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Exploring the Antimicrobial Potential of *Moringa Oleifera* Extracts Against *Acinetobacter Baumannii*

Saima Pervaiz[1*], Afshan Zia[2], Nida Javed[3], Sara Masood Cheema[3] Umaira Ahsan[4], Ikram Ul Haq[5], Saba Shamim[6] and Shah Jahan[7]

[1]Department of Immunology, University of Health Sciences, Lahore, Pakistan
[2]Department of Microbiology, University of Health Sciences, Lahore, Pakistan
[3]Department of Pathology, Azra Naheed Medical College, Lahore, Pakistan
[4]Department of Pathology, Abu Umara Medical and Dental College, Lahore, Pakistan
[5]Department of Pathology, Sahara Medical College, Narowal, Pakistan
[6]Institute of Molecular Biology and Biotechnology, University of Lahore, Lahore, Pakistan
[7]Institute of Allied Health Sciences, University of Health Sciences, Lahore, Pakistan

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***Corresponding Author:**

Saima Pervaiz
Department of Immunology, University of Health Sciences, Lahore, Pakistan
saimaprvz@gmail.com

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ABSTRACT

Background setups around the world, especially in Asian countries.Published data explained the situation of extreme drug resistance and left patients with very few antibiotic options against this particular pathogen. Plant and their extracts are well known for their antimicrobial properties. *Moringa oleifera* is one of the prime plants with multiple applications in industries, especially in health care. **Objective:** To determine the antimicrobial activity of *Moringa oleifera* plant extracts against *Acinetobacter baumannii*. **Methods:** In this experimental study, ethanol extracts of *Moringa oleifera* root, stem, and leaves were prepared and tested against *Acinetobacter baumannii* by the well diffusion method and minimum inhibitory concentration method. Four concentrations of all three extracts were prepared as 5mg/ml, 10mg/ml, 15mg/ml, and 20mg/ml. All experiments were tested three times. **Results:** No inhibition was seen at 5mg/ml, a 6mm zone diameter was observed at 10mg/ml, and 11mm was seen at 15mg/ml. **Conclusions:** It was concluded that *Moringa oleifera* showed good inhibition activity against *Acinetobacter baumannii.* This study does not include the quality analysis of extracts; after quality analysis and precise concentrations could create a huge opportunity in dealing with this extremely drug-resistant pathogen.

INTRODUCTION

Infectious microbes, especially bacteria, are the leading cause of death worldwide. The excessive use of antibiotics to treat various illnesses poses a serious threat to health globally, resulting in the development of super-bacteria that are more resistant to antibiotics.These super-resistant bacteria result in multiple epidemics.Some scientific survey predicts that we are now entering in "post-antibiotic era".The misuse of antibiotics results in detrimental effects. Furthermore, bacteria are developing different mechanisms to cope with a broad spectrum of antibiotics [1]. Among those multidrug-resistant bacteria, *Acinetobacter baumannii* is a well-known opportunistic pathogen causing infections in healthcare settings [2].A significant risk to public health because it is becoming more resistant to multiple antibiotics [3].It is a gram-negative bacterium causing nosocomial infections.This

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microbe causes a wide range of infections, including ventilator-associated pneumonia, meningitis, urinary tract infections, and skin-related issues.*A. baumannii* is declared the leading microbe for antibiotic research as it has gained resistance to broad-spectrum antibiotics.It is one of the most important bioweapons due to its vast range of infections, facilitated by diverse mobility of genetic elements, genetic plasticity, integration of foreign determinants, and evolution [4, 5].Recently, there has been an increasing fascination with investigating alternative therapeutic approaches to address infections caused by multidrug-resistant bacteria such as *Acinetobacter baumannii*. From ancient times, a few plants have been suggested to act as medicinal plants due to their therapeutic properties and to treat various illnesses.Even fossil records suggest that humans were using medicinal plants a long time ago. The curative nature of these plants is due to the various organic and inorganic chemicals produced by them. Such plants produce or release a few natural drugs as a result of various metabolic processes. In the current era, medicinal plants have gained special attention due to the antibiotic crisis, i.e. bacteria have developed multidrug resistance.These medicinal plants have a wide range of metabolites, including flavonoids, phenolic compounds, terpenoids, and alkaloids [6, 7]. Among these medicinal plants, a native plant with the old name of "Sohanjna" is a plant that naturally grows in tropical and subtropical areas and is well-known for its wide range of medicinal properties [8].*Moringa oleifera* has its importance in medicine and industry due to the prime compounds produced by its leaves.Because of its high nutritional value, therapeutic properties, and dual function as a vegetable and a seed, the plant is widely capitalized in a variety of cultural traditions. Its significance in increasing the taste and flavor of food is particularly noticeable [9-10]. The *M. oleifera* fresh leaves are edible or otherwise cooked, these can also be stored as dried powder [11]. Previously published studies enlightened the nutritional importance, pharmaceutical importance, and industrial importance of the *Moringa oleifera* plant [12].A well-known property of plants is antimicrobial activity through the production of different antimicrobial chemicals for their defense system, such as alkaloids [13].Alkaloids and phenolic compounds are secondary metabolites of plants with significant antibiotic properties. In a previously published study, the antimicrobial activity of *Moringa oleifera* was successfully evaluated against *Escherichia coli* and *Staphylococcus aureus* [14].The primary reason for the spread of bacterial resistance to antibiotics worldwide is the excessive and unreasonable use of antibiotics.This has resulted in a decline in the efficiency of many common antibiotics against harmful bacteria.The ongoing development of this issue has required the investigation of new antibacterial chemicals.Recent research has emphasized the significance of medicinal plants as a valuable reservoir of natural antimicrobial compounds. Such plant extracts can act directly as antimicrobial agents or may increase the synthetic antibiotic activity via a synergistic effect [5]. A plant of prime importance cultivated for commercial purposes in Egypt as well as other African, American, and many other Asian countries.

Despite increasing global concern regarding multidrug-resistant *Acinetobacter baumannii*, there remains a limited body of research evaluating standardized plant-based alternatives with clearly defined concentrations and reproducible antimicrobial outcomes. Most existing studies on *Moringa oleifera* focus on general antimicrobial effects without detailed comparison of different plant parts or correlation with MIC values and clinical isolates of highly resistant strains. Additionally, mechanistic understanding and standardized extract profiling are still insufficient, particularly in relation to clinically relevant MDR pathogens. The current problem is the rising burden of antibiotic resistance in *A. baumannii*, where conventional antibiotics are losing effectiveness, creating an urgent need for alternative therapeutic agents. This study aims to assess the efficacy of *M. oleifera* for the inhibition of *Acinetobacter baumannii* and its pathogenic effects. More precisely, this study finds the mechanism of *M. oleifera* extract as an antimicrobial agent against *A. baumannii* and its pathogenesis inhibition. Finding out the antibacterial properties of *M. oleifera* against *A. baumannii* could have great significance for developing a prime therapeutic action plan to manage the rising incidence of multidrug-resistant bacterial infections.

METHODS

This was applied research. Laboratory experiments were performed at the Department of Microbiology, Institute of Molecular Biology and Biotechnology (IMBB), University of Lahore, from July 2023 to Nov 2023. An approval letter was taken from the biosafety management committee of the Institute of Molecular Biology and Biotechnology (IMBB) (CriMM/23/Research/30), University of Lahore, for taking the bacterial samples. *Moringa oleifera* was collected from a nearby nursery to collect fresh stems, leaves, and roots. The plant was washed properly with sterilized distilled water for dirt and debris removal. Later on, it was dried at room temperature in the shade. Stem, Root, and leaves of *M. oleifera* are shown in Figure 1.

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Figure 1: Stem, Root, and Leaves of *M. Oleifera*

Dried parts of the plants, leaves, stems, and roots were converted into powder separately and shown in figure 2.

Figure 2: Dried Powder of Stem, Root, and Leaves of *M. Oleifera*

For the extraction of alkaloids from powder of all three parts, a quantity of 10 grams of powdered *Moringa oleifera* was immersed in 50 mL of ethanol (70%) and left to soak for 24 hours at room temperature.The mixture was agitated periodically to promote optimal extraction [15].Finding shows the mixture of powdered stems, roots, and leaves of *M. oleifera* in 70% ethanol, as shown in figure 3.

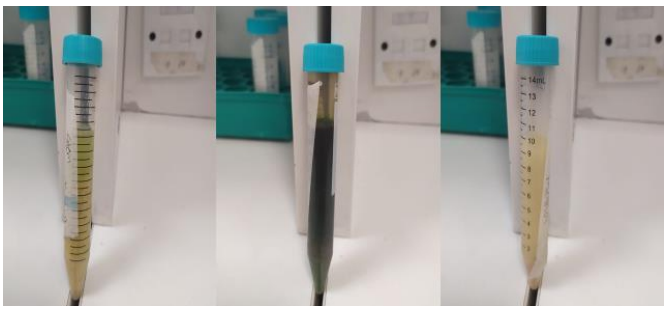

Figure 3: Mixture of Powdered Stem, Root, and Leaves of *M. Oleifera* in 70% Ethanol

Following an average of 24 hours, the extracts underwent filtration using Whatman filter paper with a pore size of 0.22 µm to eliminate any particulates.The liquid samples were condensed by removing excess pressure using a rotating evaporator at a temperature of 40°C [16]. A total of 10 isolates of *Acinetobacter baumannii* were used in this study.For this experiment, the strain of *Acinetobacter baumannii* known as ATCC 19606 was utilized.This strain was obtained from the American Type Culture Collection (ATCC). To get colonies that were free of contamination, the bacterial strain was grown on Mueller-Hinton agar plates and then incubated at a temperature of 37 degrees Celsius for twenty-four hours.To create a bacterial suspension with a concentration of roughly 1.5 × 10^8 CFU/mL, a single colony was introduced into Mueller-Hinton broth and incubated at 37°C with shaking at 150 rpm for 18 hours. Two different methods were used to check antimicrobial activity against *Acinetobacter baumannii.*Well diffusion method and serial dilution method. Well diffusion method: Already isolated *Acinetobacter baumannii* was diluted in sterile normal saline to prepare a 0.5% MacFarland standard. Suspension was spread on Muller Hinton agar by a sterile swab; this is called inoculating. 4 wells were made in each Muller Hinton Plate. 4 dilutions of each extract were prepared, like 25 µl, 50 µl, 75 µl, and 100µl in one ml sterile saline each. One drop was poured into each well of the already loaned MH plate. 4th well was poured with normal saline as a control. After incubation for 24 hours, activity was checked. Serial Dilution Method: Serial dilution tubes were sub-cultured on blood agar plates for growth testing, the highest inhibition was seen on leaves, with no growth after 50 µg/ml, and the least activity was seen on the stem in where 50 µg/ml was not sufficient concentration to inhibit the growth. Descriptive statistics were used to estimate the suitable concentration of *Moringa oleifera's* different parts. Reading and results were obtained in triplets then the results were expressed as mean ± standard deviation(SD).

RESULTS

A total of 10 *Acinetobacter* isolates were obtained from clinical samples such as urine, pus. Wound swab, tracheal aspirate, and blood. The clinical samples used for the isolation of 10 isolates of *A. baumannii* along with the total count in terms of percentage, is given in table 1.

Table 1: Clinical Samples of *A. baumannii Isolates*

Clinical Samples	n (%)
Urine	2 (20%)
Pus	2 (20%)
Wound Swab	1(10%)
Tracheal Aspirate	4 (40%)
Blood	1(10%)
Total	10 (100%)

As per CLSI, the cut-off range for inhibition was as Amikacin=<4.0 µg/mL, Tobramycin=<4.0 µg/mL, Imipenem =<2.0 µg/mL, Meropenem=<2.0 µg/mL, Piperacillin-tazobactam<4.0 µg/mL, Ciprofloxacin = <1.0 µg/mL, Minocycline = <4.0 µg/mL and Tigecycline was from EUCAST guideline as <4.0 µg/mL. Ciprofloxacin was sensitive in only one strain, showing a susceptibility rate of 5%, and was the least sensitive drug, while maximum sensitivity was shown by tigecycline, with 10 sensitive strains, as shown in table 2.

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Table 2: MIC results for *A. baumannii* Isolates

Amikacin	Tobramycin	Tigecycline	Imipenem	Meropenem	Pipracillin/Tazobactam	Ciprofloxacin	Minocycline
0.75	1	0.5	8	8	32	256	256
16	16	4	16	16	256	256	256
2	1	0.75	2	2	1	2	0.5
32	8	0.5	256	256	256	256	8
256	32	0.5	8	4	16	256	1
256	256	8	8	4	16	256	8
1	2	16	16	4	32	256	256
32	16	2	8	8	256	256	8
256	8	1	1	0.75	1	256	0.25
256	256	1	256	256	256	256	256

MIC unit µg/mL

Well Diffusion Method: After 24 hours of incubation, no antibacterial activity was shown by extracts, the highest activity was shown by leaves with a minimum of 50 µg/ml with an inhibition zone of 22 ± 9 mm, and the least activity was shown by the stem with a minimum 75 µg/ml with inhibition zone 16.5 ± 11 mm. Root extract showed a zone of 19±8 mm. Figure 4 shows the experimentation of the diffusion method for the Inhibition effect of *M. Oleifera* suspension on *A. baumannii* are shown in Figure 4.

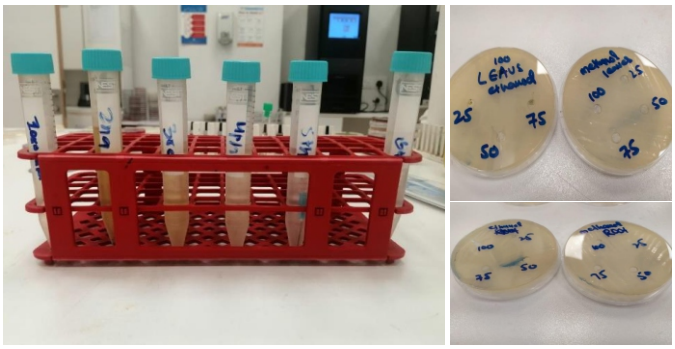

Figure 4: Inhibition Effect of *M. Oleifera* Suspension on *A. Baumannii*

This table narrates the effect of different concentrations of leaves, roots, and stems on 10 different isolates of *A. baumannii* (isolated from different sources as mentioned in methodology) (Results are expressed in descriptive stats, i.e. mean ± Standard deviation). The zone of inhibition is given in Table 3.

Table 3: Effect of Different Concentrations of Leaves, Roots, and Stems of *M. Oleifera* On *A. Baumannii* Isolates

Bacterial #	Leaves				Root				Stem			
	25µg/ml	50µg/ml	75µg/ml	100µg	25µg/ml	50µg/ml	75µg/ml	100µg	25µg/ml	50µg/ml	75µg/ml	100µg
1	0 ± 0	31 ± 0.8	36 ± 0.5	40 ± 0.08	0 ± 0	28 ± 0.4	32 ± 0.3	40 ± 0.2	12 ± 0.5	20 ± 0.3	24 ± 0.3	27 ± 0.2
2	0 ± 0	25 ± 0.6	31 ± 0.08	40 ± 0.3	0 ± 0	16 ± 0.2	21 ± 0.08	40 ± 0.2	0 ± 0	15 ± 0.6	18 ± 0.4	31 ± 0.1
3	0 ± 0	17 ± 0.08	23 ± 0.1	40 ± 0.2	0 ± 0	15 ± 0.3	17 ± 0.2	40 ± 0.08	0 ± 0	13 ± 0.1	15 ± 0.4	28 ± 0.2
4	0 ± 0	16 ± 0.1	18 ± 0.2	40 ± 0.08	0 ± 0	21 ± 0.2	24 ± 0.3	40 ± 0.3	12 ± 0.3	13 ± 0.3	12 ± 0.6	23 ± 0.3
5	0 ± 0	30 ± 0.2	36 ± 0.08	40 ± 0.3	13 ± 0.3	25 ± 0.08	27 ± 0.1	40 ± 0.1	12 ± 0.5	12 ± 0.5	13 ± 0.3	16 ± 0
6	13 ± 0.4	16 ± 0.08	21 ± 0	40 ± 0.1	18 ± 0.3	17 ± 0.6	18 ± 0	40 ± 0.3	0 ± 0	0 ± 0	19 ± 0.3	21 ± 0.4
7	11 ± 0.5	21 ± 0.4	24 ± 0.3	40 ± 0.3	0 ± 0	15 ± 0.5	20 ± 0.2	40 ± 0.08	0 ± 0	12 ± 0.6	15 ± 0.3	16 ± 0.5
8	0 ± 0	24 ± 0.1	27 ± 0.2	40 ± 0.08	11 ± 0.2	20 ± 0	23 ± 0.1	40 ± 0.2	0 ± 0	16 ± 0	19 ± 0.3	23 ± 0.5
9	0 ± 0	21 ± 0.1	23 ± 0.2	40 ± 0.2	12 ± 0.4	17 ± 0.3	21 ± 0.8	40 ± 0.3	12 ± 0.6	15 ± 0.5	18 ± 0.2	19 ± 0.4
10	0 ± 0	19 ± 0.1	21 ± 0.08	40 ± 0.2	12 ± 0.3	16 ± 0.3	18 ± 0.2	40 ± 0.08	12 ± 0.5	17 ± 0.2	12 ± 0.5	13 ± 0.1

The Bar graph shows antimicrobial activity at 25µg/ml vs zone of inhibition. Not much microbial activity was observed from leaves, root and stem extracts, except in isolate 6, root extract shows the maximum inhibition zone, while lesser or no activity was observed in other isolates, as shown in Figure 5.

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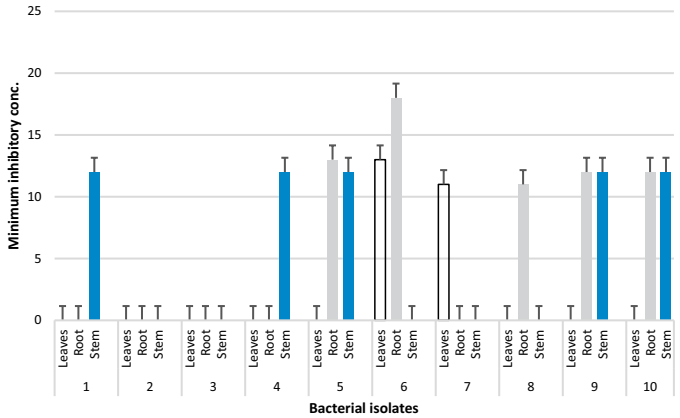

Figure 5: Antimicrobial Activity at 25µg/mL vs Zone of Inhibition

The Bar graph shows antimicrobial activity at 50µg/ml vs zone of inhibition.The leaf extract shows the maximum inhibition zone in all 10 bacterial isolates.Hence, 50µg/ml is declared as the minimum inhibitory concentration for bacterial growth in this work and shown in Figure 6.

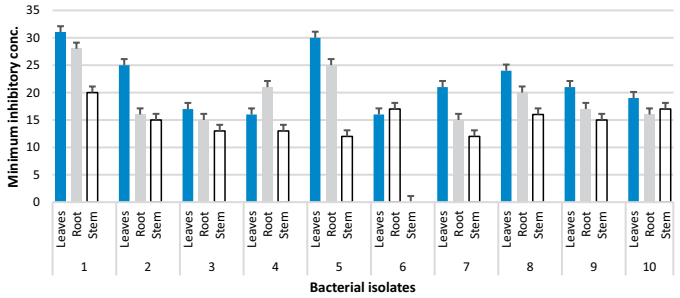

Figure 6: Antimicrobial Activity at 50 µg/ml vs Zone of Inhibition

The Bar graph shows antimicrobial activity at 75µg/ml vs zone of inhibition.Here, the stem extracts show the least antimicrobial activity, while leaves and root extracts show more antimicrobial activity, as shown in Figure 7.

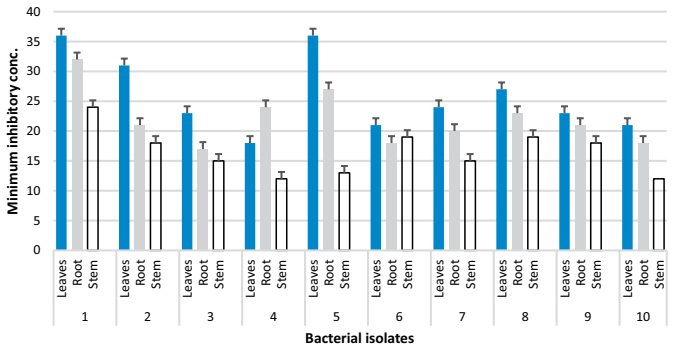

Figure 7: Antimicrobial Activity at 75 µg/ml vs Zone of Inhibition

The Bar graph shows antimicrobial activity at 100µg/ml vs zone of inhibition. Bacterial isolates are inhibited by leaves and root extracts, however, much lesser activity was observed in case of stem extracts, as shown in Figure 8.

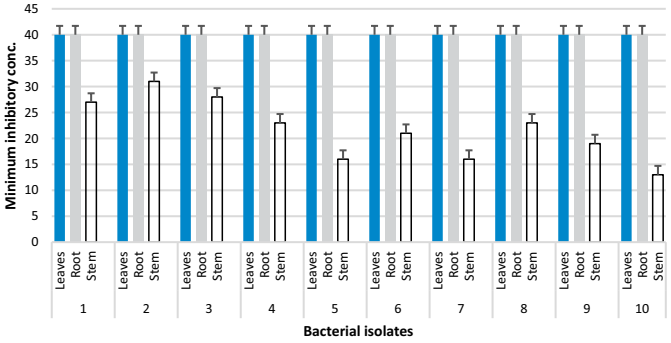

Figure 8: Antimicrobial Activity at 100 µg/ml vs Zone of Inhibition

Serial dilution tubes were sub cultured on blood agar plates for growth testing, highest inhibition was seen on leaves with no growth after 50 µg/ml plate, and the least activity was seen on the stem in which 50 µg/ml was not sufficient concentration to inhibit the growth as shown in figures given below. After 24 hours of incubation, growth was observed only on a 50µl dilution plate. There was no bacterial growth on other dilutions. Finding shows the Serial dilution inhibition effect on blood agar in Figure 9.

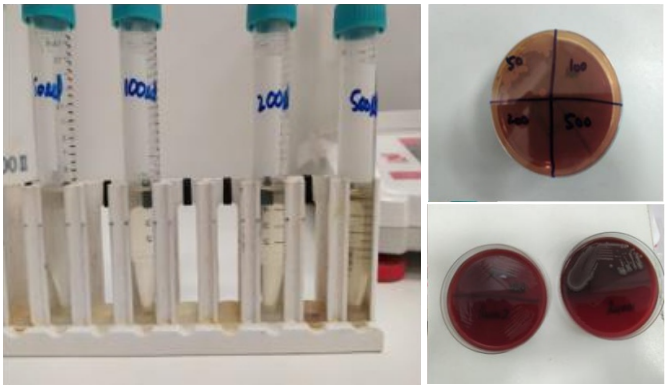

Figure 9: Serial Dilution Inhibition Effect on Blood Agar

DISCUSSION

The present study investigated the antibacterial activity of extracts from different parts of a plant against clinical isolates of *Acinetobacter baumannii,* along with their susceptibility to commonly used antibiotics.This study contributes to finding some alternate sources of antibiotics to overcome the synthetic antibiotics load and minimize drug resistance.The results of this research explained the antimicrobial activity of different parts of the *Moringa oleifera* plant. The extracts obtained from leaves had the most significant action, with a minimum inhibitory concentration (MIC) as low as 50 micrograms per milliliter (µg/ml) and an inhibition zone measuring 22 ± 9 millimeters. On the other hand, stem extracts exhibited the lowest level of activity, indicating a greater concentration (at least 75µg/ml) to achieve inhibition.The root extracts exhibited moderate activity, as evidenced by an inhibition zone measuring 19 ± 8 mm.The same findings were reported in

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previously published studies about plant extract inhibition effects against *A. baumannii* [17]. Our investigations find increased antibiotic resistance among the present study isolates of *A. baumannii.* These isolates were showing resistance against multiple drugs, as shown in Table 1; some were very potent antibiotics like imipenem, meropenem, and ciprofloxacin. Some of these antibiotics, like amikacin and tigecycline, showed good susceptibility. Still, there was some variation among the isolates regarding susceptibility patterns. These findings showed similarity with the common pattern of multidrug resistance observed in isolates of *Acinetobacter baumannii* [18]. A significant association was found between extracts' ability from plants to inhibit bacteria and restrict these bacteria from developing drug resistance. Strains that showed higher resistance to conventional antibiotics were found to be more prone to the plant extract, indicating a possible synergistic or complementary effect between chemicals derived from plants and antibiotics. Multiple previous studies have shown that the combination of plant extracts and synthetic antibiotics enhances their antibacterial efficacy against multiple drug-resistant bacteria [19]. This study also provides valuable and prime evidence of the antimicrobial ability of plant extracts against *A. baumannii.* But still, a precise concentration is very necessary to produce a prime drug, which may require Molecular docking studies, as conducted by [20], could help identify potential targets for synergistic interactions between plant compounds and antibiotics. Additionally, clinical trials are warranted to evaluate the safety and efficacy of plant-derived therapies in human populations.This research outcome gave a way to develop a novel combination therapy for the inhibition of multidrug-resistant *A. baumannii.* Plant extracts have a special amount of flavonoids with prime applications in industry.

This study is limited by its relatively small sample size and its in vitro experimental design, which may not fully reflect in vivo biological responses. The absence of detailed phytochemical quantification and compound isolation restricts understanding of the specific active constituents responsible for antibacterial activity. Additionally, toxicity profiling and clinical validation were not performed, which limits translational application. Future research should focus on molecular docking and mechanistic studies to identify active compounds and bacterial targets. Standardization of extract concentrations, in vivo animal studies, and clinical trials are recommended to validate safety and therapeutic potential. Furthermore, evaluating synergistic effects between *Moringa oleifera* extracts and conventional antibiotics could provide stronger evidence for combination therapy against MDR pathogens.

CONCLUSIONS

It was concluded that this research described the antimicrobial activity of different parts of the Moringa oleifera plant. The extracts obtained from leaves had the most significant action with a minimum inhibitory concentration (MIC) as low as 50 micrograms per milliliter (µg/ml).

Authors' Contribution

Conceptualization: SP
Methodology: AZ, NJ, SS
Formal analysis: UA
Writing and Drafting: SMC, IUH, SJ
Review and Editing: SMC, IUH, SJ

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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