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In vitro antibacterial activity of *Moringa oleifera* leaf and seed extracts against antibiotic-resistant bacteria and clinical isolates

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Abstract

Background Resistance to antibiotic has increased extensively over the years, posing a serious threat to public health. Plants are promising sources of antibiotic-resistance inhibitors. The aim of this study is to investigate the antibacterial properties leaf and seed extracts of *Moringa oleifera* against strains of pathogenic bacteria.

Methods Eight extracts of *M. oleifera* leaves and seeds were prepared using four solvents; water, ethanol, ethyl acetate and petroleum ether, and evaluated against drug-resistant clinical isolates and standard laboratory strains of *Staphylococcus aureus*, *Escherichia coli and Pseudomonas aeruginosa* using disc diffusion method. Minimum inhibitory concentration of the extracts was determined by broth microdilution method.

Results The results show that aqueous extracts of both leaves and seeds had higher zones of inhibition for all the isolates, including Methicillin-resistant *S. aureus*, *E. coli* ESBL, *meropenem-resistant E. coli*, than the rest of the extracts, with zones of inhibition ranging from 20.0 mm to 32.5 mm. Ethyl acetate seed extract also inhibited all the isolates except Meropenem-resistant *E. coli*. Likewise, ethyl acetate leaf extract inhibited all except the *E. coli* isolates and had significantly higher zones inhibition for almost all the isolates compared to the ethyl acetate seed extract. However, aqueous seed extract showed the lowest minimum inhibitory concentration against all bacteria strains, ranging from 50 mg/ml to 200 mg/ml.

Conclusions The data indicates that aqueous and ethyl acetate extracts of *M. oleifera*, particularly aqueous seed extract, have the potential of capturing inhibitors against drug-resistant and clinical isolates of *S. aureus*, *P. aeruginosa* and *E. coli*.

Keywords *Moringa*, Antimicrobial activity, Drug-resistant bacteria

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Background

Bacterial infections are among major causes of ailments and deaths globally with much higher prevalence in developing and resource scarce countries [1]. Antibiotics have help immensely in combating these infections, improving public health and life expectancy [2]. However, these gains are under threat from development and spread of antimicrobial resistance (AMR) and multidrug resistance (MDR) arising from misuse, abuse, overuse and extensive use of antibiotics [3–5]. Multi-drug resistance refers to a situation where microbial agents are resistant to at least one drug from three or more chemical classes [6].

AMR poses a serious threat not only to the health of people of developing countries but also people in developed countries. For example, it has been estimated that more than two million people in the United States become ill with antibiotic-resistant illnesses annually with at least 23,000 associated deaths [7]. It has also been estimated that antimicrobial resistance cost more than nine billion euros annually, in Europe and yearly increases direct healthcare costs in the United States by 20 billion dollars, not to mention the related loss of productivity [8]. If the trend of development and spread of AMR is not effectively dealt with, by 2050, it has been projected that its associated annual mortality may reach about 10 million people worldwide and Africa may contribute more than 40% of such deaths [8].

The ever-increasing burden of antimicrobial resistance bring to the fore the need to search for new antimicrobials. In this vein, the appropriateness of the proposal for development of new drugs among other strategies by the World Health Organization [9], cannot be overemphasized. Traditionally, many plants have long been used in herbal medicine, particularly, by Indians and Africans and numerous studies have shown that many plants, including *Moringa oleifera* have antimicrobial activity [10–12]. Some have wide range of polypeptides, phenols and polyphenols that could provide leads to the discovery of new antibiotics [13].

Moringa oleifera is an edible plant, used to treat an array of illnesses including infectious diseases [14]. Analyses of phytochemical constituents of *Moringa oleifera* leaves and seeds indicate that in addition to these medicinal properties, the plant also contains nutritional substances such as proteins, fibbers, minerals, vitamins A, B, C, fats and amino acids, and is consumed in several countries for its nutritional value [14–16].

There is evidence that show that the seeds, stem bark, and leaves have antimicrobial activity [14]. However, little data exist on the activity of *M. oleifera* extracts on drug-resistant bacteria. The aim of this study, therefore, is to examine the activity of organic solvents and aqueous seed and leaf extracts of *M. oleifera* against drug-resistant isolates of gram-positive and gram-negative bacteria isolates.

Methods

Plant materials, bacterial isolates and study design

Moringa leaves and seeds were sampled from areas of no known chemical pollution of the University of Ghana campus (GPS: GA-522-3371) and identified by a botanist of Ghana Herbarium, with a voucher specimen (GF 1603/23) kept at the Herbarium. The plant samples were then extracted and tested for their antimicrobial properties against various resistant bacterial strains, including MRSA and *E. coli*. Control strains and clinical isolates were also used to evaluate the efficacy of the *Moringa* extracts. Clinical isolates of *E. coli* were obtained from blood and *S. aureus* isolates from dog skin lesions. All the strains were isolated and identified at the Medical Microbiology Laboratory of the University of Ghana Medical School.

Preparation of *Moringa* oleifera leaves and seeds for extraction

The leaves of *M. oleifera* were destalked and the seeds removed from the pods and dehusked. They were shade-dried for two weeks at an average room temperature with continuous turning of the leaves to prevent fungal growth. They were kept away from direct sun light to avoid destruction of active compounds. The white seed kernels and the leaves were separately ground to powdered form using a blender. The powdered seed and leaves were packaged in clean sterile air-tight containers till further processing.

Maceration and extraction

Four solvents; ethyl acetate, petroleum ether, absolute ethanol and water were used for the extractions. The powdered plant materials were soaked in solvents at a ratio of 1:5 (*w*/*v*) which is within the widely used range of 1:4 to 1:16 [17]. The soaked plant materials were allowed to stand for 72 h to obtain filtrates of plant compounds. The solvents were added to their respective residues to repeat the maceration procedure multiple times. The organic solvents and aqueous extracts were then dried using a rotary evaporator (Cole-Parmer) and a freeze dryer, respectively.

Antibacterial activity testing of extracts

Antimicrobial activity of the crude extracts was assayed using the agar well diffusion method. Nutrient agar was prepared according to the manufacturer's instruction and dispensed into universal bottles at 20 ml per aliquot. They were then sterilized at 121 °C for l5 minutes using an autoclave and allowed to cool to about 45 °C before dispensing into petri dishes. Following the initial

culturing, bacterial isolates were suspended, and optical density readings were adjusted to a 0.5 McFarland standard. The petri dishes were streaked with the 0.5 McFarland standard of each of the microorganism; five resistant strains each of MRSA, *P. aeruginosa*, *E. coli* ESBL and Meropenem-resistant *E. coli*, five different strains of clinical isolates of pathogenic *S. aureus* and *E. coli* as well as control strains each of *E. coli* ATCC 25,922, *S. aureus* ATCC 25,923 and *P. aeruginosa* ATCC 29,853. A sterile corkborer was used to bore equidistant wells of about 5 mm in diameter into the agar and filled with the *M. oleifera* extracts. Antibiotics, amikacin and linezolid were used as positive controls for gram-negative (*E. coli* and *P. aeruginosa*) and gram-positive (*S. aureus*) bacteria, respectively, at 30 µg/ml. Dimethyl sulfoxide (DMSO) and distilled water were also used as negative controls for organic and aqueous extracts, respectively. The plates were then incubated at 35 °C ± 2 °C for 18–24 h. Effect of the extract was assessed by measuring the diameters of zones of inhibition to the nearest millilitre.

Determination of the minimum inhibitory concentration (MIC)

To access the degree of antibacterial activity of the extracts, the MIC of extracts with activity against most bacteria strains, aqueous and ethyl acetate extracts, were determined using the broth dilution method. Twofold serial dilutions of extracts were made using distilled water and DMSO, respectively, with the concentration ranging from 400 mg/ml to 3.125 mg/ml.

According to the inoculum standardization, 100 µl of the bacterial isolates were added to 100 µl of the serially diluted extracts in 96-well microtiter plates. The plates were then examined for visible growth or turbidity to determine the MIC after 24 h of incubation at 37 °C. The lowest final well concentration of diluted extract fractions with no visible growth was regarded the MIC.

Statistical analyses

Data was entered into excel spreadsheets and exported into GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) for analysis. T-test was employed to compare the mean zones of inhibition, except for Meropenem-resistant bacteria and clinical isolates, where non-parametric Tukey test was used. $P < 0.05$ is considered significant. Mean of zones of inhibitions and the percentage of each isolate inhibited were presented in tables.

Results

Proportions of isolates inhibited by the extracts

Table 1 shows the proportion of the resistant and clinical isolates inhibited by eight different extracts, over the 18–24 h incubation period. All isolates were susceptible to the control antibiotics. Interestingly, petroleum ether extracts for both seed and leaf showed no antibacterial activity and, therefore, had no zones of inhibition. The same was observed for both control and clinical isolates of all types of bacteria investigated. Aqueous extracts of both seed and leaf inhibited all (100%) bacterial isolates, including resistant and control strains as well as clinical isolates. Except for Meropenem-resistant *E. coli*, all resistant isolates of each bacteria type were inhibited by the ethyl acetate seed extracts. Additionally, ethyl acetate seed extracts inhibited all control and clinical isolates for all types of bacteria investigated. All isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA) (100%) were inhibited by ethyl acetate leaf extracts, while some resistant isolates of *P. aeruginosa* (60%) were inhibited by the ethyl acetate leaf extracts. None of the isolates of *E. coli* ESBL and Meropenem-resistant *E. coli* were inhibited by the ethyl acetate leaf extract. Except for *E. coli*, all control and clinical isolates were inhibited by ethyl acetate leaf extract. Lastly, ethanolic seed extract inhibited only 20% of *S. aureus* isolates and no activity against all resistant strains. A similar trend was observed for the ethanolic leaf extract, except that all MRSA isolates were inhibited.

Table 1 Proportion of resistant and clinical bacterial isolates inhibited by extracts

Bacteria	Proportion of Isolates Inhibited (%)							
	Absolute ethanol		Petroleum ether		Water		Ethyl acetate	
	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf
Resistant strains								
MRSA ($n=5$)	0	100	0	0	100	100	100	100
P. aeruginosa ($n=5$)	0	0	0	0	100	100	100	60
E. coli ESBL ($n=5$)	0	0	0	0	100	100	100	0
Meropenem-resistant *E. coli* ($n=5$)	0	0	0	0	100	100	0	0
Clinical isolates								
S. aureus ($n=5$)	20	0	0	0	100	100	100	100
E. coli ($n=5$)	0	0	0	0	100	100	100	0

The values are expressed as a percentage of isolates treated. Antibiotics - amikacin was used as positive control for *E. coli* and *P. aeruginosa*, and Linezolid for *S. aureus*. All isolates were inhibited by the positive controls.

Inhibitory activity of seed and leaf extracts compared

Overall, aqueous extracts had better activity than the rest of the extracts. It had activity against all the isolates. The best zones of inhibition were also observed in aqueous extracts, with values ranging from 20.0 to 32.5 mm. This was followed by ethyl acetate extracts. Ethyl acetate seed extract had effect on all the isolates except Meropenem-resistant *E. coli*, with most isolates having zones of inhibition greater than 20 mm. Ethyl acetate leaf extract, on the other hand, inhibited all the MRSA and *P. aeruginosa* isolates but none of the *E. coli* strains. Ethanolic seed extract had no effect on any of the resistant strains but inhibited the clinical and the standard isolates of *S. aureus*. It also inhibited the standard strain (ATCC 25922) of *E. coli.* The ethanolic leaf extract, on the other hand inhibited the MRSA, and ATCC 25,923 *S. aureus* and ATCC 29,853 *P. aeruginosa* standard strains. The positive controls (amikacin and Linezolid) had zones of inhibition ranging from 18.7 mm to 25.8 mm, at 30 µg/ml.

The least activity was observed for petroleum ether extracts, which had no inhibitory effect on all the isolates (Table 2). Against MRSA, aqueous seed (26.20 mm), ethyl acetate seed (24.60 mm) and aqueous leaf (21.40 mm) extracts had stronger activity than the other extracts, as revealed by their zones of inhibitions. For *Pseudomonas aeruginosa*, zones of inhibition suggest that the activity of the extracts was in the order: aqueous leaf extract (26.60 mm) > aqueous seed extract (23.60 mm) > ethyl acetate seed extract (22.60 mm). The best inhibitory effect of extracts against *E. coli* ESBL was observed with aqueous leaf (28.40 mm), aqueous seed (25.00 mm), and ethyl acetate seed (16.20 mm) extracts (Table 2).

The zones of inhibition of the extracts were also compared with those of the positive control. The results revealed that the aqueous seed extract and the positive control antibiotics had similar zones of inhibition against most isolates, except for *P. aeruginosa* and clinical isolates of *S. aureus*, for which the aqueous seed extracts had significantly higher zones of inhibition ($p = 0.042$ and $p = 0.001$, respectively). In contrast, the zones of inhibition of the aqueous leaf extract were significantly higher than those of the positive controls ($p < 0.05$) for most isolates analysed (Table 3). However, against MRSA, the positive control had a higher zone of inhibition compared to the aqueous leaf extract ($p = 0.036$), while no significant difference was observed against meropenem-resistant *E. coli* ($p = 0.134$). Similar to the aqueous seed extract, the ethyl acetate seed extract had significantly higher zones of inhibition against *P. aeruginosa* and clinical isolates of *S. aureus* than the positive controls. Conversely, the positive controls had higher zones of inhibition compared to the ethyl acetate leaf extracts for all the isolates (Table 3).

Comparing seed and leaf extracts, the strength of activity against most isolates did not differ significantly between aqueous seed and aqueous leaf extracts; however, aqueous leaf extract had significantly higher zones of inhibition against *E. coli* ESBL ($p = 0.033$) and Meropenem- resistant *E. coli* ($p = 0.009$) (Table 4) compared to the seed extract. With regards to ethyl acetate extracts, the seed extract had significantly stronger inhibition against all isolates compared to the leaf extract, except against Meropenem-resistant *E. coli*, where both ethyl acetate extracts had no inhibitory effect (Table 5).

Table 2 Zone of Inhibition for resistant, control and clinical strains of bacteria

Bacteria	Zone of Inhibition ± SEM (in millimeters)								
	Antibiotic*	Absolute ethanol		Petroleum ether		Water		Ethyl acetate	
	(30 µg/ml)	Seed	Leaf	Seed	Leaf	Seed	Leaf	Seed	Leaf
Resistant strains									
MRSA ($n = 5$)	25.8 ± 0.6	0	12.6 ± 0.7	0	0	26.2 ± 0.8	21.4 ± 2.8	24.6 ± 0 0.7	13.0 ± 0.5
P. aeruginosa ($n = 5$)	18.7 ± 1.9	0	0	0	0	23.6 ± 1.6	26.6 ± 0.6	22.6 ± 1.0	8.6 ± 3.6
E. coli ESBL ($n = 5$)	23.1 ± 1.5	0	0	0	0	25.0 ± 1.2	28.4 ± 0.5	16.2 ± 0.6	0
Meropenem-resistant *E. coli* ($n = 5$)	21.4 ± 1.1	0	0	0	0	20.4 ± 0.2	22.8 ± 0.2	0	0
Clinical isolates									
S. aureus ($n = 5$)	19.7 ± 0.9	6.0 ± 3.6	0	0	0	32.0 ± 4.0	32.5 ± 0.5	31.0 ± 1.0	15.5 ± 0.5
E. coli ($n = 5$)	22.4 ± 0.8	0	0	0	0	24.0 ± 1.0	26.5 ± 1.5	19.5 ± 3.5	0
Control strains									
ATCC 25,923 *S. aureus*	25.6	13.0	18.0	0	0	20.0	21.0	30.0	12.0
ATCC 29,853 *P. aeruginosa*	20.0	0	14.0	0	0	25.0	26.0	19.0	11.0
ATCC 25,922 *E. coli*	19.3	20.0	0	0	0	26.0	26.0	20.0	0

Where applicable, the values are the mean ± SEM of parallel measurements. MRSA: Methicillin-resistant *Staphylococcus aureus*, SEM: standard error of the mean. *Antibiotics- amikacin and Linezolid were used as positive control for *E. coli* and *P. aeruginosa*, and *S. aureus*, respectively.

Table 3 Zone of Inhibition of positive control compared with those of extracts

	Zone of Inhibition ± SEM (in millimetres)								
Bacteria	**Antibiotic***	**Water**				**Ethyl acetate**			***P*-value a vs. e**
Resistant strains	**(30 µg/ml)[a]**	**Seed[b]**	***P*- value a vs. b**	**Leaf [c]**	***P*- value a vs. c**	**Seed[d]**	***P*- value a vs. d**	**Leaf [e]**	
MRSA (*n* = 5)	25.8 ± 0.6	26.2 ± 0.8	0.890	21.4 ± 2.8	**0.036**	24.6 ± 0 0.7	0.794	13.0 ± 0.5	**0.001**
P. aeruginosa (*n* = 5)	18.7 ± 1.9	23.6 ± 1.6	**0.042[#]**	26.6 ± 0.6	**0.014[#]**	22.6 ± 1.0	**0.049[#]**	8.6 ± 3.6	**0.001**
E. coli ESBL (*n* = 5)	23.1 ± 1.5	25.0 ± 1.2	0.057	28.4 ± 0.5	**0.039[#]**	16.2 ± 0.6	**0.002**	0	ND
Meropenem-resistant *E. coli* (*n* = 5)	21.4 ± 1.1	20.4 ± 0.2	0.768	22.8 ± 0.2	0.134	0		0	ND
Clinical isolates									
S. aureus (*n* = 5)	19.7 ± 0.9	32.0 ± 4.0	**0.001[#]**	32.5 ± 0.5	**0.001[#]**	31.0 ± 1.0	**0.001[#]**	15.5 ± 0.5	**0.002**
E. coli (*n* = 5)	22.4 ± 0.8	24.0 ± 1.0	0.063	26.5 ± 1.5	**0.046[#]**	19.5 ± 3.5	0.079	0	ND
Control strains									
ATCC 25,923 *Staph. aureus*	25.6	20.0	ND	21.0	ND	30.0	ND	12.0	ND
ATCC 29,853 *P. aeruginosa*	20.0	25.0	ND	26.0	ND	19.0	ND	11.0	ND
ATCC25922 *E. coli*	19.3	26.0	ND	26.0	ND	20.0	ND	0	ND

Where applicable, the values are the mean ± SEM of parallel measurements. MRSA: Methicillin-resistant *Staphylococcus aureus*, SEM: standard error of the mean, ND: Not determined. *Antibiotics- amikacin and Linezolid were used as positive control for *E. coli* and *P. aeruginosa*, and *S. aureus*, respectively. [#]Comparison with significantly higher zone of inhibition for extract compared to positive control.

Table 4 Comparison of zone of Inhibition between aqueous seed and leaf extracts

	Zone of Inhibition (95% CI) or ± SEM (in millimetres)		
	Seed	**Leaf**	***P*-value**
Resistant strains			
MRSA (*n* = 5)	26.0 (24.5, 28.0)	24.0, (14.5, 25.0)	0.114
P. aeruginosa (*n* = 5)	24.0 (20.0, 27)	26.0 (25.5, 28.0)	0.171
E. coli ESBL (*n* = 5)	26.0 (22.0, 27.5)	28.0 (27.5, 29.5)	**0.033**
Meropenem- resistant *E. coli* (*n* = 5)	20.0 (20.0, 21.0)	23.0 (22.5, 23.0)	**0.006**
Clinical isolates			
S. aureus (*n* = 5)	32.0 ± 4.0	32.5 ± 0.5	0.9013
E. coli (*n* = 5)	24.0 ± 1.0	26.5 ± 1.5	0.7794
Standard strains			
ATCC 25,923 *Staph. aureus*	20	21	ND
ATCC 29,853 *P. aeruginosa*	25	26	ND
ATCC25922 *E. coli*	26	26	ND

Where applicable, the values are the mean ± SEM of parallel measurements. MRSA: Methicillin-resistant *Staphylococcus aureus*, SEM: standard error of the mean, CI: Confidence interval, ND: Not determined.

Table 5 Comparison of zone of Inhibition between Ethyl acetate seed and leaf extracts

	Zone of Inhibition (95% CI) or ± SEM (in millimetres)		
	Seed	**Leaf**	***P*-value**
Resistant strains			
MRSA (*n* = 5)	24.0 (23.5, 26)	13.0 (12.0, 14.0)	**0.008**
P. aeruginosa (*n* = 5)	22.0 (21.0, 24.5)	12.0 (0, 15.5)	**0.009**
E. coli ESBL (*n* = 5)	16.0 (15.0, 17.0)	0	N/A
Meropenem-resistant *E. coli* (*n* = 5)	0	0	N/A
Clinical isolates			
S. aureus (*n* = 5)	31.0 ± 1.0	15.5 ± 0.5	**0.0390**
E. coli (*n* = 5)	19.5 ± 3.5	0	N/A
Standard strains			
ATCC 25,923 *Staph. aureus*	30	12	ND
ATCC 29,853 *P. aeruginosa*	19	11	ND
ATCC 25,922 *E. coli*	20	0	ND

Where applicable, the values are the mean ± SEM of parallel measurements. MRSA: Methicillin-resistant *Staphylococcus aureus*, SEM: standard error of the mean, CI: Confidence interval, ND: Not determined, N/A: Not applicable; ND: Not determined.

Minimum inhibitory concentration (MIC) of extracts with activity against most bacteria strains

Generally, aqueous seed extracts showed the best activity against all tested strains, with MIC values ranging from 50 mg/ml to 200 mg/ml, followed by ethyl acetate leaf extracts with MIC either 100 mg/ml or 200 mg/ml. The best activity of aqueous seed extract was observed against all strains of *E. coli* and *P. aeruginosa*, with MIC of 50 mg/ml (Table 6). The activity of the aqueous seed extract was low with the lowest observed against MRSA, with MIC values of 200 mg/ml. Ethyl acetate leaf extracts showed no activity against all strains of *E. coli* and low activity against *S. aureus* and *P. aeruginosa* isolates (MIC > 200 mg/ml). Also, low activity was observed against *S. aureus* and *P. aeruginosa* isolates, with MIC values of 200 mg/ml and 100 mg/ml or 200 mg/ml, respectively. Though aqueous leaf and ethyl acetate seed extracts showed activity in their pure form in the disc diffusion method, low activity was exhibited by the aqueous leaf extract against all the bacteria isolates in the broth dilution system (MIC > 400 mg/ml).

Table 6 Minimum inhibitory concentration (MIC) for extracts with high zones of Inhibition

Bacteria	MIC (mg/ml)			
	Water		Ethyl acetate	
	Seed	Leaf*	Seed*	Leaf
Resistant strains				
MRSA	200	> 400	> 400	200
P. aeruginosa	50	> 400	> 400	100
E. coli ESBL	50	> 400	> 400	N/A
Meropenem-resistant *E. coli*	50	> 400	> 400	N/A
Clinical isolates				
S. aureus	100	> 400	> 400	200
E. coli	50	> 400	> 400	N/A
Control strains				
ATCC 25,923 *S. aureus*	100	> 400	> 400	200
ATCC 29,853 *P. aeruginosa*	50	> 400	> 400	200
ATCC 25,922 *E. coli*	50	> 400	> 400	N/A

MRSA: Methicillin-resistant *Staphylococcus aureus*, N/A: No activity even in the pure form. *Had activity in the pure form but no activity at 400 mg/ml which was the starting concentration.

Discussion

Antibacterial activity of crude aqueous, ethanolic, ethyl acetate and petroleum ether extracts of dried leaves and seeds of *Moringa oleifera* were investigated using clinical and resistant isolates of two gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, and one gram-positive bacterium, *Staphylococcus aureus*.

Comparing the zones of inhibition of various plant extracts to positive controls, revealed interesting patterns of effectiveness. The aqueous seed extract showed similar activity compared to the positive control against most isolates but outperformed it against *P. aeruginosa* and clinical *S. aureus* isolates. In contrast, the aqueous leaf extract demonstrated significantly higher activity than the positive control against most isolates, except against MRSA, where the positive control was more effective. The ethyl acetate seed extract mirrored the performance of the aqueous seed extract, exhibiting higher activity against *P. aeruginosa* and clinical *S. aureus* isolates than the positive control. Conversely, the positive controls had higher activity compared to the ethyl acetate seed extracts, all ethanolic and petroleum ether extracts, for all the isolates. Whereas these results do not imply that any of the leaf and seed extracts of *M. oleifera* is more effective against the tested bacteria species than the control antibiotics, they clearly demonstrate that aqueous and ethyl acetate extracts of leaf and seed of *M. oleifera* have notable antibacterial activity against the selected isolates of Gram-positive and Gram-negative bacteria.

Comparisons of activity were also made among the extracts using the diameter of zone of inhibition. The results indicate that aqueous extracts of both leaves and seeds had better activity against all the isolates than the organic solvent extracts. In categorization of activity of antibacterial agents using zone of inhibition, inhibition diameter > 20 mm was categorized as having very strong antibacterial activity, 15–20 mm as strong, 10–14 mm as moderate and 9–7 mm as weak, whereas zone of inhibition < 7 mm was categorized as having no antibacterial activity [18]. This implies that aqueous extracts, having inhibition zones > 20 mm, had very strong activity against the isolates.

These findings not only confirm the activity of the aqueous leaf and seed extracts of *M. oleifera* [11, 14] but also revealed that cold aqueous extracts worked better than the organic extracts investigated. Previous studies have not clearly demonstrated that cold water extract of *M. oleifera* leaves and seeds have better activity against the tested bacteria species than any organic solvent extracts. On the contrary, some studies have indicated that organic solvent extracts of *M. oleifera* had equal or even better antibacterial activity against many species, including species of bacterial isolates used in this study [10–12, 19, 20]. These discrepancies can be due to differences in the extraction systems, bacteria strains investigated as well as the number of experimental replicates used. Studies have shown that the amount and types of bioactive substances composition of extracts differ depending on the solvent used [12] and it is not surprising that variations exist in the antibacterial activity of the extracts examined. The findings corroborate the report that, though many studies avoid the use of aqueous fractionation entirely, there are exceptional water-soluble compounds that are usually more effective as inhibitors of pathogen than those extracted in commonly used techniques [13].

Apart from MDR *S. aureus*, against which aqueous leaf extract of *M. oleifera* was found to have some inhibitory effect, albeit very low [19], no report had convincingly demonstrated the efficacy of aqueous extracts of *Moringa* against MDR. However, few reports have shown some antimicrobial activity of organic solvent extracts (ethanol, methanol, chloroform, dichloromethane and acetone) against MDR [19, 21]. In this study, ethyl acetate seed extract also, with most inhibition zones > 20 mm, showed very strong activity against most of the isolates. Findings exist that show that ethyl acetate extracts have activity against some species of the bacterial isolates used in this study [10, 20, 22] and in one study, the ethyl acetate leaf extract was shown to have higher antibacterial activity compared to other extracts, including aqueous extract contrary to the current findings [10]. However, these were based on a disc diffusion analysis, which does not reveal the true degree of activity of the extracts, as it is affected by the rate at which the active compounds can diffuse through the agar.

Further analysis using broth culture revealed that the crude extracts did not have strong antibacterial activity as expected but aqueous seed extract had the best antibacterial activity among the four extracts, with MIC ranging from 50 mg/ml to 200 mg/ml (Table 6). Ethyl acetate leaf extract had low activity (MIC = 100 mg/ml or 200 mg/ml) and was inactive against all *E. coli* strains, but its activity was better than that of aqueous leaf extract (MIC > 400). Ethyl acetate seed extract, like aqueous leaf extract, had MIC > 400 against all the isolates. These indicates that plant part may determine the choice of solvent for extraction and the consequent antimicrobial activity.

Both seeds and leaves extracts of petroleum ether had no antibacterial activity against any of the bacterial isolates. This result corroborates a report that showed that petroleum ether leaf extract did not exhibit any antibacterial activity [19]. The ineffectiveness of the absolute ethanol and petroleum ether extracts might not be an issue of polarity of solvent used only but also the phytochemical composition of the extract. For instance, ethanol is next to water in terms of polarity but ethyl acetate, with a lower polarity, had a higher activity than ethanol. However, these results suggest that the compounds responsible for this activity are more or less water-soluble compounds.

Comparing the zones of inhibition between seed and leaf extracts indicates that the antimicrobial activity of aqueous extract against most isolates did not differ significantly between the two, but aqueous leaf extract had significantly higher zones of inhibition against *E. coli* ESBL and Meropenem- resistant *E. coli* compared to the seed extract. A report also suggests that aqueous leaf extract of *Moringa* had a higher antibacterial activity against *E. coli* than the aqueous seed extract [23]. However, MICs from broth microdilution method showed that the antimicrobial activity of aqueous seed extract is far better than that of aqueous leaf extract (Table 6). This implies that the observed lower activity of the aqueous seed extract compared to the aqueous leaf extract, in the disc diffusion system against *E. coli*, could be attributed to variation in the rate of diffusion of antimicrobial compounds in the extracts through the agar. This study demonstrates that *Moringa* leaf extracts from cold aqueous maceration might not be as potent as its seed equivalent against various strains of bacteria, including *E. coli*.

Similar findings were obtained for ethyl acetate extracts, in which the seed extract had significantly higher zone of inhibition against all isolates compared to the leaf extract, except against Meropenem-resistant *E. coli*, for which ethyl acetate extracts had no inhibitory effect. However, activity of the leaf extract was better than that of seed extract in the broth culture system as indicated by the MICs. It would therefore be misleading to compare the degree of activity of extracts based solely on zone of inhibition, as alluded to by other authors [24–26], and without reference to the extraction solvent.

There is scarcity of data comparing seed and leaf extracts. The discrepancies observed between this, and previous studies could be due a few factors including the strains of microbes used. Aside that the data demonstrate that for the strains of bacteria evaluated, and for aqueous and ethyl acetate extracts, *M. oleifera* seeds had higher and more effective antibacterial activity than the leaves. Some level of resistance by some trains of *E. coli* has been observed against the ethyl acetate extracts, particularly, the leaf extract. It is not clear why ethyl acetate leaf extract showed no activity against all strains of *E. coli* (even in the pure form with the disc diffusion method) and had low activity against *S. aureus* and *P. aeruginosa.*

This study has limitations, including testing only a limited number of bacterial strains, which may not be representative of the broader range of bacterial pathogens. Furthermore, the in vitro results may not necessarily reflect the same outcomes in in vivo settings due to possible bioavailability problems and pharmacological factors. To fully explore the potential of *Moringa oleifera* as a source of new antimicrobials, further research is needed, including in vivo studies, identification of specific antibacterial compounds, and elucidation of their mechanisms of action.

Conclusions

The data from the disc diffusion method suggests that aqueous extracts of *Moringa oleifera* seeds and leaves and ethyl acetate seed extracts had very strong activity against most of the selected clinical and drug-resistant Gram-positive and Gram-negative bacterial isolates, and in some cases performed even better than the control antibiotics. Aqueous extracts showed the broadest antibacterial activity, inhibiting all isolates. The best zones of inhibition were also observed in aqueous extracts, with values ranging from 20.0 to 32.5 mm. In contrast, ethanol and petroleum ether extracts showed weak to no antibacterial activity against any of the isolates, indicating that the compounds responsible for the activity are mainly water-soluble. However, the strong activity in the disc diffusion system could not reflect in the broth microdilution system as aqueous seed extract which showed the best activity overall, had MIC values ranging from 50 to 200 mg/m. Overall, the study suggests that *Moringa oleifera* seeds and leaves have antibacterial activity against clinical isolates as well as drug-resistant strains and, aqueous and ethyl acetate extracts of the seeds and leaves capture the active antibacterial compounds.

Abbreviations

AMR	Antimicrobial resistance
ESBL	Extended-spectrum beta-lactamase
MDR	Multidrug resistance

MIC Minimum Inhibitory Concentration
MRSA Methicillin-resistant Staphylococcus aureus

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Author contributions
Conceptualization: GF, ESK and MAC; design: GF, ESK, SAT and MAC; validation: GF, ESK, SAT, M-MO and MAC; resources: GF, ESK, SAT, M-MO, MAC and CAAT; laboratory work, SAT, MAC, CAAT and M-MO; data analysis: GF and SAT; writing—original draft preparation GF and SAT; writing—review and editing, GF, ESK, SAT and MAC; supervision, GF, ESK and MAC; project administration, GF and ESK; All authors read and approved the final manuscript.

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Data availability
The data analyzed during this study are available and can be obtained from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the Declaration of Helsinki and applicable institutional and national regulations. The clinical isolates used in the study are covered by ethics approvals from the Ethical and Protocol Review Committee of the College of Health Sciences (CHS), University of Ghana (CHS-Et/M.5-P4.5/2021–2022) and the Ghana Health Service (GHS) Ethics Review Committee (GHS-ERC: 005/01/23). Written informed consent was also sought from all study participants prior to inclusion in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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