International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Phytochemistry, GC-MS analysis of *Acacia nilotica* fruits and *Moringa oleifera* leaves and their Antibacterial activity on *Escherichia coli* and *Staphylococcus aureus* local strains

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International Journal of Science and Research Archive, 2024, 13(02), 3746-3755

Publication history: Received on 13 November 2024; revised on 22 December 2024; accepted on 24 December 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.13.2.2579

Abstract

Antibiotics are bacteria-control agents used to inhibit or kill bacterial cells and most of their alternative sources are the natural products. The aim of this work was to study the phytochemical composition of *Acacia nilotica* fruits and *Moringa oleifera* leaves and their antibacterial effects on *E. coli* and *Staphylococcus aureus*. The dried powders of the selected plant materials were prepared to determine the main phytochemicals, the GC-MS analysis and to estimate the inhibitory effects against the selected bacterial strains through disc diffusion method. The results showed that, *M. oleifera* contained flavonoids, saponnins and terpenoids, as same as *A. nilotica* which included also glycosides and tannins. The main compound from *A. nilotica* fruits was (1,2,3-Benzenetriol compound was the main component; 85.11%), while that of *M. oleifera* leaves was (1,3-propanediol, 2-(hydroxymethyl)-2nitro-; 13.68%). Also, *E. coli* was relatively more susceptible to *A. nilotica* fruits extract than *M. oleifera* leave, but *M. oleifera* leaves show promising alternative to penicillin. *S. aureus* was relatively more susceptibility to *M. oleifera* leave than *A. nilotica* fruits and penicillin than *E. coli* local strain. More pharmaceutical investigation on these plant parts should be done in order to get maximum benefit of these products to control different bacterial strains.

Keywords: Phytochemistry; *Acacia nilotica*; *Moringa oleifera*; antibacterial; *E. coli*; *Staphylococcus aureus*

1. Introduction

Escherichia coli is a gram-negative (its cell wall composed of a thin peptidoglycan layer), facultative anaerobic, typically rod-shaped, and are about 2.0 µm long and 0.25–1.0 µm in diameter, with a cell volume of 0.6–0.7 µm^3 (Yu *et al.*, 2014). It is commonly found in the lower intestine of human (Tenaillon *et al.,* 2010), but some are pathogenic and can cause serious food poisoning, and food contamination incidents (Vogt *and Dippold*, *2005)*. Most strains are harmless or even beneficial to humans (e.g., some strains of *E. coli* producing vitamin K_2) or preventing colonization of the intestine by pathogenic bacteria (Bentley and Meganathan, 1982). *E. coli* constitute about 0.1% of gut microbiota, and is expelled within fecal matter to the environment (Eckburg *et al.,* 2005). It can survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination. Fecal–oral transmission is the major route through which pathogenic *E. coli* cause disease. Environmentally persistent *E. coli* that can survive for many days and grow outside a host has been studied (Montealegre *et al.,* 2018).

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E. coli virulent strains can cause gastroenteritis, urinary tract infections, meningitis and hemorrhagic colitis. In rarer cases, virulent strains are also responsible for tissue death and perforation without progressing to hemolytic-uremic syndrome (Son and Taylor, 2021).

Staphylococcus aureus is a bacillota, anaerobic, gram-positive, and are sometimes found in the upper respiratory tract and on the skin. Although it acts usually as a commensal microorganism, it can also become an opportunistic (can cause skin abscesses, respiratory infections, and food poisoning). It is often produced certain factors that binds and inhibit antibodies, though it considered one of the leading pathogens for emergence of antimicrobial resistance. No vaccine for *S. aureus* has been introduced yet (Masalha *et al.,* 2001). An estimated 20-30% of the human population are carriers of *S. aureus* (Turista and Puspitasari, 2019), which also can be found in female's lower reproductive tract (Hoffman, 2012). Skin infections are the most common form of *S. aureus* infection. It is still one of the most common causes of hospital-wound infections following surgery (Bowersox, 1999), and it responsible for deaths associated with antimicrobial resistance during 2019 (Murray *et al.,* 2022).

Acacia nilotica (family Fabaceae), the gum Arabic tree, is a flowering tree that is native to Africa, the Middle East and the Indian subcontinent, and an invasive species in Australia, as well as a noxious weed in the United States (Kyalangalilwa *et al.,* 2013). This plant is used directly as a food, or to treat sore throat, chest pains and caught in India and Malaysia, while it was used against dysentery, pile and stomach ulcers in Nigeria (Christopher *et al.,* 2002). The phytochemical screening carried out on different extracts of *A. nilotica* fruit cover showed high amount of glycosides, flavonoids and terpenoids (Abdalla *et al.,* 2020).

Moringa oleifera (family Moringaceae) is a fast-growing tree, widely cultivated and used as vegetables and for traditional herbal medicine, because it is drought-resistant tree. It is native to the Indian subcontinent. It is also used for water purification (Olson, 2010). Consuming certain compounds in the bark and roots may cause adverse effects. Supplementation with *M. oleifera* leaf extract is potentially toxic (more than 3 g/kg, but safe below 1 g/kg of body weight) (Asare and Nyarko, 2012).

Edible parts of *M. oleifera* plant include the whole leaves, stems, immature green fruits or seed pods, flowers, and roots (Lim, 2012). The phytoconstituents of the leaves revealed the presence of alkaloids, flavanoids, glycosides, terpenoids, tannins, saponnins, steroids etc. which could be a source for the industrial manufacture of useful drugs in treatment of various diseases (Gupta *et al.,* 2014).

The antibiotic is a natural or synthetic substances that has potential control or inhibit activity against bacteria (either kill or inhibit the growth of bacteria). Some antibiotics are also antiprotozoal, antivirus or antifungal activity (Gallagher and MacDougall, 2012). Antibiotics may be given as a preventive measure to populations with a weakened immune system, those taking immunosuppressive drugs, cancer patients, and those having surgery. Antibiotics are also used to prevent infection in cases of neutropenia particularly cancer-related (Flowers *et al.,* 2013).

Some antibiotics may damage the mitochondrion of bacteria and human cells which has been suggested as a mechanism for side effects from fluoroquinolones (Marchant, 2018). The bactericidal activity of antibacterials may depend on the bacterial growth phase, and it often requires ongoing metabolic activity and division of bacterial cells (Mascio *et al*., 2007).

Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. Most target bacterial functions or growth processes. Some antibiotic target the bacterial cell wall (penicillin and cephalosporin) or the cell membrane (polymyxins), or interfere with essential bacterial enzymes (rifamycins, quinolones). Narrow-spectrum antibiotics target (Cunha, 2009). The antibiotic-resistant bacteria is mainly caused by the overuse/misuse and it led to a global health threat (Sample, 2018).

2. Material and methods

The leaves of *M. oleifera* (L.) were collected from the garden of the main campus of University of Gezira, while the fruits of *A. nilotica* ((L.) P.J.H.Hurter & Mabb) were brought from the local market of Wad Medani City, Gezira State, Sudan. The pure *E. coli* (O25:H4) and *S. aureus* (SO-1977) strains were brought from the Microbiology Department, Central Medial Laboratory, University of Gezira.

2.1. Preparation of Extracts

Five grams of each plant dried powder were dissolved separately in 15 ml ethanol in a conical flask for 24 hours. Each extract was filtered in a clean Petri-dish, and the clear solution were let to evaporate under room temperature. The difference in weight of the empty and extract-containing Petri-dish of each product were used to calculate the stock concentration of the hydroethanol extract referring to the amount (in ml) of the distilled water added.

2.2. Phytochemical Screening Tests

Phytochemical screening was done according to the method described by Gupta *et al.* (2014):

- Alkaloids: To 3 ml ethanol extract, 2 drops of dilute HCl were added and then filtered, then treated with 2 drops of Dragendroff's reagent; positive result was indicated by the formation of the orange brown precipitate.
- Flavonoids: To 3 ml aqueous plant extract and 5 ml of dilute ammonia solution, 2 drops of concentrated H2SO4 were added. The formation of yellow color indicates the presence of flavonoids.
- Glycosides: Mixture of 3 ml ethanol plant-extract and 15 ml diluted H_2SO_4 was boiled for 15 minutes. Then cooled and neutralized with 10% NaOH, then 3 ml of Fehling solution was added. Glycosides is detected by the formation of brick-red precipitate.
- Terpenoids: To 5 ml ethanol plant-extract, 2 ml of chloroform and 2 ml concentrated H2SO4 was added carefully. Terpenoids is detected by the formation of reddish-brown layer.
- Tannins: To 5 ml diluted aqueous extract, 4 drops of 10% $FeCl_3$ were added. The presence of blue or green color indicates tannins.
- Saponnins: To 1 g of plant powder 5 ml of water were added and shacked well for 15 minutes. The formation of foam froth indicates the presence of saponnins.
- Steroids: To 3 g plant powder and 2 drops of concentrated H2SO4 and 5 ml chloroform were added. Steroids is detected by the formation of red color in chloroform layer.

2.3. The GC-MS Test

The ethanol extract (5 ml) of each product was analyzed using GC-MS techniques (GCMS-QP2010 Ultra, Shimadzu Europa GmbH, Library: NIST 11s.lib) at the Central Laboratory, University of Gezira. The test revealed the Retention time, compound name, molecular formula and concentration (%).

2.4. Media Preparation

2.4.1. For S. aureus strain

Weight of 28 g of nutrient agar powder (0.5% of peptone, 0.3% yeast extract, 1.5% agar, and 0.5% NaCl) was suspended in 1.0 L distilled water. This mixture was heated, stirred to fully dissolve all components, and autoclaved at 121 ℃ for 15 minutes. Then allowed to cool but not solidify. When the mixture cooled to 45-50 °C, 5% (v/v) sterile defibrinated blood (that has been warmed to room temperature) was added and mixed well. Air bubbles were avoided and pH was adjusted to 7.2 – 7.6. The whole mixture was then dispensed into sterile plates before solidified (Turista and Puspitasari, 2019).

2.4.2. For E. coli strain

Ten g tryptone, 5 g yeast extract, 5 g NaCl, and 15 g agar were added and the volume was adjusted to 1000 ml with distilled water. The mixture was autoclaved in conical flask at 121 °C for 30 minutes, let to cool to about 60 °C and then poured into sterile plastic petri dishes. The plates were allowed to cool and solidify at room temperature and pH of 7.0 (Son and Taylor, 2021).

Each media was then poured in sterile Petri dishes (size of 100 mm × 15 mm), left to solidify and incubated at 37 °C under aerobic conditions. Each bacteria strains was cultured in its proper media before the antibacterial agents were added, in Biosafety Level 2 (BSL-2) cabinet, provided with fan, ultra violet lamp and flame. The prepared plates was stored at 4 °C when not used.

2.5. Antibacterial Activity Test

It was run using Disc diffusion method. Five diluted concentrations (w/v) of each product were prepared from the stock concentration, in addition to penicillin (the synthetic antibiotics) as +ve control. Using sterile cotton wood swab, then *S. aureus* and *E. coli* strains were cultured at the surface of the prepared media. After 10 minutes, the excessive solution whenever noticed was aspirated completely out of each Petri-dish. Small disk papers (5 mm diameter) soaked in only

one concentration of the prepared concentrations were placed onto the media seeded with the tested bacteria strain and one disc embedded in distilled water was placed as negative control, while penicillin soaked disc papers were used as positive control. These procedures were performed under strict aseptic condition. Three replicates were done. Each Petri-dish was incubated at 37 ℃ for 48 h. The diameter (mm) of each inhibition zone was measured by transparent ruler.

2.6. Statistical Analysis

The main classes of the phytochemicals were expressed as (+) for detected, and (-) for those not detected, while the GC-MS identified compounds were expressed as compound names, conc. %, molecular formula, and retention time. The antimicrobial activity was expressed as inhibition zone (in mm) and the Least Significant Difference (LSD) was used to evaluate the obtained results.

3. Results

3.1. The Phytochemical Screening Tests

Table (1) showed that, *M. oleifera* contained flavonoids, saponnins and terpenoids, as same as *A. nilotica* which included also glycosides and tannins. It was also noticed that, both plant product did not contain alkaloids and steroids, while *M. oleifera* also lack glycosides and tannins.

Table 1 Main phytochemicals detected in *M. oleifra* leaves and *A. nilotica* fruits

Main class	***M. oleifera* leaves**	***A. nilotica* fruits**
Alkaloids	-	-
Flavonoids	+	+
Glycosides	-	+
Saponnins	+	+
Steroids	-	-
Tannins	-	+
Terpenoids	+	+

- mean that: the main class was not detected; + mean that: the main class was detected

3.2. GC-MS analysis

Table (2) and Figure (1) showed the detected compounds from the ethanol extract of *A. nilotica* fruits and their corresponding molecular formula and concentrations (%). The main constituent was 1,2,3-Benzenetriol (85.11%), followed by 3-O-Methyl-d-glucose (7.64%), Tetrahydro-4H-pyran-4-ol (2.39%), Catechol (2.16%) and some other traces.

Table 2 GC-MS analysis for the ethanol extract of *A. nilotica* fruits

No.	**R. time**	**Compound name**	**Area%**	**Mol. Formula**
1	7.820	Tetrahydro-4H-pyran-4-ol	2.39	$C_5H_{10}O_2$
2	8.412	Catechol	2.16	$C_6H_6O_2$
3	11.490	1,2,3-Benzenetriol	85.11	$C_6H_6O_3$
4	16.070	3-O-Methyl-d-glucose	7.64	$C_7H_{14}O_6$
5	16.441	1-Octanol, 2.2-dimethyl-	0.13	$C_{10}H_{22}O$
6	17.406	Phthalic acid, isobutyl octadecyl ester	0.10	$C_{30}H_{50}O_4$
7	18.299	Eicosanoic acid	0.20	$C_{20}H_{40}O_2$

8	18.582	Acetic acid, 2-hydroxy-2-(3-methyl-	0.81	$C_9H_{10}O_3$
9	20.539	2-methyltetracosane	0.45	$C_{25}H_{52}$
10	23.421	Hexadecanoic acid, 2-hydroxy-1-	0.24	$C_{19}H_{38}O_4$
11	23.784	Di-n-octyl phthalate	0.78	$C_{24}H_{38}O_4$

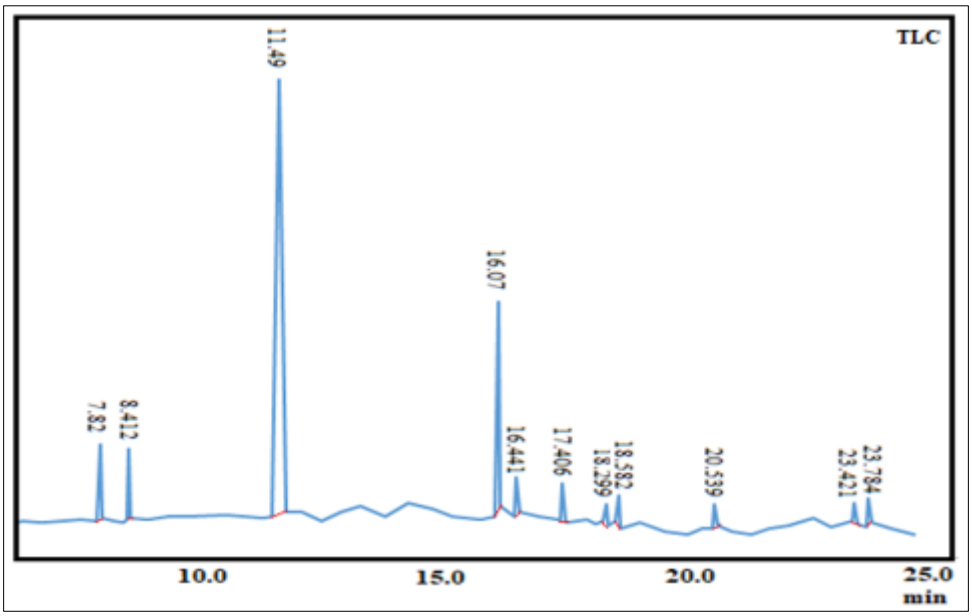

Figure 1 GC-MS chromatogram of the ethanol extract of *A. nilotica* fruits

The detected compounds from the ethanol extract of *M. oleifera* leaves and their corresponding molecular formula and concentrations (%) were presented in Table (3). The main constituent was Eicosane (15.79%), followed by 1,3-propanediol, 2-(hydroxymethyl)-2nitro- (13.68%), 2,4,6-Cycloheptrien-1-one,4-methyl (8.68%), Beta-D-glucopyranose, 1,6-anhydro (8.49%), di-n-octyl phthalate (5.12%) and Phytol (3.64%) and some other traces (Figure, 2).

Table 3 GC-MS analysis for the ethanol extract of *M. oleifera* leaves

No.	**R.time**	**Compound name**	**Area%**	**Mol.formula**
1	4.135	Butane, 1,1-diethoxy-3-methyl-	2.67	$C_9H_{20}O$
2	4.781	Diglycerol	2.11	$C_6H_{14}O_5$
3	4.985	2-4-Butyl-4-methyl-5-oxo[1,3]dioxalane	1.54	$C_9H_{14}O_5$
4	5.760	2,4,6-Cycloheptrien-1-one,4-methyl	8.68	C_8H_{80}
5	6.537	Nonanal	1.42	$C_9H_{18}O$
6	8.755	Benzofuran,2,3-dihydro-	1.39	C_8H_8O
7	11.290	Hexadecane	2.02	$C_{16}H_{34}$
8	12.179	1,3-propanediol, 2-(hydroxymethyl)-2-nitro-	13.68	$C_4H_9NO_5$
9	12.575	3-Isopropoxy-1,1,7,7,7-hexamethyl-3,5,5-	1.77	$C_{18}H_{52}O_7Si$
10	12.843	Beta-D-glucopyranose, 1,6-anhydro-	8.49	$C_6H_{10}O$
11	13.040	Phenol, 2,6-bis(1,1-dimethylethyl)-	2.17	$C_{14}H_{22}O$
12	13.233	8-Decen-2-one, 9-methyl-5-methylene-	3.81	$C_{12}H_{20}O$

13	13.430	4-Chloro-1,7-dioxaspiro[5,5]undecane	0.79	$C_9H_{16}Cl_4O_2$
14	13.720	Isoquinoline, 3-methyl-	1.61	$C_{10}H_9N$
15	14.038	Eicosane	15.97	$C_{20}H_{42}$
16	14.140	2-Phenylpyrimidine	2.18	$C_{10}H_8N_2$
17	14.769	1,2-dihydro-8-hydroxylinalool	1.21	$C_{10}H_{20}O_2$
18	15.060	Carbonic acid, dodecyl, 2,2,2-trichloroethyl	1.10	$C_9H_{13}Cl_3O_3$
19	15.275	Tetradecane	1.74	$C_{14}H_{41}$
20	15.425	3-Buten-2-one,4-(4-hydroxy-2,2,6-trimethyl	2.50	$C_{13}H_{20}O_3$
21	15.571	Cyclohexanol, ethyl-	2.10	$C_8H_{16}O$
22	16.115	Heptadecane, 3-methyl-	2.29	$C_{18}H_{38}$
23	16.910	Phytol acetate	0.56	$C_{22}H_{42}O$
24	17.675	2-(4,5-Dihydro-3-methyl-5-oxo-1-phenyl-4-	1.12	$C_{17}H_{13}N_5O_5$
25	18.588	Octadecane	3.10	$C_{16}H_{38}$
26	19.818	Phytol	3.64	$C_{20}H_{40}O$
27	22.321	Hexacosane	1.44	$C_{26}H_{54}$
28	23.413	Glycerol 1-palmitate	3.77	$C_{19}H_{38}O$
29	23.780	di-n-octyl phthalate	5.12	$C_{24}H_{38}O_4$

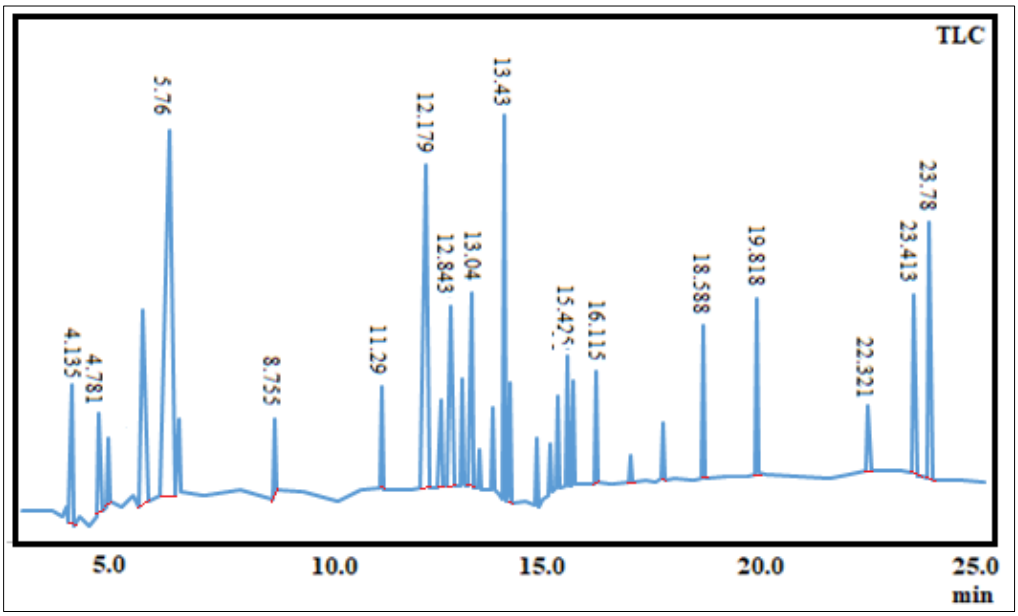

Figure 2 GC-MS chromatogram of the ethanol extract of *M. oleifera* leaves

3.3. Antibacterial Tests

3.3.1. Against E. coli bacteria

Five different concentrations (1.97, 7.88, 31.52. 126.06 and 504.25 mg/ml) of the hydroethanol extract of *A. nilotica* fruits was tested at against *E. coli* strain. The respected mean inhibition zones were (6.4, 6.5, 7.1, 7.8 and 8.0 mm). The hydroethanol extract of *M oleifera* leaves was tested at 5 different concentration (1.32 - 338 mg/ml) on *E. coli* strain.

The resulted mean inhibition zones ranged between (6.2 - 7.0 mm). Penicillin was tested at two concentrations (125 and 500 mg/ml) which resulted in means of 6.5 and 8.0 mm inhibition zones (Table, 4)

Table 4 Inhibition zone (mean ± SE) of the hydroethanol extracts of *A. nilotica* fruits and *M. oleifera* leaves and penicillin antibiotic on *E. coli* using disc diffusion method

A. nilotica fruits	Conc (mg/ml)	1.97	7.88	31.52	126.06	504.25
	Inhibition (mm)	6.4 ± 0.08c	6.5 ± 0.12c	7.1 ± 0.33b	7.8 ± 0.12a	8.0 ± 0.08a
M. oleifera leaves	Conc (mg/ml)	1.32	5.28	21.13	84.50	338
	Inhibition (mm)	.62 ± 0.12c	.63 ± 0.33c	6.4 ± 0.12c	6.6 ± 0.08c	7.0 ± 0.33b
Penicillin	Conc (mg/ml)	125		500		
	Inhibition (mm)	6.5 ± 0.33c		8.0 ± 0.12a		

Different significant levels are marked with different letters

3.3.2. Against S. aureus bacteria

The hydroethanol extract of *A. nilotica* fruits (at same concentrations used against *E. coli*; 1.97 - 504.25 mg/ml) against *S. aureus* resulted in mean inhibition zones ranged from 6.3 mm to 8.7 mm, whereas that of *M. oleifera* leaves at concentrations ranged between (1.32 - 338 mg/ml) resulted in mean inhibition zones ranged between (8.0 - 9.2 mm). Penicillin was tested at two concentrations (125 and 500 mg/ml) resulted in mean of 9.0 and 10.5 mm inhibition zones (Table, 5).

Table 5 Inhibition zone (mean±SE) of the hydroethanol extracts of *A. nilotica* fruits and *M. oleifera* leaves and penicillin antibiotic on *S. aureus* using disc diffusion method

A. nilotica fruits	Conc (mg/ml)	1.97	7.88	31.52	126.06	504.25
	Inhibition (mm)	6.3 ± 0.23d	6.7 ± 0.33d	6.7 ± 0.33d	7.5 ± 0.12c	8.7 ± 0.33b
M. oleifera leaves	Conc (mg/ml)	1.320	5.281	21.125	84.50	338
	Inhibition (mm)	8 ± 0.08c	8.5 ± 0.33bc	8.7 ± 0.12bc	8.9 ± 0.12b	9.2 ± 0.23b
Pencillin	Conc (mg/ml)	125		500		
	Inhibition (mm)	9 ± 0.33 b		10.5 ± 0.12 a		

Different significant levels are marked with different letters

4. Discussion

Natural products have historically played a crucial role in drug discovery and development, offering a vast array of bioactive compounds that serve as direct therapeutic agents or as templates for synthetic modifications. The antibacterial potential of these compounds is increasingly important in the context of rising multidrug-resistant bacterial strains. The diverse mechanisms of action of natural products include disruption of bacterial cell walls, interference with protein synthesis, and inhibition of key metabolic pathways (Zhao et al., 2022).

In this study, the phytochemical profiles and antibacterial activities of *M. oleifera* leaves and *A. nilotica* fruits were investigated. The GC-MS analysis revealed that *M. oleifera* contains significant amounts of flavonoids, saponnins, and terpenoids, while *A. nilotica* also includes glycosides and tannins. These phytochemicals are known for their wide-ranging pharmacological activities. For instance, terpenoids, which are the main components of essential oils, possess notable antimicrobial properties. They enhance the effectiveness of antibiotics through synergistic or additive actions, reducing the required dose and minimizing side effects while broadening the antibacterial spectrum (Stan *et al*., 2021).

Terpenoids as the main components of the essential oils are known for their antimicrobial properties (they increase activity through synergistic or additive action, reduce required doses, reduce both cost and side effects, and increase the spectrum of activity). In bacteria, the cell membrane is a very important target for essential oil components, such as terpenoids, which could interfere with the phospholipid bilayers of the cytoplasmic membrane. These are phenolic monoterpene compounds and are particularly attractive to cell membrane structures due to their lipophilic nature.

These two compounds are able to dissolve the outer membrane of bacteria and release the lipopolysaccharide components, which increases the permeability of adenosine triphosphate in the cytoplasmic membrane and consequently alters the passive permeability of the cell. Some essential oils showed promising antibacterial activity against most of the bacteria studied and had an inhibition zone diameter > 11 mm (Stan *et al.,* 2021).

The ability of essential oils to disrupt bacterial cell membranes, particularly through interactions with the phospholipid bilayer, is well documented. This interaction increases membrane permeability, leading to the leakage of cellular contents and ultimately cell death. In this study, terpenoids from both *M. oleifera* and *A. nilotica* demonstrated similar effects, suggesting a plausible mechanism for their observed antibacterial activities (Stan et al., 2021). Additionally, flavonoids, another major group of compounds identified in the extracts, exhibit antibacterial properties by inhibiting nucleic acid synthesis and disrupting bacterial energy metabolism (Shamsudin *et al*., 2022).

The antibacterial assays showed a concentration-dependent inhibition of both *Escherichia coli* and *Staphylococcus aureus*. *E. coli* was more susceptible to the *A. nilotica* extract, likely due to the combined action of its phytochemical constituents, including glycosides and tannins. On the other hand, *S. aureus* showed greater susceptibility to the *M. oleifera* extract at its highest concentration (338 mg/ml), which resulted in an inhibition zone comparable to that of penicillin at 500 mg/ml. This suggests that *M. oleifera* could be a viable alternative to conventional antibiotics, especially against gram-positive bacteria, which lack the additional outer membrane found in gram-negative bacteria like *E. coli* (Rota *et al*., 2018).

To combat multidrug-resistance in bacteria, natural products (e.g., alkaloids, terpenoids, flavonoids, saponnins, tannins), can be used because of their versatile pharmacological effects. Flavonoids (as polyphenolic compounds) showed antibacterial property due to its tendency to inhibit the growth of many microorganisms and drug-resistant bacteria (Shamsudin *et al.,* 2022).

A. nilotica fruit detected 11 different compounds through the GC-MS technique, but none of them contain any N-containing compounds (hence, no alkaloids). Catechol is known to be a genotoxic compound, while Eicosanoic acid (the arachidic acid) and Hexadecanoic acid, 2-hydroxy-1- are fatty acids. Di-n-octyl phthalate is a colorless odorless oily substance, but 3-O-Methyl-d-glucose is consider as glucose analogs. From the 29 compounds detected within the ethanol extract of *M. oleifera* leaves, Eicosane (15.79%), Octadecane (3.10%) and Tetradecane (1.74%) are alkanes, Glycerol 1-palmitate (3.77%) and Diglycerol (2.11%) are alkylglycerols, Nonanal (1.42%) which is a colorless component of perfumes, while 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- is usually used as a disinfectant agent. Phenol, 2,6-bis(1,1-dimethylethyl) is a phenolic compound, and Isoquinoline, 3-methyl- (1.61%) is an aromatic constituent. Phytol acetate (0.65%) which has a waxy odor and usually used as flavor and fragrance agent. The detected compounds also contain Phytol (3.64%) which is a hydrogenated diterpene and it considered as a precursor for Vitamin E and K synthesis. The GC-MS analysis also showed 4 different N-containing compounds, two different Cl-containing compounds and one Si-containing compound.

It was clear that, the inhibition zone was concentration dependent, *E. coli* was relatively more susceptible to *A. nilotica* fruits than *M. oleifera* leave hydroethanol extract, and this may be due greatly to the phytochemical mixture detected in each extract. In a study conducted during 2020, *A. nilotica* fruit showed antimicrobial activity against some strains; e.g. *S. aureus and E. coli* (Abdalla *et al.,* 2020).

It was also clear that, *M. oleifera* leaves at its maximum concentration (338 mg/ml) which is less than that of penicillin (500 mg/ml) show promising alternative (9.2 mm inhibition zone compared to 10.5 for penicillin). *S. aureus* was relatively more susceptibility to *M. oleifera* leave than *A. nilotica* fruits hydroethanol extract. Also, *S. aureus* (as a gram-positive) showed relatively more susceptibility to penicillin than local stain *E. coli* as a gram-negative bacteria (Yu *et al.,* 2014). This may be due to the outer cover structure and to the antibiotic susceptibility of each stain.

The development of drug-resistance to current antibiotics, triggered the needs to develop new compounds to combat them (with low toxicity, specificity and availability). Plant compounds (e.g., terpenoids, saponnins, tannins and flavonoids) recorded to have antimicrobial and antiviral activities, and each had its own mechanism of action against bacterial strains (Stan *et al.,* 2021).

The global rise in antibiotic resistance necessitates the exploration of novel antimicrobial agents with unique mechanisms of action. Plant-derived compounds such as terpenoids, saponnins, tannins, and flavonoids offer promising alternatives due to their multi-targeted effects on bacterial cells. These phytochemicals can inhibit enzyme activity, disrupt cell membranes, and interfere with genetic material, making them effective against a wide range of pathogens, including drug-resistant strains (Stan *et al.,* 2021). Future studies should focus on isolating and characterizing the most

active compounds from these extracts, assessing their efficacy in vivo, and exploring their potential synergistic effects with existing antibiotics.

5. Conclusion

In conclusion, the findings from this study demonstrate that *Moringa oleifera* leaves and *Acacia nilotica* fruits contain bioactive compounds with significant antibacterial activity against both *E. coli* and *S. aureus*. The potential of these extracts to serve as complementary or alternative therapies to conventional antibiotics is promising, particularly in the fight against antibiotic resistance. Further research into the pharmacodynamics, toxicity, and clinical efficacy of these phytochemicals is warranted to fully realize their therapeutic potential.

Compliance with ethical standards

Acknowledgments

The authors extend their appreciation to the University of Gezira (Central, Microbiology, Applied Chemistry and Central Medical) Laboratories for technical support.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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