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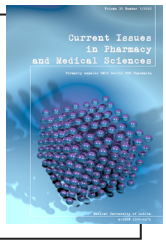

Phytochemical analysis and evaluation of antibacterial activity of *Moringa oleifera* extracts against gram-negative bacteria: an *in vitro* and molecular docking studies

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ABSTRACT

Moringa oleifera seed and leaf are used traditionally for the treatment of various health problems (among others, hypertension, scrapes, skin infection, diabetes, genitourinary illnesses), and to boost the immune system, as well as to act as a contraceptive. In this study, the antibacterial activity of seed and leaf *M. oleifera* extracts on three-gram negative bacteria was investigated, and phytochemical analysis for the association of antibacterial activity with the active constituents in the plant was determined. Moreover, understanding of the mechanism of action was achieved by applying the Auto Dock Vina technique. The phytochemical screening of *M. oleifera* seed and leaf extracts exhibited the presence of alkaloids, carbohydrates, cardioactive glycosides, flavonoids, tannins, phenols, steroids and terpenoids. In silico results revealed that compounds (4-O-caffeoyl quinic acid, 4-(α-L-rhamnopyranosyloxyl)-benzylisothiocyanate); (Isoquercitrin, 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate); and (Astragalin, 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate) from leaf and seed have the highest binding affinity and very good interactions with Transcriptional Activator Protein (LasR), *Klebsiella pneumoniae* carbapenemase (KPC), and Malonyl-CoA-acyl carrier protein transacylase (FabD), respectively.

INTRODUCTION

The increasing occurrence of antibiotic-resistant strains of bacteria and the spread of multidrug-resistance with reduced sensitivity to antimicrobial agents has compelled intensive searches for effective methods to overcome the therapeutic problem, as well as to discover new infection-fighting approaches [1]. One of the strategies to reduce the resistance to antimicrobial agents is to use antibiotic resistance inhibitors such as that found in traditional medicine [2]. Many plant-based agents used in traditional medicine are considered to be potential or actual therapeutic substitutes to antibiotics, and are considered natural, harmless and inexpensive, yet still effective [3]. *Moringa oleifera* or "Drumstick", globally, is a beneficial multifunctional and miracle tree since all parts of the plant are used as food, for water decontamination, medicine, and for a variety of industrial and cosmetic purposes [4]. Drumstick is a small, strong-growing perennial tree that usually grows up to 10 to 12 m in height. According to Amabye and Tadesse [5], the plant is native to the Himalayas region, India, Pakistan, the Pacific and Caribbean Islands, Africa, Arabia, South Asia, and South America. It is traditionally employed in the treatment of hypertension, dysentery, toothache, colitis, scrapes, sores, skin infection, diabetes, anemia and typhoid fever [6].

Drumstick contains various biologically active secondary plant metabolites, among others, flavonoids (Rutin, Quercetin, isoquercetin, Kaempferol, apigenin, Daidzein, Epicatechin, Procyanidins), phenolic acids (Gallic acid, Gentisic acid, Ellagic acid, Ferulic acid, Caffeic acid, p-Coumaric acid, |Chlorogenic acid, Cryptochlorogenic acid), terpenes (13-z-Lutein, 15-z-β-Carotene, E-Zeaxanthin), alkaloids and sterols (Niazimicin, marumoside A, marumoside B, β-sitosterol) [7-9]. Of note, quercetin possesses antioxidant activity 4 to 5 times greater than vitamin E and vitamin C [10].

Research has shown that leaf extracts of *M. oleifera* inhibit the propagation of B16F10 melanoma cells through apoptosis induction at the sub-G1-area and stimulation of cell arrest at the G2/M phase [11]. Moreover, a study

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indicated that hexane and methanol seed extracts of *M. oleifera* displayed pronounced inhibition of waterborne pathogens, chiefly toward *Escherichia coli, Salmonella typhi*, and *Vibrio cholera* [12]. Other research has demonstrated that *M. oleifera* aqueous leaves extract combat hyperglycemia in diabetic rats by elevating insulin levels and reducing insulin resistance [13]. Numerous studies have also revealed that different parts of the plant-like seed, bark, leaf, flower and root, exhibit antimicrobial activity against several pathogens such as enteric *Escherichia coli*, *Vibrio cholerae,* and also others [14,15].

Many Gram-negative bacteria communicate through "quorum sensing" molecules and can regulate pathogenic virulence factor production and antimicrobial resistance. *Pseudomonas aeruginosa* is an opportunistic human pathogen annually responsible for the death of thousands of cystic fibrosis sufferers and many other immunocompromised individuals. Quorum sensing inhibitors (Transcriptional Activator Protein or LasR) can attenuate the pathogenicity of *P. aeruginosa* [1,16]. *Klebsiella pneumoniae* is a Gram-negative bacterium, which is an important causal agent for nosocomial infections. Prolonged exposure to β-lactam antibiotics leads to the development of resistance. The major reason for the β-lactam resistance in *K. pneumoniae* is the secretion of the enzyme *Klebsiella pneumoniae* carbapenemase (KPC) [17]. In *Escherichia coli*, Malonyl-CoA-acyl carrier protein transacylase (FabD) is a key enzyme in the fatty acid biosynthesis pathway, catalyzing the transfer of a malonyl moiety from malonyl-CoA to holo acyl carrier protein (ACP), generating malonyl-ACP and free CoASH. Malonyl-ACP is the product of this reaction and the key building block for *E. coli* [18].

The objective of this study was to screen the phytochemical content responsible for their biological activity, to assess the bactericidal effect of M. oleifera seed and leaf extracts against gram-negative bacteria, and to test the mechanism of action by applying the AutoDock Vina method.

MATERIALS AND METHODS

Plant collection and extract preparation

M. oleifera leaves and seeds were collected from the herbal store in Erbil city. The identity of the plant was authenticated by the Department of Pharmacognosy, College of Pharmacy, Hawler Medical University (Voucher No. 22). Four hundred grams of dried powdered leaves and seeds of M. oleifera were extracted through 1 L ethanol (Chem-Lab, Belgium) using an ultrasonic-assisted extractor (LUC-405, Korea) for 1hr at 40°C. After filtration and drying, the extract was redissolved in 10% ethanol and fractionated by diethyl ether (Chem-Lab, Belgium), and ethyl acetate (Chem-Lab, Belgium) [19,20]. The obtained fractions were stored at 4°C until their usage for phytochemical screening and evaluation of their biological activity.

Phytochemical analysis

The crude diethyl ether and ethyl acetate extracts of leaves and seeds of *M. oleifera* were investigated for the presence of primary and secondary metabolites according to standard phytochemical methods [21-23].

1. Dragendorff's test for alkaloids

For 5 mL extracts, 1 mL of Dragendorff's reagent (Thomas Baker PVT., India) was added. The appearance of an orange-red precipitate confirms the presence of alkaloids.

2. Molish test for carbohydrates

For 5 mL extracts, 2-3 drops of alcoholic α-naphthol (Merck KGaA, Germany) solution and 5 mL of concentrated H_2SO_4 (Chem-Lab, Belgium) along the side of the test tube were added. The appearance of violet rings at the junction confirms the presence of carbohydrates.

3. Foam test for saponins

In a test tube, different parts of plant extracts (0.5 g) were separately shaken with 10 mL distilled water. Following warming, in a water bath, the formation of foaming which persists for 5 min, confirms the presence of saponins.

4. Keller-killiani test for cardioactive glycosides

For 5mL extracts, 5 mL glacial acetic acid (Scharlau Chemie S.A., European Union) containing a few drops of ferric chloride (Thomas Baker PVT., India) was added, followed by 3 mL H_2SO_4 along the side of the test tube. The appearance of a brown ring at the interface and bluish green at the upper layer confirms the presence of cardiac glycoside.

5. Lead acetate test for flavonoids

For 5mL extracts, a few drops of lead acetate solution (Thomas Baker PVT., India) was added. The formation of yellow color precipitate confirms the presence of flavonoids.

6. Ferric chloride test for phenol and tannins

For 5 mL extracts, 3-4 drops of (1%) ferric chloride solution were added. The formation of bluish-black color confirms the presence of phenols.

7. Salkowski test for steroids and terpenoids

In a test tube for 2 ml extracts 2 ml of chloroform (Chem-Lab, Belgium) were added, followed by the addition of concentrated 2 ml H_2SO_4 along the side of the test tube. The appearance of a reddish-brown color at the interface confirms the presence of steroids and terpenoids.

8. Xanthoproteic test for Proteins

For 5mL extracts, a few drops of concentrated nitric acid (Chem-Lab, Belgium) was added. The formation of yellow color confirms the presence of proteins.

Microorganisms

Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 13883 were used in this current study.

Disc diffusion assay

The antimicrobial susceptibility test was conducted by applying the agar disc diffusion method on Muller-Hinton agar. Firstly, the bacterial isolates were suspended in peptone broth and incubated at 37°C for 4 hrs. The turbidity of the broth was then adjusted to 0.5 McFarland. Muller-Hinton

agar plates were subsequently inoculated with various strains of bacteria separately using sterile swabs, then sterilized discs of Whatman filter paper were saturated with different concentrations (50, 100, and 200 mg) of the extracts. These discs were placed on a streaked Muller-Hinton agar plate surface, and were afterwards, incubated at 37°C for 24 hrs. The antimicrobial activity was detected by measuring zones of inhibition [24].

Preparation of proteins and plant extract compounds

In this part of the research, to assess the crystal structures for proteins; Transcriptional Activator Protein (**LasR**) (PDB ID: 2UV0) [25], *Klebsiella pneumoniae* carbapenemase (**KPC**) (PBD ID: 2OV5) (Malathi *et al.*, 2019), and Malonyl-CoA-acyl carrier protein transacylase (ACP) (**FabD**) (PBD ID: 2G1H) [18], retrieved from Protein Data Bank (PDB) (http://www.rcsb.org). Discovery Studio 4.1 (http://accerys.com) [26] and Molecular Graphics Laboratory (MGL) Tools 1.5.6 (http://mgltools.scripps.edu) were employed to prepare a .pdbqt file of the proteins for molecular docking to supply AutoDock Vina [27].

In the present study, all available 27 selected phytochemicals (Figure 1) from *M. oleifera* leaves and seeds were investigated for the target proteins. Included in the work were: compounds from leaves 1-19: Moringine (1), Marumoside A (2), Marumoside B (3), Pyrrolemarumine 4-O-α-L-rhamnopyranoside (4), 4-(α-L-rhamnopyranosyloxy) benzylcarbamate (5), Kaempferol (6), Rhamnetin (7), 3-hydroxy-4-phenylchromen-4-one (8), Quercetin (9), Isoquercitrin (10), Kaempferitrin (11), D-(6''-O-malonyl)-glucoside (12), Astragalin (13), Kaempferol 3-O-β-D-(6''-O-malonyl)-glucoside (14), 4-O-(4-O-α-D-glucopyranosyl) caffeoyl quinic acid (15), 4-O-(3-O-α-D-glucopyranosyl) caffeoyl quinic acid (16), Chlorogenic acid (17), 4-O-caffeoyl quinic acid (18), 5-O-caffeoyl quinic acid (19), and compounds from seeds 20-27: O-Methyl-4-(4'-O-acethyl-α-L-rhamnosyloxy)benzyl thiocarbamate (20), Octacosanoic acid (21), 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate (22), Isothiocyanatomethylbenzene (23), 4-(4'- Oacethyl-α-L-rhamnosyloxy)-benzylisothiocyanate (24), 4-(α-L-rhamnopyranosyloxyl)-benzylisothiocyanate (25), 4-(β-D-glucopyranosyl-1→4-α-L-rhamnopyranosyloxy)-benzyl isothiocyanate (26), β-sitosterol-3-O-β-D-glucopyranoside (27) [28].

The 2D compound structures (Fig. 1) were constructed using ChemDraw Professional ver. 18.2, (http://www.cambridgesoft.com/), then converted to .pdb by way of Discovery Studio 4.1 (http://accerys.com) [26], and Open Babel graphical user interface (GUI) (http://openbabel.org/) [29]. In previous studies, O'Boyle *et al*. saved the files in .pdbqt format, and then applied Discovery Studio 4.1 and UCSF Chimera Ver. 1.10.1 program (http://www.cgl.ucsf.edu/chimera/) [30]. The work of Pettersen *et al*, was used as a guideline for visualizing the interactions of the compounds with the active site of the proteins.

AutoDock Vina

After specifying the protein active sites using Discovery Studio 4.1 (http://accerys.com) [26], the initiation of the colored cube around the active site was achieved by employing Molecular Graphics Laboratory (MGL) Tools 1.5.6 (http://mgltools.scripps.edu). The grid box described in the configuration file and the dimensions of the line cube for all studied proteins was 18 $Å^3$, while covering the active sites with grid point spacing of 1.0 Å. We found the center grid boxes dimensions in X, Y and Z to be (24.977, 16.282, 80.221) for LasR, (55.11, -23.9, -1.089) for KPC, and (73.406, 27.024, 27.533) for FabD, respectively. All related information was saved in the text configuration file (conf.txt) as this is a requirement for supplying AutoDock Vina 1.1.2 [27]. The molecular docking was performed on the Windows 10 operating system, Cori 7, with 8 CPUs and 12 GB of RAM. All compounds were docked three times to the binding site of the studied proteins.

RESULTS

Phytochemical analysis of *Moringa oleifera*

The yield of the diverse extracts of *M. oleifera* is given in Table 1. The greatest quantity of the plant metabolite was extracted by diethyl ether solvent (12.8% and 1.38%). The amount of active constituents extracted from the seed of *M. oleifera* was 12.8% by diethyl ether and 6.02% by ethyl acetate – which was higher than that of the leaf (1.38% by diethyl ether and 1.26% by ethyl acetate).

Table 1. The percentage yield of seed and leaf extracts of *M. oleifera* by using different solvent polarities

Moringa oleifera	Solvents	
	Diethyl ether	Ethyl acetate
Seed	12.8%	6.02%
Leaf	1.38%	1.26%

The qualitative screening of seed and leaf extracts of *M. oleifera* revealed the presence of numerous constituents responsible for important pharmacological activity. We found alkaloids, carbohydrates, cardioactive glycosides, steroids and triterpenoids to be present in both the seed and leaf extracts, and we noted flavonoids, phenols and tannins in ethyl acetate extracts of both parts, as well as in the diethyl ether extract of the leaf. In contrast, saponin glycosides and proteins were found to be absent in both parts (Tab. 2).

Table 2. Phytochemical analysis for the different solvent extracts of *Moringa oleifera*

Phytochemical compounds	Qualitative Chemical Tests	Seed		Leaf	
		DEE	EA	DEE	EA
Alkaloids	Dragendorff's test	+Ve	+ Ve	+ Ve	+ Ve
Carbohydrates	Molisch's test	+ Ve	+ Ve	+ Ve	+ Ve
Saponin glycoside	Foam test	- Ve	- Ve	- Ve	- Ve
Cardioactive glycosides	Keller-kiliani tests	+ Ve	+ Ve	+ Ve	+ Ve
Flavonoids	Lead acetate test	- Ve	+Ve	+ Ve	+ Ve
Phenols and tannins	Ferric chloride test	- Ve	+Ve	+ Ve	+ Ve
Steroids and terpenoids	Salkowski test	+ Ve	+ Ve	+ Ve	+ Ve
Proteins	Xanthoproteic Test	- Ve	- Ve	- Ve	- Ve

DEE: Diethyl Ether; EA: Ethyl acetate, +Ve = Presence; –Ve = Absence

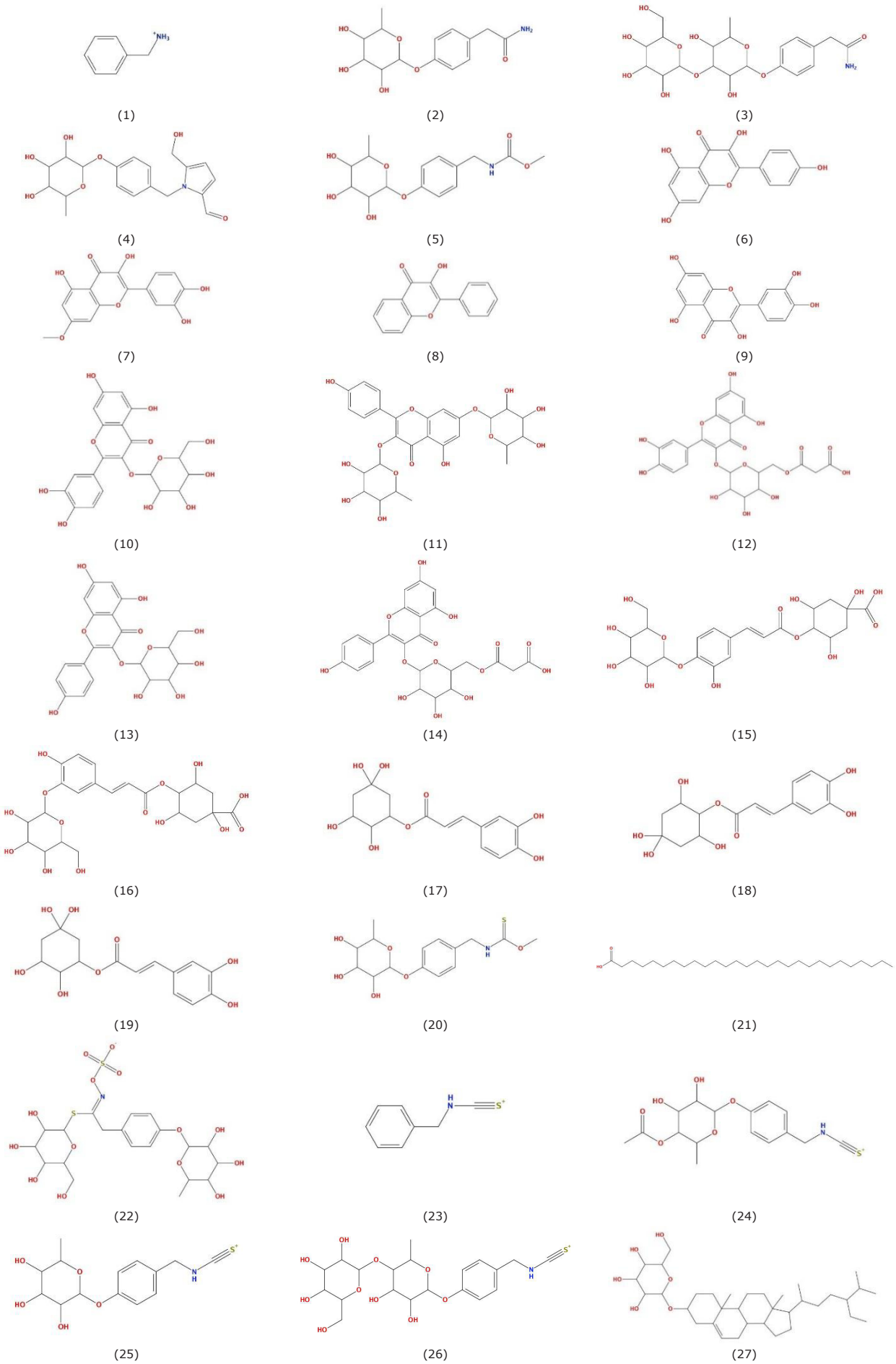


Figure 1. Diagrams showing 2D structures of the studied compounds

Antimicrobial activity of *Moringa oleifera* leaves and seeds extracts against microorganisms

The antibacterial activity of three concentrations (50 mg/ml, 100 mg/ml, and 200 mg/ml) of the diethyl ether and ethyl acetate extracts of *M. oleifera* leaves and seeds against pathogenic gram-negative bacteria are shown in Table 3 and 4. The results revealed that the antibacterial effect of the tested extract showed a variable zone of inhibition ranging from 10.33±0.577 to 19.33±1.154 mm according to the kind of pathogenic bacteria. *Moringa* diethyl ether seed extracts showed a high inhibition effect on the growth of *K. penumoniae* compared to other tested bacteria while having low antibacterial activity against *P. aeruginosa*.

Table 3. Antibacterial activity of *Moringa oleifera* leaf extracts against some pathogenic bacteria

Bacteria strain	Diethyl ether leaf (DEEL) Zone of inhibition (mm)			Ethyl acetate leaf (EAL) Zone of inhibition (mm)		
	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
E. coli	0	12.33 ±0.577	12.66 ±1.154	11.66 ±0.577	12.66 ±0.577	13.66 ±0.577
K. penumoniae	0	11.33 ±0.577	14.33 ±0.577	10.33 ±0.577	14.33 ±0.577	15 ±1
P. aeruginosa	0	11 ±0.577	11.66 ±0.577	0	0	11.33 ±0.577

Values are presented as mean ± SD of triplicate experiments. 0 = Growth

Table 4. Antibacterial activity of *Moringa oleifera* seed extracts against some pathogenic bacteria

Bacteria strain	Diethyl ether Seed (DEES) Zone of inhibition (mm)			Ethyl acetate Seed (EAS) Zone of inhibition (mm)		
	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
E. coli	15.33 ±1.527	17 ±1	19.33 ±1.154	0	0	0
K. penumoniae	17 ±1	18 ±0	18.33 ±0.577	0	0	12.66 ±0.577
P. aeruginosa	0	11.66 ±1.527	13.66 ±0.577	0	0	12.33 ±0.577

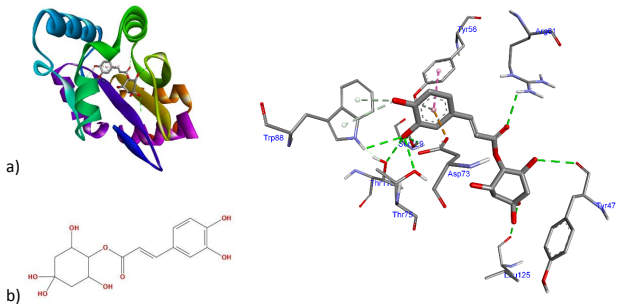
Values are presented as mean ± SD of triplicate experiments. 0 = Growth

The studied compounds (1-27) were well docked into the binding site of proteins. In our study, AutoDock Vina software was used to perform three runs per compound and the average docking scores were calculated. The docking results shown in (Tab. 5) are a list of studied compounds with their binding energy or binding affinity to docked proteins. Generally, compounds with values of lower energy (highly negative) state (the more negative the energy is) display the most fitting conformations with high affinity within the active site (Figs 5-7).

Table 5. Average of binding affinity for studied compounds docked to protein (LasR, KPC, and FabD)

No.	Compound Name	Average of Docking Scores (kcal/mol) lowest energy		
		LasR	KPC	FabD
1	Moringine	-6.7	-5.0	-4.2
2	Marumoside A	-8.4	-7.8	-7.7
3	Marumoside B	-7.7	-7.9	-7.9
4	Pyrrolemarumine 4-O-α-L-rhamnopyranoside	-7.9	-8.2	-7.5
5	4-(α-L-rhamnopyranosyloxy) benzylcarbamate	-8.9	-7.6	-8.0
6	Kaempferol	-10.5	-8.1	-7.9
7	Rhamnetin	-10.6	-8.0	-8.3
8	3-hydroxy-4-phenylchromen-4-one	-11.2	-7.9	-7.2
9	Quercetin	-10.5	-8.2	-8.0
10	Isoquercitrin	-7.6	-8.6	-8.1
11	Kaempferitrin	-0.5	-7.8	-8.6
12	D-(6''-O-malonyl)-glucoside	-0.6	-7.5	-8.5
13	Astragalin	-7.6	-8.6	-8.8
14	Kaempferol 3-O-β-D-(6''-O-malonyl)-glucoside	-2.6	-7.5	-8.5
15	4-O-(4-O-α-D-glucopyranosyl) caffeoyl quinic acid	-7.3	-8.0	-8.2
16	4-O-(3-O-α-D-glucopyranosyl) caffeoyl quinic acid	-7.8	-7.7	-8.2
17	Chlorogenic acid	-9.7	-8.1	-7.5
18	4-O-caffeoyl quinic acid	-11.2	-8.2	-7.7
19	5-O-caffeoyl quinic acid	-10.8	-8.5	-8.3
20	O-Methyl-4-(4'-O-acethyl-α-L-rhamnosyloxy)benzyl thiocarbamate	-9.4	-7.1	-7.8
21	Octacosanoic acid	-7.0	-5.1	-5.1
22	4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate	-6.3	-8.8	-8.6
23	Isothiocyanatomethylbenzene	-7.2	-5.2	-4.6
24	4-(4'- Oacethyl-α-L-rhamnosyloxy)-benzylisothiocyanate	-8.0	-7.7	-7.2
25	4-(α-L-rhamnopyranosyloxyl)-benzylisothiocyanate	-9.6	-7.9	-7.5
26	4-(β-D-glucopyranosyl-1→4-α-L-rhamnopyranosyloxy)-benzyl isothiocyanate	-7.6	-7.3	-7.9
27	β-sitosterol-3-O-β-D-glucopyranoside	2.1	-8.0	-7.0

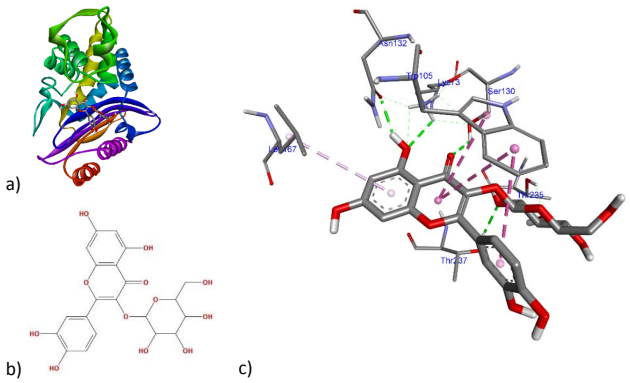
The binding mode of compound (18) with LasR involved hydrogen bonds with TYR[47, 56], ARG[61], THR[75,115], TRP[88], LEU[125] and hydrophobic interactions with TYR[56], in addition to electrostatic interaction with ASP[73] (Fig. 2).



a) The protein and the compound inactive site, b) 2D structure of the compound, c) The residues of a protein involved in interactions with the compound

Figure 2. Compound (18) docked to LasR

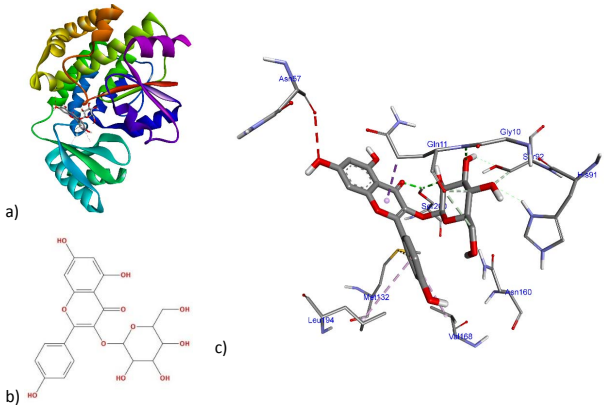
Compound (10) interacted with KPC, forming hydrogen bonds with LYS[73], SER[130], ASN[132], THR[235,237] and hydrophobic interactions with TRP[105] and LEU[167] (Fig. 3).



a) The protein and the compound inactive site, b) 2D structure of the compound, c) The residues of a protein involved in interactions with the compound

Figure 3. Compound (10) docked to KPC

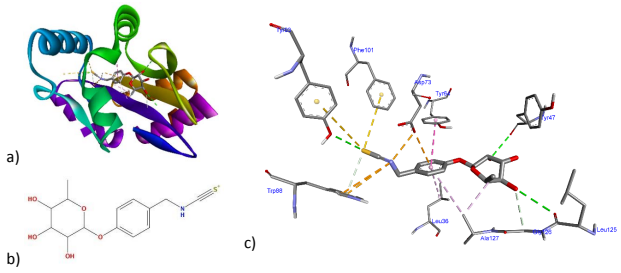
Compound (13) was found to bind with FabD, forming hydrogen bonds with GLN11, HIS[91], GLY[94], SER[92,200], ASN[160] and hydrophobic interactions with GLN[11], VAL[168], LEU[194] (Fig. 4).



a) The protein and the compound inactive site, b) 2D structure of the compound, c) The residues of the protein involved in interactions with the compound

Figure 4. Compound (13) docked to FabD

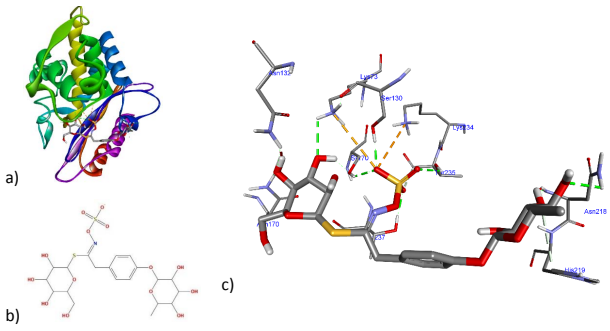
Compound (25) interaction with LasR involved hydrogen bonds with TYR[47, 93], LEU[125], GLY[126] and hydrophobic interactions with LEU[36], TYR[64], ALA[127], in addition to electrostatic interactions with ASP[73], TRP[88] and other interactions with TYR[93] and PHE[101] (Fig. 5).



a) The protein and the compound inactive site, b) 2D structure of the compound, c) The residues of the protein involved in interactions with the compound

Figure 5. Compound (25) docked to LasR

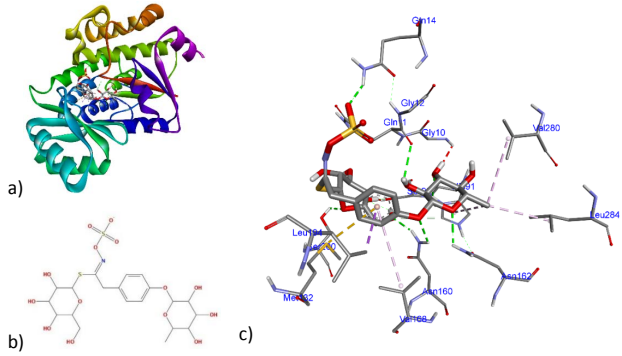
Compound (22) interacted with KPC, forming hydrogen bonds with LYS[73], SER[70,130], ASN[170,218], HIS[219], THR[235,237], while electrostatic interactions were observed with LYS[73,234] (Fig. 6).



a) The protein and the compound inactive site, b) 2D structure of the compound, c) The residues of a protein involved in interactions with the compound

Figure 6. Compound (22) docked to KPC

Compound (22) interacted with FabD, forming hydrogen bonds with GLN[11,14], HIS[91], SER[92,200], ASN[160,162] and hydrophobic interactions with HIS[91], LEU[194,284], VAL[168,280] (Fig. 7).



a) The protein and the compound inactive site, b) 2D structure of the compound, c) The residues of a protein involved in interactions with the compound

Figure 7. Compound (22) docked to FabD

DISCUSSION

Plant metabolites are commonly used as defense mechanisms towards herbivores, insects and microorganisms. In the literature, the number of isolated secondary metabolites have been assessed at <10% of the total actually present in plants [31]. In our study, different phytochemical compounds were detected in different parts of *M. oleifera*. Among others, these included carbohydrates, cardioactive glycosides, steroid and triterpenoids, flavonoids, phenols and tannins. Our results were supported by previously recorded data [32,33], while the presence of alkaloids and absence of saponins were in contrast with the opinion of the same studies. According to the previously recorded data, the identified compounds are known to have various therapeutic importances. For example, flavonoids such as astragalin, Isorhamnetin, Quercetin, Kaempferol and their derivatives have been reported as powerful antibacterial compounds against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis* and *Escherichia coli* [34]. Moreover, β-sitosterol-3-O-glucoside was found to be active against *S. aureus*, Methicillin-Resistant *Staphylococcus aureus, P. mirabilis, S. typhi, K. pneumoniae, E. coli,* and *B. subtilis,* at a dose (200 µg/ml) with a zone of inhibition ranging between (24-34 mm) [35]. Mabhiza *et al.* [36] stated that the alkaloid extracted from *Callistemon citrinus* and *Vernonia adoensis* exhibited broad-spectrum activity against both Gram-positive and Gram-negative bacteria. According to study, chlorogenic acid, which we found in Drumstick, and which is the main phenolic compound in coffee, exerts its antibacterial activity by increasing the permeability of the outer and plasma membranes, which leads to a loss of barrier function, even causing slight leakage of nucleotides and cytoplasmic contents [37].

The extracts of *M. oleifera* displayed varying antibacterial activity on a wide variety of pathogenic bacteria [14]. Both leaf and seed extract were effective in countering the effects of pathogenic microorganisms. The chance to find antibacterial activity was further apparent in diethyl ether than in ethyl acetate extracts of the same shrubberies. Over

all, it can be said that *M. oleifera* could be a basis for making new antimicrobial agents [38]. The results we obtained demonstrate that all concentrations of diethyl ether extracts of *M. oleifera* had high inhibitory effects on *K. pneumoniae* and *E. coli,* but had lower inhibitory effects on the *P. aeruginosa* strain. The variance in bacterial retort to the plant extracts was perhaps due to the nature of the pathogenic bacterial species. Previous data indicate that *K. pneumoniae* and *E. coli* revealed obvious sensitivity against phytochemical constituents that can be extracted with nonpolar solvents [39]. The *M. oleifera* extract showed notable antibacterial activity against Gram-negative bacteria. These results are comparable to other researchers' findings who stated that most plant extracts have activity against Gram-negative pathogenic bacteria [14,15].

The docking study provides a computable or computational energetic measure that confirms that the studied compounds can inhibit the studied proteins. Compounds (18, 10, 13) from leaf extracts showed the lowest docking scores (-11.2, -8.6, -8.8) kcal/mol binding with LasR, KPC, and FabD respectively (Figures 2-4), while compounds (25, 22, 22) from seed extracts produced (-9.6, -8.8, -8.6) kcal/mol binding with the studied proteins (Figs. 5-7 and Tab. 5). All compounds were found to bind to the proteins via hydrogen bonds and hydrophobic interactions. The binding energy according to AutoDock Vina, is the summation of the intermolecular forces acting upon the complex protein compound, and this intermolecular force generates a scoring derived based on the distance and type of bonding between the active site of a protein and the docked compound.

CONCLUSIONS

In conclusion, the seed extract of *M. oleifera* has greater antimicrobial activity against various gram-negative bacteria such as *Escherichia coli* and *K. pneumoniae* than did leaf extracts, this could be due to the phytochemical compounds present in it. Among the studied plant extracts, compounds (18, 25), (10, 22), and (13, 22) have excellent binding interactions with LasR, KPC, and FabD proteins, respectively. Further laboratory work on the separate phytochemical components will propose the actual compound responsible for its inhibitory effects.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest regarding the publication of the manuscript.

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