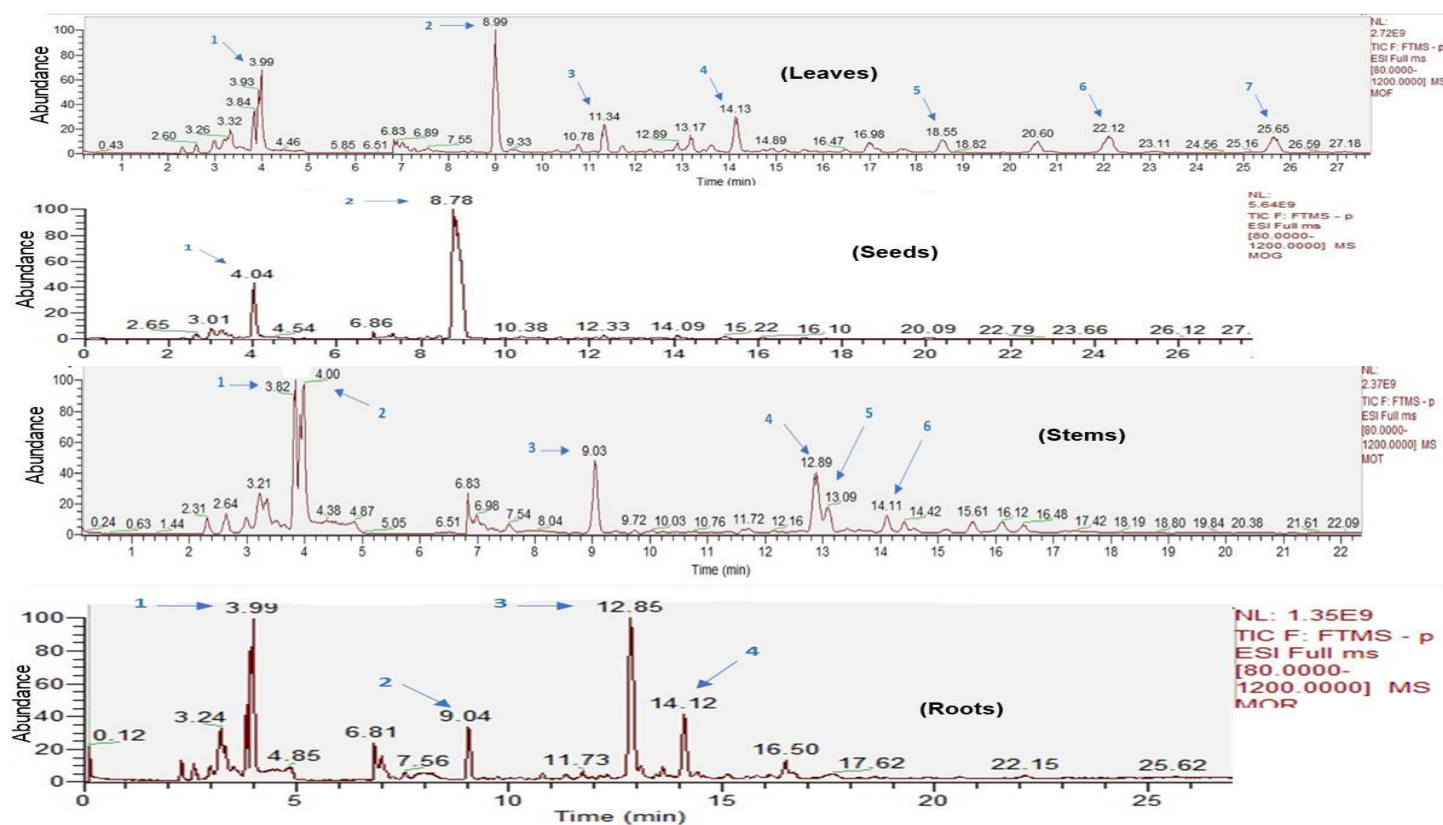
#### Determination of the MBC/MIC ratios

The ratio obtained aimed to conclude whether extracts tested were bacteriostatic or bactericidal. When this ratio is greater than 4, the substance is said to be bacteriostatic. If this ratio is less than 4, the substance is considered to be bactericidal (Abedini, 2013; Okou et al., 2018).

## RESULTS

#### Chromatographic analyses and mass spectral determination

Figure 1 shows the mass spectra obtained of extracts respectively from leaves, seeds, roots and stems of *M. oleifera*. These different profiles were obtained by negative ionization. Many chemical compounds have been identified and are presented in Table 1. Some of them have been identified in several parts of the plant. For example, Saccharose (glycoside) and Glucomoringinin (Glucosinolate) were identified in leaves, seeds, stems and roots extracts. The O-acetylshanzhiside methyl ester was identified in leaves, stems and roots extracts. The Glucotropaeolin was identified in stems and roots extracts. On the other hand, some compounds have only been identified in certain specific parts of the plant. This is for example Neochlorogenic acid, Isoquercetin, Kaempferol-3-O-glucoside and Kaempferol acetyl glucoside which were identified only in leaves extracts. It is the same for Quinic acid and Catechin which were found only in stems extracts (Table 1).



**Figure 1** LC/MS profile of the crude methanol extract of leaves, seeds, stems and roots of *M. oleifera* (spectrum (-))

Compounds identified are of 7 different classes (Table 1). They are heteroside, glucosinolate, phenolic acid, irridoid, 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 irridoid identified was found in leaves, in stems and in 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 only in stems (Table 1).

#### Extract's minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

For each of the 2 bacterial species *E. coli* and *S. aureus*, the mean values of MICs and MBCs obtained with the extracts of each part considered of *M. oleifera* were calculated. They are presented in Figure 2. It is noted that when *E. coli* is present, the MICs varied from 0.0045 mg/mL to 1.25 mg/mL. The lowest value was recorded with the leaves extract, and the greatest with the seed extract. With this bacterium, the MBCs varied from 0.078 to 1.5 mg/mL. The smallest value was also recorded with the leaves extract (Figure 2). With *S. aureus*, the MICs value varied from 0.0195 mg/mL (leaves and seeds) to 0.321 mg/mL (stem and roods). The

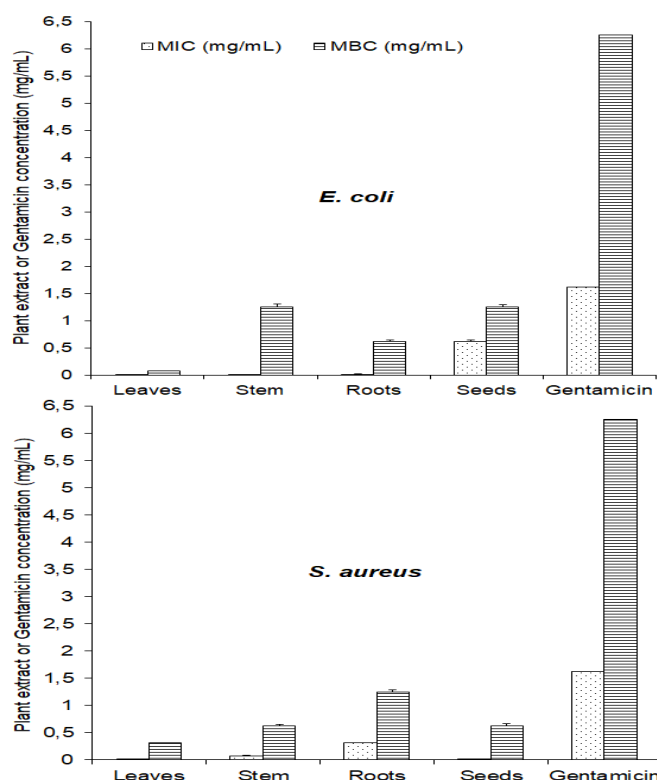
MBCs varied from 0.312 mg/mL (leaves) to 1.25 mg/mL (stem, seeds and roots) (Figure 2).

Gentamicin, which is taken as the reference antibiotic, has a MIC of 1.625 mg/mL and a CMB of 6.25 mg/mL for the 2 bacterial species. These values are higher than those recorded using the extracts of the 4 parts of the plant. Against *E. coli*, the leaf

extract is relatively more effective than extracts from other parts of the plant. And vis-à-vis *S. aureus*, the extracts of the leaves and seeds are relatively more effective than the extracts of the other parts of *M. oleifera*.

**Table 1** Phytochemical profile of MeOH extracts of each part considered of *M. oleifera* (comparison with analytical standard or according literature data)

N°	Rt (min)	Part of the plant	Compound name	Compound formula	M-H <sub>exp</sub> (m/z)	Class of molecule	References
1	3.99	Leaves	Saccharose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	341.1086	Heteroside	Standard
2	8.99	Leaves	Glucomoringinin	C <sub>20</sub> H <sub>29</sub> NO <sub>14</sub> S <sub>2</sub>	570.0956	Glucosinolate	Xiong et al., 2022
3	11.34	Leaves	Neochlorogenic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	353.0875	Phenolic acid	Standard
4	14.13	Leaves	O-acetylshanzhiside methyl ester	C <sub>19</sub> H <sub>28</sub> O <sub>12</sub>	447.1508	Iridoid	Lin et al., 2019
5	18.55	Leaves	Isoquercetin	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	463.0883	Flavonoid	Standard
6	22.12	Leaves	Kaempferol-3-O-glucoside	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	447.0931	Flavonoid heteroside	Standard
7	26.65	Leaves	Kaempferol acetyl glucoside	C <sub>23</sub> H <sub>22</sub> O <sub>12</sub>	489.1037	Flavonoid heteroside	Xu et al., 2019
8	4.04	Seeds	Saccharose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	341.1093	Heteroside	Standard
9	8.78	Seeds	Glucomoringinin	C <sub>20</sub> H <sub>29</sub> NO <sub>14</sub> S <sub>2</sub>	570.0966	Glucosinolate	Xiong et al., 2022
10	3.85	Stems	Quinic acid	C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>	191.0547	Carboxylic acid	Standard
11	4.00	Stems	Saccharose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	341.1046	Heteroside	Standard
12	9.03	Stems	Glucomoringinin	C <sub>20</sub> H <sub>29</sub> NO <sub>14</sub> S <sub>2</sub>	570.0955	Glucosinolate	Xiong et al., 2022
13	12.89	Stems	Glucotropaeolin	C <sub>14</sub> H <sub>28</sub> NO <sub>9</sub> S <sub>2</sub>	408.0426	Glucosinolate	Xu et al., 2019
14	13.09	Stems	Catechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	289.0718	Flavonoid	Standard
15	14.11	Stems	O-acetylshanzhiside methyl ester	C <sub>19</sub> H <sub>28</sub> O <sub>12</sub>	447.1506	Iridoid	Lin et al., 2019
16	3.99	Roots	Saccharose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	341.1087	Heteroside	Standard
17	9.04	Roots	Glucomoringinin	C <sub>20</sub> H <sub>29</sub> NO <sub>14</sub> S <sub>2</sub>	570.0956	Glucosinolate	Xiong et al., 2022
18	12.85	Roots	Glucotropaeolin	C <sub>14</sub> H <sub>28</sub> NO <sub>9</sub> S <sub>2</sub>	408.0426	Glucosinolate	Xu et al., 2019
19	14.12	Roots	O-acetylshanzhiside methyl ester	C <sub>19</sub> H <sub>19</sub> O <sub>12</sub>	447.1507	Iridoid	Lin et al., 2019



**Figure 2** Variation of the MICs and MBCs values of leaves, seeds, stem and roots extracts of *M. oleifera* and those of Gentamicin, against the bacteria *E. coli* and *S. aureus*

**Bactericidal or bacteriostatic activity of each extract**

From the values of the MBC/MIC ratios, the antibacterial properties of each extract on each of microorganisms considered were determined. These different properties are presented in Table 2. It emerges from this that against *E. coli*, the leaves, stems and roots extracts are bacteriostatic whereas those from seeds are bactericidal. Against *S. aureus*, the seeds, stems and roots extracts are bacteriostatic whereas those from leaves are bactericidal. The bactericidal or bacteriostatic properties of the extract varied depending on the part of the plant and the microorganism considered (Table 2).

**Comparison amongst the extracts MICs and MBCs**

A comparison of the MICs and MBCs among the extract from the 4 parts of the plant extracts for each bacterial species was made. It has been noted that the MBCs of both bacteria varied significantly (P<0.05) from one part of the plant to another. However, MICs do not differ significantly from one part of the plant to another (P>0.05) (Table 3).

**Table 2** Effect of the extract of each part (leaves, seeds, stem and roots) of the plant *M. oleifera* against each of the microorganism used

Microorganism used	Nature of the extracts activity			
	Leaves	Seeds	Stem	Roots
<i>E. coli</i>	Bs	Bc	Bs	Bs
<i>S. aureus</i>	Bc	Bs	Bs	Bs

**Legend :** Bc – bactericidal, Bs - bacteriostatic



**Table 3** “P” values of the MIC and MBC values of the 4 parts of the plant Comparison for each considered bacteria species

Bacteria species	Extracts concentrations	
	MIC	MBC
<i>E. coli</i>	P= 0.146	P=0.036*
<i>S. aureus</i>	P= 0.089	P= 0.023*

\* : Significant difference (P < 0.05)

## DISCUSSION

This study showed that the magnitude of the extract’s activity varies according to the part of the plant used as well as according to the germ present. This activity would result from various interactions between the chemical compounds contained in the extract and the microorganisms present. Most of the chemical compounds found in this study were also isolated in the same plant extracts by other authors (Kuethe, 2010, 2017; Willams, 2019; Xiong et al., 2022). These molecules (quinic acid, isoquercetin, glucomoringin, neochlorogenic acid, O-acetylshanzhiside methyl ester) have demonstrated antimicrobial effects.

Glucomoringin has been found in most parts of the plant studied. The antibiotic and antiviral activity of this glucosinolate on *S. aureus* and H1N1 virus respectively, has been reported (Galuppo et al., 2013; Xiong et al., 2022). Its bioactivation with myrosinase enzyme leads to the increasing power of the compound (Galuppo et al., 2013). Some glucosinolates as those from the plants of genus *Brassica* can be converted into bioactive compounds known to induce phase II enzymes which may decrease the risk of cancers (Mullaney et al., 2013). Interactions between glucosinolates and most bacteria can result in various metabolites produced (Sikorska-Zimny and Beneduce, 2021). According to Nurul Ashikin Abd et al. (2016) and Maina et al. (2020), the chemopreventive effects of *M. oleifera* are expected due to the existence of glucosinolates which has the inhibition ability of carcinogen activation and induction of carcinogen detoxification, anti-inflammatory, and anti-tumor cells proliferation (Nurul Ashikin Abd et al., 2016; Maina et al., 2020). The availability and bioactivity of those glucosinolates are directly proportional to their contents at the source, which may be affected by the methods of processing and extraction (Maina et al., 2020). O-acetylshanzhiside methyl ester which is an iridoid has been detected in leaves, stems and roots extracts. Cytotoxic activities of iridoids against cancer cell lines have also been indicated (Liu et al., 2017). Those isolated from *Nepeta* species have been indicated as displaying activity against food-borne pathogens such as *E. coli* and *S. aureus* with MIC varying from 0.0050 to 0.2 mg/mL and from 0.02 to 0.2 mg/mL respectively, and with MBC varying from 0.01 to 0.4 mg/mL and from 0.04 to 0.4 mg/mL respectively (Anicic et al., 2021). Although the MBCs obtained by these authors were relatively lower for the 2 bacteria compared to the present study, it can be noted that the MICs they obtained for *S. aureus* were relatively higher.

According to some authors, the antibacterial activity of some plants extracts could be related particularly to quercetin or isoquercetin. This flavonoid inhibits the growth of several bacteria (Macedo et al., 2021; Ribeiro et al., 2022). Changes in the cell membrane, inactivation of essential enzymes, and/or modification of the functions of genetic material are some mechanisms that allow phenolic compounds to suppress microbial growth (Kuethe, 2017; Silva et al., 2020; Ribeiro et al., 2022). Flavonoids extracted from *M. oleifera* seed coat also displayed antibiofilm potential against *S. aureus* (Onsare and Arora, 2015). Iridoids and flavonoids detected in 3 different parts (leaves, stems, roots) of 3 plants species of the *Barleria* genus were noted as responsible for the biological activities observed against *Candida albicans*, although the inhibitory activity of leaves extracts was higher than others (Amoo et al., 2011).

Neochlorogenic acid which is a phenolic acid has been found only in leaves extracts. It is a natural polyphenolic compound with antioxidant, antibacterial, antiviral activities, and it also inhibit microglial (the resident innate immune cells that sense pathogens and tissue injury in the central nervous system) activation (Kim et al., 2015). During experiments on the antibacterial activity of extracts obtained from the flowering herbs of *Carduus acanthoides*, Kozyra et al (2017) noted that the diameter of growth inhibition of *S. aureus* due to phenolic acids extracts was relatively higher than that of *E. coli* and other *Staphylococcus* species. Other authors working on antibacterial activity of *Xanthoxylum bungeanum* leaves against *S. aureus* measured a MIC of 5 mg/mL due to neochlorogenic acid (Chang et al., 2018). The antimicrobial activity of phenolic compounds might be due to their ability to form a complex with bacterial cell wall thus inhibiting the microbial growth (Kozyra et al., 2017). The inhibitory activity against different  $\beta$ -lactamases produced by bacteria and which are the key enzymes that disable the common

antibiotics has also been indicated (Bouarab-Chibane et al., 2019; Fast and Sutton, 2013).

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 (Xie et al., 2015; Ngoufack Tagousop et al., 2018; Bouarab-Chibane et al., 2019; Biharee et al., 2020). Against Gram positive bacteria, lipophilicity seems to be a key factor for flavonoids and the cell membrane seems to be the main site of flavonoids which likely involves the damage of phospholipid bilayers, the inhibition of the respiratory chain or the ATP synthesis, or some others (Yuan et al., 2021). Thurow (2012) noted that chlorogenic and neochlorogenic acids found in prune have anticarcinogenic effects on the human adenocarcinoma, Caco-2 cell line (a type of cancer that develops in the glands lining organs like, breast, stomach, prostate, lung, pancrea and colon (Bernstein, 2021). They inhibit cells proliferation and their activities result in carcinogen uptake and metabolism modification, and changes in cell morphology and surface properties.

Quinic acid is the only carboxylic acid found. It was isolated only in stem extracts. It is a natural organic acid with potent antibacterial activity against *S. aureus*. It decreases the intracellular pH, reduced succinate dehydrogenase activity and caused a significant decrease in intracellular ATP concentration. It could remarkably reduce the DNA content of *S. aureus* and directly interacts with genomic DNA (Bai et al., 2018).

Chemicals compounds found in extracts tested act differently against most microorganisms or by mechanisms similar to those of the other compounds above. According to Nourbakhsh et al. (2021), targeting specific phospholipids of bacterial membrane by natural product has the potential of being effective against both Gram-positive and Gram-negative bacteria. A highly important stage in the mechanism of activity of these natural compounds involved their incorporation into membranes, leading to change in membrane integrity, permeability, and causing loss of vitally important cellular material (Nourbakhsh et al., 2021).

It is indicated that most of compounds found in extracts from various parts of the plant and which have natural antibacterial activities are composed of a variety of molecules. Those molecules could have varied intensity activities among themselves (Bouarab-Chibane et al., 2019; Kabeer Farha et al., 2020; Lee et al., 2021). It is also indicated that the microorganisms considered in this study, despite the common constitution of their wall, have different metabolic pathways and resistance systems (Reygaert, 2018; Ghannoum and Rice, 2020; Bhattacharya et al., 2020). This would partly explain the variations in recorded MICs and MBCs, as well as the variation in the effect of activity of each extract from one microorganism to another. In addition, there was variation in the abundance of the same compounds from one part of the plant to another.

The two bacterial species considered in this study (*E. coli* and *S. aureus*) have in return appropriate mechanisms to fight against molecules with antimicrobial purpose. *S. aureus* resistance can either develop by horizontal transfer of resistance determinants encoded by mobile genetic elements via plasmids, transposons and the staphylococcal cassette chromosome or by mutations in chromosomal genes (Pantosti et al., 2007; Foster, 2017). Horizontally acquired resistance can occur by one of the following mechanisms: (i) enzymatic drug modification and inactivation, (ii) enzymatic modification of the drug binding site, (iii) drug efflux, (iv) bypass mechanisms involving acquisition of a novel drug-resistant target, (v) displacement of the drug to protect the target. Acquisition of resistance by mutation can result from (i) alteration of the drug target that prevents the inhibitor from binding, (ii) derepression of chromosomally encoded multidrug resistance efflux pumps and (iii) multiple stepwise mutations that alter the structure and composition of the cell wall and/or membrane to reduce drug access to its target (Pantosti et al., 2007; Foster, 2017; Guo et al., 2020). *E. coli* has evolved different mechanisms of resistance against antimicrobial compounds. These include (a) Inactivation of antimicrobial compounds of the beta-lactams class by the production of beta-lactamases; (b) decreased penetration of the antimicrobial compounds to the target site; (c) alteration of target site penicillin-binding proteins; and (d) efflux from the periplasmic space through specific pumping mechanism by the production of a group of enzymes referred as the “ $\beta$ -lactamases” (Allocati et al., 2013; Galindo-Méndez, 2020).

The saccharose has been found in the four considered parts of the *M. oleifera*. It can be benefit to the growth of bacteria. Although it has been suggested by Nigam (2015) that drug transporters may be part of a larger system of remote communication (‘remote sensing and signalling’) between cells, the origin of some differences noted in MICs and MBCs amongst extracts from different parts of *M. oleifera* could be linked to the presence of this carbohydrate. In addition, the use

of crude *M. oleifera* in antimicrobial treatment would come from the degradation of the powder. As the powder degrades there will be an increase in the concentration of nutrients in the medium, since many parts of this plant are of high protein content (Lewerenz et al., 2020). This increase in nutrient concentration has been shown to support the regrowth of surviving bacteria (Williams, 2019). This would partly explain the variations in recorded MICs and MGCs, as well as the variation in the effect of activity of each extract between the two microorganisms.

## CONCLUSION

Various classes of chemical compounds having antimicrobial effects are found in the plant species *Moringa oleifera*. The MICs and MBCs varied from one part of the plant extract to another. Against *E. coli*, the leaves, stems and roots extracts are bacteriostatic whereas those from seeds are bactericidal. Against *S. aureus*, the seeds, stems and roots extracts are bacteriostatic whereas those from leaves are bactericidal. This would be linked in part to the diversity of molecules with antimicrobial effects in various parts of the plant, to the variation of their concentrations from one part of the plant to another, as well as to the resistance properties of each microorganism considered. The extract from each part of the plant could therefore be used for a specific antimicrobial purpose. Additional antibacterial tests using purified compounds will help to identify active molecule(s) responsible for the observed activities of the extract of each part of the plant.

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