



HAL
open science

Minimum inhibition and bacterial concentrations of the plant *Moringa Oleifera* extracts against the bacteria *Escherichia coli* and *Staphylococcus aureus*

Claire Stéphane Metsopkeng, Mireille Ebiane Nougang, Antoine Tamsa Arfao, Fanny Perrière, Luciane Marlyse MOUNGANG, Olive Vivien Noah Ewoti, Florence Donnadiou, Paul Alain Nana, Geneviève Bricheux, Jean Samuel Eheth, et al.

► To cite this version:

Claire Stéphane Metsopkeng, Mireille Ebiane Nougang, Antoine Tamsa Arfao, Fanny Perrière, Luciane Marlyse MOUNGANG, et al.. Minimum inhibition and bacterial concentrations of the plant *Moringa Oleifera* extracts against the bacteria *Escherichia coli* and *Staphylococcus aureus*. *Bacterial Empire*, In press, 10.36547/be.484 . hal-03865237

HAL Id: hal-03865237

<https://hal.science/hal-03865237>

Submitted on 23 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Claire Stéphane Metsopkeng¹, Mireille Ebiane Nougang², Antoine Tamsa Arfao¹, Fanny Perrière³, Luciane Marlyse Moungang¹, Olive Vivien Noah Ewoti¹, Florence Donnadiou³, Paul Alain Nana⁴, Geneviève Bricheux³, Jean Samuel Eheth¹, Edith Brunelle Moufao Tammou¹, Isabelle Ripoche⁵, Pierre Chalard⁵, Téséphore Sime-Ngando³, Moïse Nola^{1,*}

Address (es):

¹ University of Yaounde I, Faculty of Sciences, Laboratory of Hydrobiology and Environment, P.O. Box 812 Yaounde, Cameroon.

² Zoology laboratory, Faculty of Sciences, University of Maroua, P.O. Box 814 Maroua, Cameroon.

³ Laboratoire « Microorganismes: Génome et Environnement », UMR CNRS 6023, Université Clermont Auvergne, Campus Universitaire des Cézeaux, 1 Impasse Amélie Murat CS 60026, 63178 Aubière Cedex, France.

⁴ Department of Oceanography and Limnology, Institute of Fisheries and Aquatic Sciences, University of Douala, P.O.Box 7236, Douala, Cameroon.

⁵ ICCF UMR 6296, SIGMA Clermont, 20 avenue Blaise Pascal - TSA 62006, 63178 Aubière Cedex, France.

*Corresponding author: moise.nola@yahoo.com

<https://doi.org/10.36547/be.484>

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, β -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 ± 2 °C) for 30 days. The dried seeds were pulped and the almond also dried at laboratory temperature (25 ± 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+NH₄, 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 2×10^8 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 BaCl₂ (1.17% w/v BaCl₂ · 2H₂O) added to 99.5mL of 0.18M H₂SO₄ (1% v/v)), i.e. 1.5×10^8 CFU/mL for bacteria and 2×10^8 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).

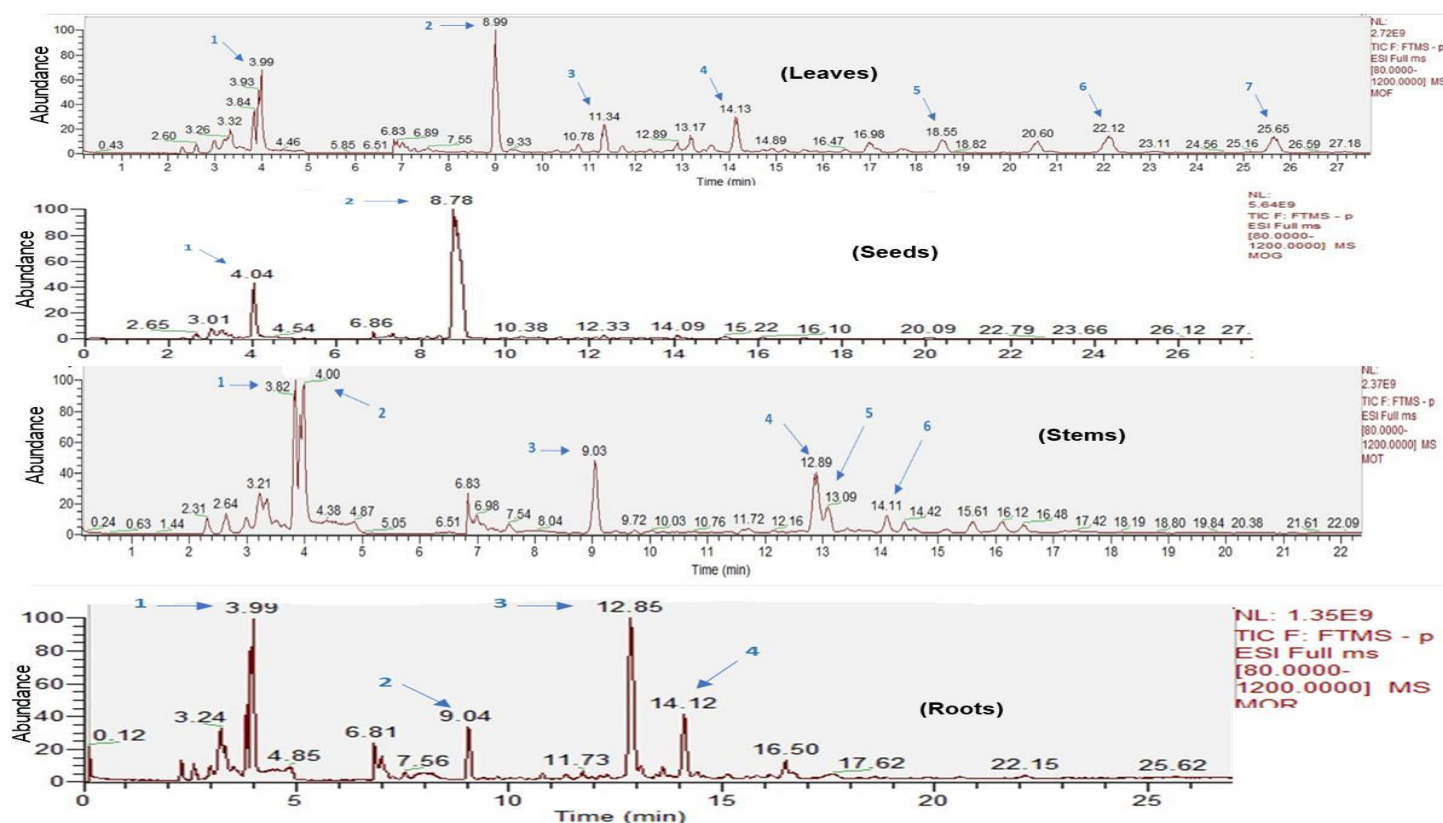


Figure 1 LC/MS profile of the crude methanol extract of leaves, seeds, stems and roots of *M. oleifera* (spectrum (-))

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

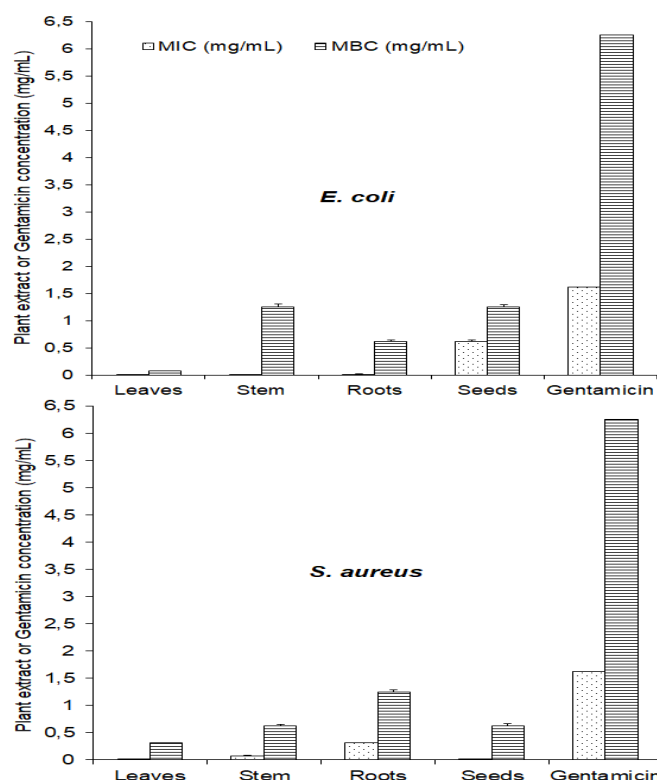


Figure 2 Variation of the MICs and MBCs values of leaves, seeds, stem and roots extracts of *M. oleifera* and those of Gentamicin, against the bacteria *E. coli* and *S. aureus*

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 in 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 β -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 “ β -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.

REFERENCES

- Abedini, A. (2013). Evaluation biologique et phytochimique des substances naturelles d'*Hyptis atrorubens* Poit. (Lamiaceae), sélectionnée par un criblage d'extraits de 42 plantes. Thèse Université du Droit et de la Santé-Lille II, France 211 p.
- Al-Mayah, W. Th., Shawardi, A. O., & Salman R. M. (2017). Microbiological quality assessment of drinking water at Al-Hayy City, Southern Iraq. *Wasit Journal for Science & Medicine*, 10(2), 20-38.
- Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). *Escherichia coli* in Europe: An Overview. *International Journal of Environmental Research and Public Health*, 10, 6235-6254. <https://doi.org/10.3390/ijerph10126235>
- Amoo, S. O., Ndhala, A. R., Finnie, J. F., & Van Staden, J. (2011). Antifungal, acetylcholinesterase inhibition, antioxidant and phytochemical properties of three *Barleria* species. *South African Journal of Botany*, 77, 435-445. <https://doi.org/10.1016/j.sajb.2010.11.002>
- Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of antimicrobial chemotherapy*, 48 (Suppl. S1), 5-16.
- Ani, E., Amove, J., & Igbadul, B. (2018). Physicochemical, microbiological, sensory properties and storage stability of plant-based yoghurt produced from *Bambaranut*, *Soybean* and *Moringa oleifera* Seed Milks. *American Journal of Food and Nutrition*, 6(4), 115-125. [Doi:10.12691/ajfn-6-4-4](https://doi.org/10.12691/ajfn-6-4-4)
- Anicic, N., Gasic, U., Lu, F., Ciric, A., Ivanov, M., Jevtic, B., Dimitrijevic, M., Andelkovic, B., Skoric, M., Nestorovic Zivkovic, J., Mao, Y., Liu, J., Tang, C., Sokovic, M., Ye, Y., & Mistic, D. (2021). Antimicrobial and immunomodulating activities of two endemic *Nepeta* species and their major iridoids isolated from natural sources. *Pharmaceuticals*, 14, 414. <https://doi.org/10.3390/ph14050414>
- Arora, D. S., Onsare, J.G., Kaur, H. (2013). Bioprospecting of *Moringa* (Moringaceae): Microbiological perspective. *Journal of Pharmacognosy and Phytochemistry* 6(1), 193-215.
- Aubert, P., Guinobert, I., Blondeau, C., Bardot, V., Ripoche, I., Chalard, P., & Neunlist M., 2019. Basal and spasmolytic effects of a hydroethanolic leaf extract of *Melissa officinalis* L. on intestinal motility: An ex vivo study. *Journal of Medicinal Food*, 22 (7), 653-662. <https://doi.org/10.1089/jmf.2018.0154>
- Bai, J., Wu, Y., Zhong, K., Xiao, K., Liu, L., Huang, Y., Wang, Z., & Hong, G. H. (2018). A comparative study on the effects of quinic acid and shikimic acid on cellular functions of *Staphylococcus aureus*. *Journal of Food Protection*, 81(7), 1187-1192. <https://doi.org/10.4315/0362-028x.jfp-18-014>
- Bautista-Trujillo, G. U., Solorio-Rivera, J. L., Renteria-Solorzano, I., Carranza-German, S. I., Bustos-Martinez, J. A., Arteaga-Garibay, R. I., Baizabal-Aguirre, V. M., Cajero-Juarez, M., Bravo-Patino, A., & Valdez-Alarcon, J. J. (2013). Performance of culture media for the isolation and identification of *Staphylococcus aureus* from bovine mastitis. *Journal of Medical Microbiology*, 62, 369-376. <https://doi.org/10.1099/jmm.0.046284-0>
- Bernstein, S. (2021). Adenocarcinoma. <https://www.ncbi.nlm.nih.gov/pubmed/35111111> (accessed 2nd may 2022)
- Bhattacharya, S., Sae-Tia, S., & Frries, B. C. (2020). Candidiasis and mechanisms of antifungal resistance. *Antibiotics*, 9(6), 312. <https://doi.org/10.3390/antibiotics9060312>
- Biharee, A., Sharma, A., Kumar, A., & Jaitak, V. (2020). Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. *Fitoterapia*, 146, 104720. <https://doi.org/10.1016/j.fitote.2020.104720>
- Biswas, D., Nandy, S., Mukhejee, A., Pandey, D. K., & Dey, A. (2020). *Moringa oleifera* Lam. and derived phytochemicals as promising antiviral agents: A review. *South African Journal of Botany*, 129, 272-282. <https://doi.org/10.1016/j.sajb.2019.07.049>
- Bouarab-Chibane, L., Forquet, V., Lantéri, P., Clément, Y., Léonard-Akkari, L., Oulhal, N., Degraeve, P., & Bordes, C. (2019). Antibacterial properties of polyphenols: characterization and QSAR (quantitative structure-activity relationship) models. *Frontiers in Microbiology*, 10, 829. <https://doi.org/10.3389/fmicb.2019.00829>
- Chang, S. Y., Zhang, J. Q., Zhong, K., Grosu, E., Gao, Z., Wu, Y. P., & Gao, H. (2018). Antibacterial and antibiofilm effects of *Zanthoxylum bungeanum* leaves against *Staphylococcus aureus*. *NPC Natural Product Communications*, 13(8), 1001-1006.
- CA-SFM/EUCAST, 2021. Comité de l'Antibiogramme (CA-SFM) de la Société française de Microbiologie./European committee on antimicrobial susceptibility testing. Recommandations 2021, Vol 1.0. Jehl F, edit., Strasbourg.
- CHU-PS. (2003). Bactériologie DCEM1. Université Paris-VI Pierre et Marie Curie, Faculté de Médecine Pitié-Salpêtrière. Service de Bactériologie, 122 pages.
- Elgamily, H., Moussa, A., Elborae, A., El-Sayed, H., Al-Moghazy, M., & Abdalla, A. (2016). Microbiological assessment of *Moringa Oleifera* extracts and its incorporation in novel dental remedies against some oral pathogens. *Open Access Macedonian Journal of Medical Sciences*, 4(4), 585-590. <https://doi.org/10.3889/oamjms.2016.132>
- Fast, W., & Sutton, L. D. (2013). Metallo-beta-lactamase: Inhibitors and reporter substrates. *Biochimica Et Biophysica Acta-Proteins and Proteomics*, 1834(8), 1648-1659. <https://doi.org/10.1016/j.bbapap.2013.04.024>
- Foster, T. J. (2017). Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. *FEMS Microbiology Reviews*, 41(3), 430-449. <https://doi.org/10.1093/femsre/fux007>
- Fuglie, L. J. (2001). Combating malnutrition with Moringa. Pp. 117-136 in The miracle tree: the multiple attributes of Moringa. Lowell Fuglie, J. ed. CTA Publication, Wageningen, the Netherlands
- Gainche, M., Ogeron, C., Ripoche, I., Senejoux, F., Cholet, J., Decombat, C., Delort, L., Berthon, J.-Y., Saunier, E., Caldefie Chezet, F., & Chalard, P. (2021). Xanthine oxidase inhibitors from *Filipendula ulmaria* (L.) Maxim. and their efficient detections by HPLC and HPLC analyses. *Molecules*, 26, 1939. <https://doi.org/10.3390/molecules26071939>
- Galindo-Méndez, M. (2020). Antimicrobial Resistance in *Escherichia coli*. In *E. coli infections - Importance of early diagnosis and efficient treatment*. Rodrigo L. edit., *Oviedo*. [Doi:10.5772/intechopen.93115](https://doi.org/10.5772/intechopen.93115)
- Galuppo, M., De Nicola, G. R., Iori, R., Utri, P. D., Bramanti, P., & Mazzou E. (2013). Antibacterial activity of glucomoringin bioactivated with myrosinase against two important pathogens affecting the health of long-term patients in hospitals. *Molecules*, 18, 14340-14348. <https://doi.org/10.3390/molecules181114340>
- Ghannoum, M.A., & Rice, L. B. (2020). Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. *ASM Journals_Clinical Microbiology Reviews*, 12(4). <https://doi.org/10.1128/CMR.12.4.501>
- Gorniak, I., Bartoszewski, R., & Krolczewski, J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews*, 18, 241-272. <https://doi.org/10.1007/s11101-018-9591-z>
- Guo, Y., Song, G., Sun, M., Wang, J., & Wnag, Y. (2020). Prevalence and therapeutic of antibiotic-resistance in *Staphylococcus aureus*. *Frontiers in Cellular and Infection Microbiology*, 10, Article 107. <https://doi.org/10.3389/fcimb.2020.00107>
- Hala, M. N. T., Azza, A. E., Gamelat, K. F., Doaa, A. M., & Hend, A. E. (2022). Immunological effect of *Moringa Oleifera* leaf extract on vaccinated and non-vaccinated Hubbard chickens experimentally infected with Newcastle virus. *Saudi Journal of Biological Sciences*, 29(1), 420-426. <https://doi.org/10.1016/j.sjbs.2021.09.012>
- Hamilton, K., Reyneke, B., Waso, M., Clements, T., Ndlovu, T., Khan, W., DiGiovanni, K., Rakestraw, E., Montalto, F., Haas, C. N. & Ahmed W. (2019). A global review of the microbiological quality and potential health risks associated with roof-harvested rainwater tanks. *npj Clean Water*, 2:7. <https://doi.org/10.1038/s41545-019-0030-5>

- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (2000). *Bergey's manual of determinative bacteriology*. Lipponcott Williams and Wilkins, Philadelphia.
- Houssou, R., Fatondji, F., Gbaguidi, Kpoviessi, S., Sonounameto, E., Latifou, L., Sanni, A., Moudachirou, M. J. P., & Accrombessi, G. C. (2010). Synthesis, characterisation and study of antimicrobial activities of the semicarbazone and the thiosemicarbazone of the carvone. *Journal of Society Ouest-Africa Chemistry*, 30, 11-17.
- Houssou, R. F., Kpoviessi, S., Gbaguidi, F., Chabi, S. K., Gbenou, J., Coffi, G., Accrombessi, Moudachirou, M., & Poupaert, J. (2018). Synthesis, characterization and antimicrobial properties of Some 1,3,4-thiadiazolines. *American Journal of Applied Chemistry*, 6(2), 64-70. <https://doi.org/10.11648/j.ajac.20180602.15>
- Kabeer Farha, A., Yang, Q. Q., Kim, G., Li, H. B., Zhu, F., Liu, H. Y., Gan, R. Y., & Corke, H. (2020). Tannins as an alternative to antibiotics. *Food Bioscience*, 38, 100751. <https://doi.org/10.1016/j.fbio.2020.100751>
- Kim, M., Choi, S.-Y., Lee, P., & Hur, J. (2015). Neochlorogenic acid inhibits lipopolysaccharide-induced activation and pro-inflammatory responses in BV2 microglial cells. *Neurochemical Research*, 40(9), 1792-8. <https://doi.org/10.1007/s11064-015-1659-1>
- Kozyra, M., Biernasiuk, A., & Malm, A. (2017). Analysis of phenolic acids and antibacterial activity of extracts obtained from the flowering herbs of *Carduus acanthoides* L. *Acta Poloninae Pharmaceutica-Drug Research*, 74(1), 161-172.
- Kuete, V. (2010). Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Medica*, 76, 1479-1491. [Doi.org/10.1371/journal.pone.0078085](https://doi.org/10.1371/journal.pone.0078085)
- Kuete, V. (2017). *Medicinal spices and vegetables from Africa: Therapeutic potential against metabolic, inflammatory, infectious and systemic diseases*. Academic Presse edit.
- Kurosu, M., Siricilla, S., & Mitachi, K. (2013). Advances in MRSA drug discovery: where are we and where do we need to be? *Expert Opinion on Drug Discovery*, 8, 1095-1116. <https://doi.org/10.1517/17460441.2013.807246>
- Latifa, A. A. H., & Muneera, D. F. A. (2016). Impact of *Moringa* aqueous extract on pathogenic bacteria and fungi in vitro. *Annals of Agricultural Science*, 61, 247-250. <http://dx.doi.org/10.1016/j.aaos.2016.06.003>
- Laxminarayan, R., Matsuoka, P., Pant, S., Brower, C., Rottingen, J. A., Klugman, K., & Davies, S. (2016). Access to effective antimicrobials: a worldwide challenge. *Lancet*, 387(10014), 168-75. [https://doi.org/10.1016/s0140-6736\(15\)00474-2](https://doi.org/10.1016/s0140-6736(15)00474-2)
- Lee, Y., Puumala, E., Robbins, N., & Cowen, L. E. (2021). Antifungal Drug Resistance: Molecular Mechanisms in *Candida albicans* and beyond. *Chemical Reviews*, 121, 6, 3390-3411. <https://doi.org/10.1021/acs.chemrev.0c00199>
- Lewerenz, L., Heinrichs, H., Hornbacher, J., Papenbrock, J., Nguyen-Thanh, B., & Selmar, D. (2020). Protein content and glucosinolates from *Moringa oleifera* Lam. -New insights into an auspicious commodity. *Journal of Applied Botany and Food Quality*, 93, 257-265. [Doi :10.5073/JABFQ.2020.093.032](https://doi.org/10.5073/JABFQ.2020.093.032)
- Lin, H., Zhu, H., Tan, J., Wang, H., Wang, Z., Li, P., Zhao, C., & Liu, J. (2019). Comparative analysis of chemical constituents of *Moringa oleifera* leaves from China and India by Ultra-Performance Liquid Chromatography coupled with Quadrupole-Time-Of-Flight Mass Spectrometry. *Molecules*, 24(5). <https://doi.org/10.3390/molecules24050942>
- Liu, Y. H., Wu, P. Q., Hu, Q. L., Pei, Y. J., Qi, F. M., Zhang, Z. X., & Fei, D. Q. (2017). Cytotoxic and antibacterial activities of iridoids and sesquiterpenoids from *Valeriana jatamansi*. *Fitoterapia*, 123, 73-78. <https://doi.org/10.1016/j.fitote.2017.09.011>
- Macedo, S. K. S., Almeida, T. S., Alencar Filho, J. M. T., Lima, K. S. B., Liborio, R. C., Costa M.M., Rolim Neto, P. J., Rolim, L. A., & Nunes, X. P. (2021). Phytochemical identification and quantification of quercetin in *Triplaris gardneriana* wedd. Leaves by HPLC-DAD with of antimicrobial activity. *Natural Products Research*, 35(18), 3083-3088. <https://doi.org/10.1080/14786419.2019.1682573> Epub 2019 Nov 3. PMID : 31680555.
- Maina, S., Misinzio, G., bakari, G., & Kim, H. Y. (2020). Human, animal and plant health benefits of glucosinolates and strategies for enhanced bioactivity : A systematic review. *Molecules*, 25, 3682. <https://doi.org/10.3390/molecules25163682>
- Mallenakuppe, R., Homabalegowda, H., Demappa Gouri, M., Basavaraju, P. S., & Chandrashekharaiah, U. B. (2019). History, taxonomy and propagation of *Moringa oleifera*-A Review. *SSR Institute of International Journal of Life Sciences*, 5(3), 2322-2327. <https://doi.org/10.21276/ssr-ijls.2019.5.3.7>
- Matic, I., Guidi, A., Kenzo, M., Mattei, M., & Galgani, A. (2018). Investigation of medicinal plants traditionally used as dietary supplements: A review on *Moringa oleifera*. *Journal of Public Health in Africa*, 9:841. <https://doi.org/10.4081/jphia.2018.841>
- Mechergui, A., Achour, W., Mathlouthi, S., & Ben Hassen, A. (2019). Prevalence of infectious multi-drug resistant bacteria isolated from immunocompromised patients in Tunisia. *African Health Sciences*, 19(2), 2021-2025. <https://dx.doi.org/10.4314/ahs.v19i2.25>
- Mellon, F. A., Bennett, R. N., Holst, B., & Williamson, G. (2002). Intact glucosinolate analysis in plant extracts by programmed cone voltage Electrospray LC/MS: Performance and comparison with LC/MS/MS methods. *Analytical Biochemistry*, 306, 83-91. <https://doi.org/10.1006/abio.2002.5677>
- Metsopkeng, C. S., Lontsi Djimeli, C., Noah Ewoti, O. V., Mounang, L. M., Nana, P. A., Tamsa Arfao, A., Ngo Bahebeck, P., Bricheux, G., Sime-Ngando, T., & Nola, M. (2019). *Moringa oleifera* seeds extract activity on Enteropathogenic *Escherichia coli* and *Aeromonas hydrophila* cells in aquatic microcosm. *Journal of Applied Biotechnology*, 7(2), 13-30. <https://doi.org/10.5296/jab.v7i2.14917>
- Metsopkeng, C. S., Nougang, M. E., Nana, P. A., Tamsa Arfao, A., Ngo Bahebeck, P., Lontsi Djimeli, C., Eheth, J. S., Noah Ewoti, O. V., Mounang, L. M., Agbor, G. A., Perrière, F., Sime-Ngando, T., & Nola, M. (2020). Comparative study of *Moringa stenopetala* root and leaf extracts against the bacteria *Staphylococcus aureus* strain from aquatic environment. *Scientific African*, 10, e00549. <https://doi.org/10.1016/j.sciaf.2020.e00549>
- Mullaney, J. A., Mcghi, T., Ansel, J., & Kelly, W. J. (2013). Lactic acid bacteria convert glucosinolates to nitriles efficiently yet differently from Enterobacteriaceae. *Journal of Agricultural and Food Chemistry*, 61(12). <https://doi.org/10.1021/jf305442j>
- Naah, E. (1990). *Hydrologie du grand Yaéré du Nord Cameroun*. Thèse de Doctorat ès sciences, Université de Yaoundé, Cameroun.
- Naitali, M., & Dubois-Brissonnet, F. (2017). *Développement des microorganismes pathogènes dans les aliments*. In: Risques microbiologiques alimentaires. Paris, France : Lavoisier. pp. 37-86.
- Newton, S. M., Lau, C., Gurcha, S. S., Besra, G. S., & Wright, C. W. (2002). The Evaluation of forty-three plant species for in vitro antimycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. *Journal of Ethnopharmacology*, 79, 57-63.
- Ngoufack Tagouss, C., Tamokou, J. D., Endeguele Ekom, S., Ngnokam, D., & Voutquenne Nazabadioko, L. (2018). Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action. *BMC Complementary and Alternative Medicine*, 18:252. <https://doi.org/10.1186/s12906-018-2321-7>
- Nigam, S. K. (2015). What do drug transporters really do? *Nature Reviews Drug Discovery*, 14, 29-44. <https://doi.org/10.1038/nrd4461>
- Nourbakhsh, F., Lotfalizadeh, M., badpeyma, M., Shakeri, A., & Soheili, V. (2021). From plants to antimicrobials: natural products against bacterial membranes. *Phytotherapy Research*, 36, 33-52. [Doi:10.1002/ptr.7275](https://doi.org/10.1002/ptr.7275)
- Nurul Ashikin Abd, K., Muhammad Din, I., Saie Brindha, K., Yaya, R., Hazrulizawati Abd, H., & Ahmad Faizal Abdull, R. (2016). *Moringa oleifera* Lam: Targeting chemoprevention. *Asian Pacific Journal of Cancer Prevention*, 17(8), 3675-3686. <https://doi.org/10.14456/apjcp.2016.155/APJCP.2016.17.8.3675>
- Okou, O. C., Ackah, J. A. A. B., Angaman, D. M., & Djaman, A. J. (2015). Activity of *Mitracarpus scaber* on *Enterococcus faecalis*. *World Journal of Pharmaceutical Research*, 4(5): 377-385.
- Okou, O. C., Yapou S. E. S., Kporou, K. E., Baibo, G. L., Monthaut, S., & Djaman, A. J. (2018). Évaluation de l'activité antibactérienne des extraits de feuilles de *Solanum torvum* Swartz (Solanaceae) sur la croissance in vitro de 3 souches d'entérobactéries. *Journal of Applied Biosciences* 122: 12282-12290. <https://dx.doi.org/10.4314/jab.v122i1.8>
- Onsare, J. G., & Arora, D. S. (2015). Antibiofilm potential of flavonoids extracted from *Moringa oleifera* seed coat against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. *Journal of Applied Microbiology*, 118(2), 313-325. <https://doi.org/10.1111/jam.12701>
- Padulosi, S., Hodgkin, T., Williams, J. T., & Haq, N. (2002). Underutilized crops: trends, challenges and opportunities in the 21st Century. In: Managing plant genetic diversity. Engels, J. M. M., Rao, V. R., Brown, A. H. D., & Jackson, M. T. (eds). Wallingford, UK: CAB International Publishing; Rome: International Plant Genetic Resources Institute (IPGRI), pp 323-338.
- Pantosti, A., Sanchini, A., & Monaco, M. (2007). Mechanisms of antibiotic resistance in *Staphylococcus aureus*. *Future Microbiology*, 2(3). <https://doi.org/10.21217/17460913.2.3.323>
- Prasad Sha, S., Suryavanshi, M. V., Jani, K., Sharma, A. Y., & Tamang, J. P. (2018). Diversity of yeasts and molds by culture-dependent and culture-independent methods for mycobiome surveillance of traditionally prepared dried starters for the production of Indian alcoholic beverages. *Frontiers in Microbiology*, 9, Article 2237. <https://doi.org/10.3389/fmicb.2018.02237>

- Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, 4(3), 482-501. <https://doi.org/10.3934/microbiol.2018.3.482>
- Ribeiro, J., Silva, V., Aires, A., Carvalho, R., Barros, L., Gaivao, I., Igrejas, G., & Poeta, P. (2022). *Plantanus hybrida*'s phenolic profile, antioxidant power, and antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). *Horticulturae*, 8, 243. <https://doi.org/10.3390/horticulturae8030243>
- Shakeri, A., Sahebkar, A., & Javadi, B. (2016). *Melissa officinalis* L. A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, 188, 204-228. <https://doi.org/10.1016/j.jep.2016.05.010>
- Sikorska-Zimny, K., & Beneduce, L. (2021). The metabolism of glucosinolates by gut microbiota. *Nutrients*, 13, 2750. <https://doi.org/10.3390/nu13082750>
- Silva, V., Falco, V., Dias, M. I., Barros, L., Silva, A., Capita, R., Allonso-calleja, C., Amaral, J. S., Igrejas, G., Ferreira, I. C. F. R., & Poeta, P. (2020). Evaluation of the phenolic profile of *Castanea sativa* mill. By-products and their antioxidant and antimicrobial activity against multiresistant bacteria. *Antioxidants*, 9(1), 87. <https://doi.org/10.3390/antiox9010087>
- Tsague Manfo, F. P., Akono Nantia, E., & Kuete, V. (2014). Hepatotoxicity and hepatoprotective effects of African medicinal plants. In *Toxicological survey of African Medical Plant*. Kuete, V., Elsevier, ed., pp. 323-355. <https://doi.org/10.1016/B978-0-12-800018-2.00011-X>
- Thurrow, T. (2012). Effect of chlorogenic and neochlorogenic acid on human colon cancer cells. Food Science Undergraduate Honors Theses, University of Arkansas, Fayetteville, <https://scholarworks.uark.edu/fdsuht/2>
- Van Baalen, P., Van Belkum, A., Summerbell, R. C., Crous, P. W., & Thomma, B. P. H. J. (2007). Molecular mechanisms of pathogenicity: how do pathogenic microorganisms develop cross-kingdom host jumps? *FEMS Microbiology Reviews*, 31 (3), 239-277. <https://doi.org/10.1111/j.1574-6976.2007.00065.x>
- Van der Bij, A. K., & Pitout, J. D. (2012). The role of international travel in the worldwide spread of multiresistant Enterobacteriaceae. *Journal of Antimicrobial and Chemotherapy*, 67, 2090-2100. <https://doi.org/10.1093/jac/dks214>
- Williams, F. E. (2019). The efficacy of *Moringa oleifera* as a practical application for sustainable water treatment. Ph.D. thesis, the University of Adelaide, USA, 118 pages.
- Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. *Current Medicinal Chemistry*, 22, 132-149. <https://doi.org/10.2174/0929867321666140916113443>
- Xiong, Y., Shahid Riaz Rajoka, M., Zhang, M., & He Zhendan (2022). Isolation and identification of two new compounds from the seeds of *Moringa oleifera* and their antiviral and anti-inflammatory activities. *Natural Product Research*, 36(4), 974-983. <https://doi.org/10.1080/14786419.2020.1851218>
- Xu, Y. B., Chen, G. L., & Guo, M. Q. (2019). Antioxidant and anti-inflammatory activities of the crude extracts of *Moringa oleifera* from Kenya and their correlations with flavonoids. *Antioxidants*, 8, 296. <https://doi.org/10.3390/antiox8080296>
- Yuan, G., Guan, Y., Yi, H., Lai, S., Sun, Y., & Cao, S. (2021). Antibacterial activity and mechanism of plant flavonoids to gram-positive bacteria predicted from their lipophilicities. *Scientific Reports*, 11:10471. <https://doi.org/10.1038/s41598-021-90035-7>
- Zirihi, G. N., Kra, A. K. M., & Guédé-Guina, F. (2003). Évaluation de l'activité antifongique de *Microglossa pyrifolia* (Lamarck) O. Kantze (Asteracées) «PYMI» sur la croissance in vitro de *Candida albicans*, *Revue de Médecine et Pharmacie Afrique*, 17, 11-18.