

Exploitation of Phytochemical Extracts of *Moringa oleifera* as Antimicrobial Agent against Human Pathogenic Bacteria

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Abstract

The rapid rise of antimicrobial resistance to commercially available antibiotics has led researchers to search for an alternative drug source. Medicinal plants pose as a potential source of natural antimicrobial drugs that compact drug resistant microorganisms. The qualitative phytochemical analysis of *M. oleifera* leaves and flowers extracts led to the identification of an important pharmacological bioactive natural compounds. The antibacterial activity of leaves and flowers extract were determined using agar disc diffusion method and MIC assay against selected human pathogens. The ethanolic and methanolic leaves and flowers extracts yielded 19.5%, 24.38%, 15.62% and 18.02% respectively. Leaves and flowers extracts were active against bacterial strains in a dose dependent manner. The ethanolic leaves extract (100 mg/mL) possessed potential antibacterial activity against the two tested Gram-negative bacteria: *E. coli*, *A. baumannii* and three Gram-positive bacteria: *Staphylococcus aureus*, *S. saprophyticus* and *E. Faecalis* by producing 20.00±0.50, 20.67±0.84, 15.33±1.04, 20.43±0.63, 25.83±0.76 mm zone of growth inhibition respectively compared to the control. However, methanolic leaves extract exhibited considerable inhibitory efficacy against two bacterial strains *A. baumannii* and *S. saprophyticus* with 17.17±0.59 and 15.81±0.62 respectively. The tested strains were highly sensitive to 200 mg/mL where the highest zone of inhibition was 30.29±0.92 mm observed against *E. faecalis*, and the lowest zone of inhibition was against *Staphylococcus aureus* with 20.33±1.04 mm. Both ethanolic and methanolic extracts showed antimicrobial efficacy more than that exhibited by ethanolic and methanolic flowers extracts. Ethanolic and methanolic leaves extracts had MIC of 200 mg/mL against all tested strains where ethanolic leaves extract showed MIC of 100 mg/mL against three strains, *E. coli*, *A. baumannii* and *S. saprophyticus*. This study suggests that the extracts of *M. oleifera* can be used to discover antibacterial agent for developing new pharmaceuticals against human pathogens.

Keywords: Antimicrobial activity, *Moringa* leaves, *Moringa* flowers, phytochemicals, MIC.

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INTRODUCTION

Microbial infections, especially those caused by bacterial ones, continue to be a leading cause of death globally. The increasing antibiotic resistance presents a serious threat to public health across the world, resulting in the spread of superbugs that are resistant to current antibiotics. Therefore, there is a legitimate concern that we may revert to an era prior to antibiotics and face widespread outbreaks of severe epidemic diseases, particularly those produced by microbial infections [1, 2]. A plethora of scientific studies now suggest that we are entering a phase referred to as the post-antibiotic era.

These investigations have revealed that epidemics thrive under, and detrimental human activities such as antibiotic over and misuse in different sectors. Moreover, bacteria possess a remarkable ability to adapt and acquired a multitude of resistance mechanisms [3, 4]. The pharmaceutical industry's waning interest and ability to develop new antibiotics led to a 40-year period where virtually no new classes of broad-spectrum antibiotics were brought to market. Companies focused instead on modifying the chemical structures of existing antibiotic classes [5, 6]. Regrettably, the present pace of antibiotic development is inadequate, and the outlook seems bleak

unless we depart from the existing method of creating synthetic antibiotics, which quickly become ineffective against multidrug-resistant bacteria. Most antibiotics developed since then have been modifications of previously discovered classes from the golden era of antibiotics [7, 8].

The WHO has already declared a scarcity of novel antibiotics to combat the threat of antimicrobial resistance. The search for novel antimicrobials from natural sources has increased as an alternative to commercial drugs to overcome these swiftly growing drug resistance issues [9, 10]. Medicinal plants have been the main source of curative drugs since ancient times and continue to play a vital role in modern medicine [11]. Over the past three decades, numerous scientific reports have suggested that medicinal plants could be a promising alternative to ineffective antibiotics in fighting infectious diseases due to their tremendous potential [12, 13]. Medicinal plants hold promise as a potential source for new antibacterial drugs due to their abundance of bioactive phytochemical compounds such as phenolic compounds, alkaloids, saponins, and terpenoids. These compounds have exhibited noteworthy antibacterial potential, primarily through membrane-disruption mechanisms, protein binding, and interference with intermediary metabolism, anti-quorum sensing, and anti-biofilm activity [14, 15]. However, to optimize their utilization as effective antibacterial drugs, further advancements in omics technologies and network pharmacology will be required in order to identify optimal combinations among these compounds or in conjunction with antibiotics. Many medicinal plants exhibit significant efficacy in targeting bacterial virulence factors and have potential for synergy with conventional antibiotics, making them viable candidates for further research and drug development. Medicinal plants such as *Tridax procumbens*, *Zanthoxylum xanthoxyloides*, *Eugenia caryophyllata*, *Alchornea cordifolia*, *Mitracarpus scaber*, *Psidium guajava*, *Cassia alata* and *Azadirachta indica* are known to be effective against a wide range of human microbial infections [16-18]. *Moringa oleifera* (Lam). is the most widely cultivated species of a monogeneric Moringaceae family, that is native to the sub-Himalayan tracts of Indian subcontinent (Fahey, 2005) which is widely used in Ayurveda, Unani and Ayush medication for treating bacterial infection, fungal infection, anti-inflammation, sexually-transmitted diseases, malnutrition and diarrhoea. *Moringa* species have long been recognized by folk medicine practitioners as having value in the treatment of tumours [19-21]. Hence, the present study was undertaken specifically to investigate the role of aqueous and organic extraction (methanolic and ethanolic extracts) of *M. oleifera* Lam. leaves and flowers as a potential antimicrobial agent against some selected human pathogenic bacterial strains.

This review presents compelling evidence supporting the antibacterial properties of various medicinal plants and their active compounds as promising alternatives. However, comprehensive investigations will be necessary to fully understand the mechanisms of action, toxicity, and pharmacokinetics of these plant-derived compounds. Nevertheless, the findings of this review establish a foundation for the development of novel and effective plant-based antibacterial drugs that can address the urgent public health threat posed by antibiotic-resistant bacteria. In line with regulatory practices for human medicines, it is now imperative for antibacterial herbal medicines to be encompassed within a comprehensive drug regulatory framework in all countries worldwide. Undoubtedly, there is a crucial imperative for effective coordination and collaboration among prominent entities such as the WHO, the FDA, the EMA, biotechnology companies, the pharmaceutical industry, and numerous regulatory agencies worldwide. This collaborative effort should aim to establish comprehensive and unambiguous guidelines for the exploration and advancement of plant-based antibacterial drugs, harnessing the extensive potential of traditional medicine in the development of therapeutic interventions for diverse and difficult-to-treat bacterial diseases.

MATERIALS AND METHODS

Plant Materials

Fresh leaves and flowers of *Moringa oleifera* (Lam) plant were collected from the botanical garden of Osmania University (O.U.), Hyderabad, Telangana state, India, in July 2023. The fresh leaves and flowers were plucked into clean, dry, labelled plastic bags and frozen at -80°C until use. A taxonomist (Professor A. Vijay Bhaskar Reddy) from the Department of Botany, O. U, authenticated and classified the plant. A voucher specimens No. Leaves/July/2023/1, Flowers/July/2023/2 were deposited at the herbarium of O. U. The collected parts were washed and rinsed with distilled water to remove dust and impurities and then air-dried at room temperature. Both dried leaves and flowers were grinded into coarse-fine particles with the help of a mechanical blender.

Extraction

The powdered leaves (50 g) and flowers (50 g) were extracted with ethanol and methanol in a ratio of (1:5). The mixture was kept for 72 h on a shaker incubator for agitation. The extracts were filtered using Whatman filter paper (No.1). The ethanolic and methanolic filtrate was concentrated with the use of rotary evaporator and were dried at 50 to 60 °C and the yielded percentage was calculated by the following formula $\text{Yield (\%)} = \frac{W_1}{W_0} \times 100$. Where W_1 represents the final weight of the *M. oleifera* leaf extract (concentrated extract) and while W_0 represents the initial weight of *M. oleifera* dried leaf and flowers powders.

The obtained powder was stored at -20 °C in deep freezer for further use [22].

Phytochemical Screening of extracts of *M. oleifera*

Qualitative phytochemical analysis of *M. oleifera* leaves was carried out using standard procedures to identify the constituents, Alkaloids, Flavonoids, Tannins, Phenols and proteins and amino acids as described by [23].

Test for alkaloids

1% of HCL prepared and added to the *M. oleifera* extracts in test tubes and heat it for 20 min with gently shake then leave it to cool. Take 1 mL of the cooled solution and added few drops of Wagner's reagent notice a creamy brown indicate the presence of alkaloids.

Test for flavonoids

3 mL of *M. oleifera* extracts was treated with concentrated Sulphuric acid. Appearance of yellowish orange show the presence of anthocyanin's, yellow to orange colour show the presence of flavones, and orange to crimson show the presence of flavonones.

Test for tannins

2 mL of *M. oleifera* extract and put it in test tube and gently heat it for few minutes, add 3 drops of 0.1% ferric chloride observe for brownish green or a blue-black colouration indicate the presence of tannin.

Test for Terpenoids (Salkowski test)

5 mL of each extract was mixed in 2 mL of chloroform, and concentrated H₂SO₄ (3 mL) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for phenols

3 mL of *M. oleifera* extract added to 5 mL distilled water then add few drops of 5% Ferric chloride notice dark green indicate the presence of phenols.

Test for Proteins and Amino Acids

The extracts were treated with a few drops of conc. nitric acid. The formation of the yellow colour indicates the presence of proteins. Ninhydrin test, to the extract, 0.25% w/v ninhydrin reagent was added and boiled for a few minutes. The formation of blue colour indicates the presence of amino acid [23].

Bacterial strains and growth conditions

All the five bacterial strains (two Gram-positive and two Gram-negative) were ATCC strains. Which are *E. coli* (ATCC 25922), *Acinetobacter baumannii* (ATCC 17978), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus saprophyticus* (ATCC 15305). These bacteria's strains were procured from American type culture collection (ATCC), USA. All the strains were

grown in tryptic soy broth (TSB) at 37 °C with shaking at 210 rpm for 18 h then sub cultured on MacKonkey, blood agar and nutrient agar and slants were preserved.

Antimicrobial activity

Inoculum preparation

The bacterial strains were grown in trypticase soy broth to an optical density at 600 nm of 0.5 which is equivalent to that of 0.5 McFarland standard ($1-2 \times 10^8$ CFU/mL). 1 mL of the bacterial suspension was centrifuged at 3400 g for 10 min at 4 °C. The pellets were washed and suspended in cold phosphate buffer saline (pH 7.4). The bacterial suspensions were further diluted to give a final bacterial concentration of approximately 5×10^6 CFU/mL [22].

Detection of antimicrobial activity of *Meringa oleifera* ethanolic and methanolic leaves and flowers extracts

Antibacterial activity of *M. oleifera* leaf and flower extracts was determined by agar disk diffusion assay at four concentrations (50, 100, 150 and 200 mg/mL). Muller Hinton agar was prepared according to the manufacturer's instructions and the plates were seeded with appropriate bacterial strains (*Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *A. baumannii* and *Escherichia coli*). Discs of 6 mm diameter were prepared from Whatman filter paper No. 24 and sterilized. The discs were impregnated in the extracts prepared concentrations. Standard antibiotic discs were used as a positive control to validate the antibacterial activity. The plates were incubate at 37° C for 18-24 h and the zones of growth inhibition were measured and recorded. Above experiments were carried out in triplicate for their confirmation [24].

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of leave and flowers extracts (ethanolic and methanolic extracts) of *Meringa oleifera* was determined by two-fold serial dilution method. Serial Stock dilution of 500 mg/ml was prepared. Serial dilution was done for rest of the samples achieve (25, 30, 35, 40, 45, 50, 100 and 200 mg/ml) as a final concentration for MIC determination. Briefly, 100 µl of varying concentrations of samples were added into the test tubes separately, containing 9 ml of the standardized suspension of tested bacteria (10^5 CFU/mL). The test tubes were incubated at 37°C for 18-24 h. Controls were used with the test organisms, using distilled water instead of the plant extract. The least concentration of the samples with no visible growth was taken as the MIC [25, 26].

Statistical Analysis

To evaluate associations between variables (antibiotic profiles), the data were analysed statistically using Student's "t" test, showing mean + standard deviation.

RESULTS

Moringa species is widely used as a medicinal plant worldwide due to its pharmacological properties and considerable medicinal values. The most common species of *Moringa* genus is *Moringa oleifera* which has rich sources of various phytochemical compounds that has antibacterial activity. In the present study, ethanolic and methanolic leaves extracts yielded 19.5% and 24.38% respectively. Where the methanolic and ethanolic flowers extracts yielded 15.62% and 18.02%. Phytochemical analysis of leaves and flowers extracts of *M. oleifera* leaves showed the presence of various bioactive secondary metabolites such as phenol, alkaloids, flavonoids and tannin...etc., are the most prominent substances as shown in table and the result of phytochemical test is presented in (Table 1). It is interesting to note that the phytochemicals detected in the ethanolic and methanolic leaves extracts were more than those detected from ethanolic and methanolic flowers extracts (Table 1). All these phytochemicals has been reported to exhibit multiple biological effects including antimicrobial, anti-inflammatory and antitumor activities.

Antibacterial activity of the leaves extracts:

The present study was concentrated on the determining antibacterial activity of *Moringa oleifera* leaves and flowers ethanolic and methanolic extracts applying different concentrations using disc diffusion method by measuring the zone of growth inhibition formed around the discs against three Gram-positive bacterial strains (*Staphylococcus aureus*, *S. saprophyticus*, *E. faecalis*) and two Gram-negative strains (*E. coli* and *A. baumannii*). The obtained data showed that the ethanolic and methanolic leaves extracts of *Moringa oleifera* leaves has a concentration dependent antimicrobial activity forming a good zone of inhibition compared to flowers same extracts against Gram-positive bacteria than Gram-negative bacteria. On the other hand, the ethanolic leaves extract possess good antibacterial activity compared to the ethanolic leaves extract against the same tested organisms. The most considerable antibacterial activity was possessed at 150, 200 mg/mL of ethanolic leaves extract (Table 2) (Fig 1). Leaves ethanolic extracts exhibited highest zone of inhibition against *E. faecalis* with 30.29 ± 0.92 mm at 200 mg/mL, where the lowest zone of growth inhibition was against *Staphylococcus aureus* with 20.33 ± 1.04 mm at the same concentration. In case of Gram-negative bacteria, the maximum zone of inhibition against *A. baumannii* and *E. coli* was 28.57 ± 1.21 and 28.00 ± 1.00 mm respectively at 200 mg/mL of the same extract while, the lowest was 12.83 ± 0.76 mm against *S. aureus* at 50 mg/mL (Table 2). Ethanolic leaves extracts 100 mg/mL possessed excellent antibacterial efficacy against all tested human pathogens, *E. coli*, *S. aureus*, *E. faecalis*, *A. baumannii* and *S. saprophyticus* as follows 20.00 ± 0.50 , 15.33 ± 1.04 , 25.83 ± 0.076 , 20.067 ± 0.84 , 20.43 ± 0.43 respectively (Table 2) (Fig 2). Our data confirms that the

most active extract was the ethanolic leaves extract (Fig 2). Our overall observations showed that the ethanolic leaves extract exhibited antimicrobial efficacy more than the antibiotics used as control (Table 1).

In comparison with standard antibiotics used in this study, ethanolic Leaves extract of *Moringa oleifera* showed considerable antimicrobial activity against *E. coli* with 20.00 ± 0.50 mm at 100 mg/mL compare to the antibiotic control used (AX) that exhibited 16.81 ± 0.98 mm. Where in case of *S. saprophyticus* the zone of inhibition was 20.43 ± 0.63 mm compared to the control (AZ) that possessed 16.53 ± 0.43 mm a zone of inhibition. The ethanolic leaves extract expressed very good zone of inhibition against *Staphylococcus aureus* with 15.33 ± 1.04 mm compare to the control antibiotic (AZ) that showed zero mm at 100 mg/mL. These results are considered incredible results. In case of *E. faecalis* the concentration 200 mg/mL of leaves ethanolic extract exhibited 30.29 ± 0.92 mm that was exceeded the zone of inhibition produced by control antibiotic (AX) that is 28.76 ± 1.12 mm (Table 2).

The methanolic leaves extract showed highest antibacterial activity against *A. baumannii* with 23.5 ± 0.87 mm and the lowest zone of inhibition against *E. faecalis* with 10.83 ± 0.87 mm both at 200 mg/mL (Table 2) (Fig 1). The same extract at 200 mg/mL exhibited excellent antibacterial efficacy against *A. baumannii* with zone of growth inhibition 24.57 ± 0.68 mm compared to antibiotic control (GE) 20.00 ± 0.12 mm. At 200 mg/mL *E. coli* and *S. saprophyticus* possessed considerable antibacterial activities with 18.17 ± 0.76 mm and 20.47 ± 1.12 mm respectively that exceeding the zone of inhibition of the antibiotic control (AX) and (AZ) that was 16.81 ± 0.98 mm and 16.53 ± 0.43 mm respectively. The methanolic leaves extract of 200 mg showed a zone of inhibition against *Staphylococcus Aureus* of 112.28 ± 1.04 mm compared with the antibiotic control (AZ) that possessed zero mm (Table 2).

The flowers ethanolic and possessed more antibacterial activities than methanolic extracts. The 200 mg/mL of flower ethanolic showed good antimicrobial activities against *S. aureus* and *A. baumannii* with a zone of growth inhibition 28.00 ± 1.00 for both while it had 8.50 ± 0.500 against *E. coli* (Table 3) (Fig 1). The methanolic flowers extract had 24.00 ± 0.98 a maximum zone of inhibition against *E. faecalis* at 200mg/mL concentration (Table 3). Flower ethanolic extract showed better antimicrobial activity against the tested bacterial species compared to the antibiotic control used in the study while methanolic flowers extract did not possess a good activity compared to the control antibiotics (Table 3) (Fig 1 & 2).

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) is an important assay to determine the exact

concentration at which will be no growth of the bacterial strain under test. In the current study, the ethanolic and methanolic extracts of *Moringa oleifera* leaves showed good antibacterial activity compared to ethanolic and methanolic flowers extracts. On the other hand, the ethanolic leaves extract possessed considerable antimicrobial activity compared to the methanolic leaves extract (Table 4 & 5).

The ethanolic leaves extract exhibited MIC of 100 mg/mL against, *A. baumannii* and *S. saprophyticus*. Where the same extract showed MIC of 160 mg/mL

against *E. faecalis* (Table 4). The highest MIC value 200mg/mL was observed against staphylococcus aureus.

The methanolic leaves extract did not show good MIC values against the tested bacterial strains. The methanolic flowers extract showed the best MIC of 160 mg/mL against *E. faecalis* (Table 5). The same flower extract possessed MIC value of 200 mg/mL against *Staphylococcus aureus*, *S. saprophyticus*, *E. coli* and *A. baumannii* (Table 5). On the other hands, the ethanolic and methanolic flowers extracts did not possess any kind of MIC at the used ranges that is from 20-200 mg/mL as the growth was detected even at 200 mg/mL.

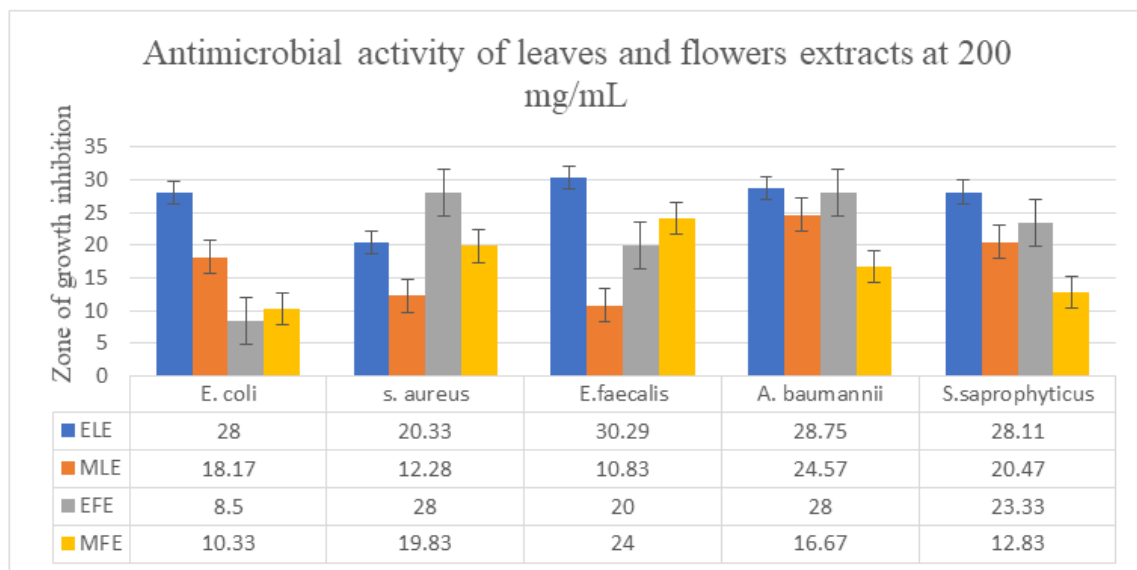


Figure 1:

ELE: Ethanolic leaves extracts, **MLE:** Methanolic leaves extracts, **EFL:** Ethanolic leaves extracts, **MFE:** Methanolic flowers extract.

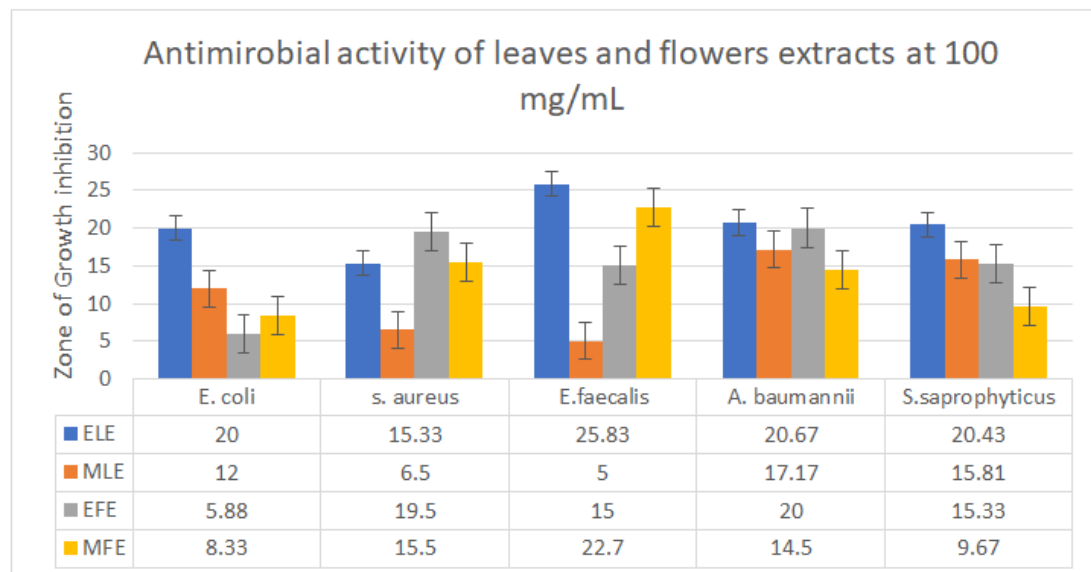


Figure 2:

ELE: Ethanolic leaves extracts, **MLE:** Methanolic leaves extracts, **EFL:** Ethanolic leaves extracts, **MFE:** Methanolic flowers extract.

Table 1: Phytochemicals analysis of *Moringa oleifera* leaves and flowers extracts

Solvent used	Alkaloids	Flavonoids	Tannins	Terpenoids	Phenols	Proteins & amino acids
Ethanolic leaves extract	++	++	++	++	++	++
Ethanolic flowers extract	+	+	+	+	+	+
Methanolic Leaves extract	++	++	++	++	++	++
Methanolic flowers extracts	+	+	+	+	+	+

+ indicates detection of phytochemical agents; - indicate non-detection or absence of phytochemical agents.

Table 2: Antimicrobial activity of *Moringa oleifera* leaves ethanolic and methanolic extracts using disc diffusion method

Bacterial species	Zone of inhibition in (mm)								
	Antibiotic discs	Different concentrations <i>M. oleifera</i> leaves ethanolic extract				Different concentrations <i>M. oleifera</i> leaves methanolic extract			
	30 µg/mL	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL
<i>E. coli</i>	AX 16.81±0.98	16.83± 1.26	20.00± 0.50	23.83± 1.50	28.00± 1.00	9.83± 1.26	12.00± 1.50	15.67± 0.58	18.17± 0.76
<i>Staphylococcus aureus</i>	AZ (0.00)	12.83± 0.76	15.33± 1.04	18.17± 0.76	20.33± 1.04	3.00± 1.00	6.5± 0.87	10.67± 0.58	12.28± 1.04
<i>E. faecalis</i>	AX 28.76±1.12	20.67± 1.15	25.83± 0.76	28.17± 0.86	30.29± 0.92	3.62± 0.77	5.00± 0.54	7.83± 0.59	10.83± 0.87
<i>A. baumannii</i>	GE 20.00±0.12	16.43± 1.11	20.67± 0.84	24.55± 0.49	28.57± 1.21	14.00± 0.54	17.17± 0.69	20.77± 0.99	24.57± 0.68
<i>S. saprophyticus</i>	AZ 16.53±0.43	16.67± 1.34	20.43± 0.63	25.00± 0.36	28.11± 0.24	12.88± 0.12	15.81± 0.62	18.61± 0.90	20.47± 1.21

Values are presented as mean ± S.E. n= triplicate experiments.

AX-Amoxicillin; AZ-Azithromycin; GE-Gentamycin

Table 3: Antimicrobial activity of *Moringa oleifera* flowers ethanolic and methanolic extracts using disc diffusion method

Bacterial species	Zone of inhibition in (mm)								
	Antibiotic discs	Different concentrations of <i>M. oleifera</i> flower ethanolic extract				Different concentrations of <i>M. oleifera</i> flower ethanolic extract			
	30 µg/mL	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL	50 mg/mL	100 mg/mL	150 mg/mL	200 mg/mL
<i>E. coli</i>	AX 15.38± 0.14	2.5± 0.50	5.88± 0.29	6.67± 0.288	8.5± 0.50	3.17± 0.76	5.16 ±0.58	8.33± 0.50	10.33±0.5 8
<i>S. aureus</i>	AZ (0.00)	16.58± 0.76	19.5± 0.87	23.83± 0.76	28.00± 1.00	12.17± 0.73	15.5 ±0.50	18.5± 0.50	19.83±0.2 9
<i>E. faecalis</i>	AX 26.91± 1.31	13.17± 1.26	15.00± 0.50	18.17± 0.76	20.00± 1.50	18.00± 0.54	20.5 ±0.51	22.67± 0.58	24.00±.98
<i>A. baumannii</i>	GE 19.48± 0.68	17.83± 1.26	20.00± 1.50	23.5± 0.87	28.00± 1.00	11.17± 1.04	12.17± 1.04	14.5± 0.50	16.67±0.7 6
<i>S. saprophyticus</i>	AZ 14.00± 0.34	13.17± 1.04	15.33± 1.24	19.33± 0.76	23.33± 0.76	4.82± 0.98	7.00± 0.53	9.67± 0.29	12.83±0.8 3

Values are presented as mean ± S.E. n= triplicate experiments.

AX-Amoxicillin; AZ-Azithromycin; GE-Gentamycin

Table 4: Minimal inhibitory concentration (MIC) activity of *Moringa oleifera* leaves ethanolic using broth dilution method

Test organisms	Different Concentrations of leaves ethanolic extracts (mg/mL)									MIC (mg/mL)
	20	40	60	80	100	120	140	160	200	
<i>E. coli</i>	+	+	+	+	-	-	-	-	-	100
<i>staphylococcus aureus</i>	+	+	+	+	+	+	+	+	-	200
<i>E. faecalis</i>	+	+	+	+	+	+	+	-	-	160
<i>S. saprophyticus</i>	+	+	+	+	-	-	-	-	-	100
<i>A. Baumannii</i>	+	+	+	+	-	-	-	-	-	100

+ indicates bacterial growth; - indicate no or absence of bacterial growth.

Table 5: Minimal inhibitory concentration (MIC) activity of *Moringa oleifera* leaves methanolic using broth dilution method

Test organisms	Different concentrations of leaves methanolic extracts (mg/mL)									MIC (mg/mL)
	20	40	60	80	100	120	140	160	200	
<i>E. coli</i>	+	+	+	+	+	+	+	+	-	200
<i>Staphylococcus aureus</i>	+	+	+	+	+	+	+	+	-	200
<i>E. faecalis</i>	+	+	+	+	+	+	+	-	-	160
<i>A. baumannii</i>	+	+	+	+	+	+	+	+	-	200
<i>S. saprophyticus</i>	+	+	+	+	+	+	+	+	-	200

+ indicates bacterial growth; - indicate no or absence of bacterial growth.

DISCUSSION

The rising prevalence of pathogenic bacteria resistant to the newly antibiotics has been expressed in the last three decades [27]. Medicinal plants have a wide range of active secondary metabolites from different parts such as leaves, seeds, flowers, fruits...etc. are often an incredible sources of pharmacological bioactive compounds that hold a medicinal and pharmaceutical application [28]. The current research reveals that *Moringa oleifera* plant shows the presence of many important secondary metabolites as phytochemical constituents such as alkaloids, flavonoids, proteins, saponins, tannins and terpenoids in different organic extracts (Table 1). The Antimicrobial activity of *Moringa oleifera* leaves and flowers extracts were expressed against different bacterial strains *E. coli*, *A. baumannii*, *Staphylococcus aureus*, *E. faecalis* and *S. saprophyticus* respectively. The ethanolic leaves extract showed maximum activity against *E. faecalis*, *S. saprophyticus*, and the methanolic leaves extract shows maximum activity against *A. baumannii*, *S. saprophyticus*, and *E. coli* respectively [Table 3] these results are in accordance with previous study [27] that showed organic extractions possessed antimicrobial activities against several bacterial species. The methanolic leaves extract showed antibacterial effect against both *A. baumannii*, *S. saprophyticus* and *E. coli* (Table 2). The results showed that the rising concentration of the methanol extracts increased the zone of inhibition. Generally, the medicinal plant *Moringa oleifera* leaves ethanolic and methanolic extracts exhibits good antibacterial activity compared to the same flowers extracts against the tested microorganisms [29].

Alkaloids, naturally occurring chemical compounds with basic nitrogen atoms, are known for their antibacterial activity. These natural bioactive compounds also possess pharmacological effects and are utilized in herbal medications [30]. Active compounds such as flavonoids and phenols have been identified to help inhibit the formation of biofilms [29, 30]. Therefore, an approach to inhibit the formation of biofilms involves identifying or extracting active compounds that act as inhibitors of biofilms. Maceration is one of the simple and inexpensive techniques for extracting the active substance from plants. This study focused on extracting phenolic and flavonoid compounds and their antibacterial activities against *P. aeruginosa*, *M. oleifera*

plants were extracted at room temperature in various methanol concentration ratios [31, 32]. Flavonoids promote the effect of vitamin C and act as antioxidants. They are also known to be biologically active against liver toxins, tumours, viruses and other microbes [33]. Tannins have revealed potential Antiviral, Antibacterial and Anti-parasitic effects. Saponins cause haemolysis of red blood cells [34]. Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, anti-neoplastic and other Pharmaceutical functions [35]. Tannins have shown potential Antiviral, Antibacterial and anti-parasitic effects. Saponins cause haemolysis of red blood cells. The antibacterial activity was screened because of their great medicinal properties towards the pathogenic organisms [36].

However, many previous results revealed that there were some studies which revealed resistance to different treatments. This resistance observed were matched from a study on the antibacterial properties of *Moringa oleifera* extracts to be ineffective. Such observations are consistent with our results where the flowers ethanolic and methanolic extracts showed less antimicrobial activity and the MIC showed no activities under the used concentrations.

CONCLUSION

This study has confirms that the leaves and flowers ethanol and methanolic extracts of *Moringa oleifera* possessed considerable degree of antimicrobial activities. Where leaf ethanolic and methanolic extracts shown to contain bioactive secondary compounds with a clear antibacterial activity, the most antimicrobial activities was expressed by ethanolic leaves extract that is capable of inhibiting the growth of Gram-positive and Gram-negative bacterial strains, *Staphylococcus aureus*, *E. faecalis*, *S. saprophyticus*, *E. coli* and *A. baumannii* respectively. The current study, achieved that *Moringa oleifera* can be used to discover more pharmacological bioactive substances which are responsible for antibacterial activity. Thus, the results in current study revealed significant inhibitory effect of methanol leaves extract of *Moringa oleifera*. The overall observations, showed that all selected microorganisms are highly susceptible to the leaves ethanolic extracts compared with the used commercially available antibiotics such as

Amoxicillin, Azithromycin, and Gentamycin. The MIC value 100 mg/mL was considered good achievable result for antimicrobial activity against three selected human pathogens using ethanolic leaves extracts. The bioactive properties of leaves and flower organic extracts make them a good candidates for potential pharmacological applications and excellent antimicrobial candidates in the nearby future.

Conflict of interest

The authors declare that they have no conflict of interests regarding the publication of this paper.

Author's contribution

R. S., wrote the manuscript and arranged the data. Mohd, developed the concept and monitor the experimental work. V. R., performed proof reading including grammatical and typographical errors. K. J., monitor the structure of manuscript.

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