Research Article

Assessment of antibacterial potential of methanol, n-hexane, ethyl acetate and chloroform *Moringa oliefera* leaf extracts

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Abstract

Bacterial infections and their increasing resistance to common antibiotics is posing serious threat to global public health. To this end, finding new alternatives and evaluating their antibacterial efficacy is always desirable. Therefore, in the present study we used methanol (MeOH), n-hexane (n-Hex), ethyl acetate (ETAC) and chloroform (CHCl₃) to prepared four different types of extracts from *Moringa oleifera* (*M. oliefera*) leaves aiming to inhibit five selected bacteria. Initially, agar well diffusion methods was used, and zones of inhibition were measured as an indicator of bacterial susceptibility. MeOH, n-Hex, ETAC and CHCl₃ leaf extracts showed highest zone of inhibition against *E. coli*, *B. cereus*, *S. pyogenes*, *S. aureus* and *S. enterica*, respectively. Moreover, highest zones of inhibition were observed at lowest incubation (24hr) and lowest zones were observed at highest incubation period (72hr) for all tested concentrations. Later, macrodilution method was used to access the antibacterial susceptibility in liquid medium. Results confirmed the susceptibility of all test bacteria with different level of IC₅₀ values ranging from 7.07 ± 0.44 to 10.91 ± 0.10 mg/ml for MeOH extract, 1.66 ± 0.08 to 2.11 ± 0.11 mg/ml for n-Hex extract, 2.58 ± 0.13 to 3.84 ± 0.21 mg/ml for ETAC extract and 3.73 ± 0.75 to 8.36 ± 0.20 mg/ml for CHCl₃ extract. Interestingly, none of the tested bacteria showed resistance against any of the tested extract in well diffusion or macrodilution method expressing the *M. oliefera* leaves extracts as potent candidates to kill bacteria in semisolid or in liquid medium to fulfill medical needs in future.

Keywords: Bacteria; *M. oleifera*; Macrodilution method; Zones of inhibition

Introduction

Antibiotics have been frequently used worldwide and saved countless lives.

However, it is evident that the success of antibiotics might only be temporary, and we now expect a long-term or perhaps never-

ending challenge to find new effective alternatives to combat increasing problem of antibiotic resistance. The number of antibiotics such as trimethoprim, nitrofurantoin, fluoroquinolones, fosfomycin, gentamicin, sulfamethoxazole, cephalixin linezolid, telavancin daptomycin, vancomycin and clindamycin are easily available. However, bacterial resistance is reported to increase continuously at an alarming rate [1, 2]. For example, *E. coli* strains are found to be resistant to ampicillin, ciprofloxacin, cephalosporins, trimethoprim-sulfamethoxazole [3], *S. aureus* strains are found to be resistant to ampicillin, penicillin [4] and, *B. cereus* strains are found to be resistant to cephalosporin, penicillin. Resistance to clindamycin, cefazolin, cefotaxime and trimethoprim-sulfamethoxazole is also reported [5]. Moreover, *S. enterica* developed resistance to macrolides, aminoglycosides, cephalosporin, tetracycline, ketolides ampicillin [6] and *S. pyogenes* strain developed resistance to most macrolides, fluoroquinolone macrolide, lincosamide and streptogramin antibiotics [7]. Therefore, search for new effective alternatives to combat microbial infections is always desirable.

Medicinal plants have been proven a good source of antibacterial therapy and have been used to treat diseases all over the world for many decades. Based on pharmacopoeias and study of medical plants in 91 countries, World Health Organization (WHO) reported almost 20,000 medicinal plant species [8]. Plants that inhibit the growth of microorganisms are important for human health and have been studied since 1926 [9, 10]. Herbal medicines being safe and environment friendly gained increasing popularity and are widespread around the globe encouraging to explore their biological properties to unmet the medical needs.

Thus, in the present study *Moringa oleifera*, one of the best known and widely distributed specie of a monogeneric family *Moringaceae* [11] is selected for the evaluation of antibacterial properties. Selected plant ranges 5 to 10 m in height [12] and found wild near the sandy beds of rivers and streams or cultivated in plains, hedges and in-house

yards. The numbers of medicinal properties have been associated to various parts of this highly esteemed tree. Plant parts including root, bark, leaf, fruit, gum, flowers and seed have been used for various ailments including infectious diseases, inflammation, gastrointestinal, cardiovascular, hematological and hepatorenal disorders [13, 14]. *Moringa* leaves are rich source of protein, vitamin C, β -carotene, calcium and potassium and have flavonoids, phenolics, carotenoids and ascorbic acid which serve as a valuable natural antioxidant and good food preserver [15, 13]. Interestingly, its leaves have been used to treat headaches, piles, sore throat, bronchitis, fevers, eye and ear infections, scurvy, diabetics as well as glandular swelling [12]. The juice from its root bark is used to put into ears to relieve earaches, placed in a tooth cavity as a pain killer and, has shown promising anti-tubercular activity [13]. Its seed extract showed protective effects by decreasing liver lipid peroxides.

Leaves of *Moringa oleifera* (*M. oleifera*) contain flavanoids and flavanol glycosides, glucosinolate and isothiocyanate, phenolic acid, alkaloid and sterol important for various biological activities including bacterial activity. Thus, in the present study we prepared methanol, n-hexane, ethyl acetate and chloroform *M. oleifera* leaf extracts and evaluated their antibacterial activity using five pathogenic bacterial strains.

Materials and methods

Selected plant and preparation of extracts

Moringa oleifera (*M. oleifera*) leaves were collected from a home garden, Mirpur, AJK, Pakistan. Selected plant part was washed 2-3 times under running tap water and air dried for 2 weeks under shade. Later, homogenized to fine powder and stored in airtight glass bottles for extract preparation. The powdered leaf sample was soaked in 1mg:10ml of methanol, n-hexane, ethyl acetate and chloroform in separate flasks for 5-7 days with 5 min shaking every day (Fig. 1). Later, filtrate was evaporated using rotary evaporator and air-dried concentrated extract was dissolved in respective solvent to prepare stock which were further diluted in broth to achieve desired concentration.

Figure 1. Different phases of *Moringa oleifera* leaf extracts

Chemicals

Methanol (MeOH), n-hexane (n-Hex), Chloroform (CHCl₃), Ethyl acetate (ETAC), 1, 1-di-phenyl-2-picryl hydrazyl (DPPH), analytical methanol, ethanol and ascorbic acid were used. Nutrient broth (Merck, Germany) and nutrient agar (OXOID CM003, UK) were used.

Bacterial strains and culture conditions

The bacterial strains *Bacillus cereus* (*B. cereus*) ATCC 10876, *Staphylococcus aureus* (*S. aureus*) ATCC 2592, *Streptococcus pyogenes* (*S. pyogenes*) ATCC 12384 and *Salmonella enterica* (*S. enterica*) were used in this study. Bacteria were streaked on agar plates, incubated at 37 °C for 17hrs. Later, plates were stored at 4 °C or used to prepare liquid culture in nutrient broth (Merck, Germany).

Evaluation of antibacterial susceptibility by well diffusion method

To evaluate the antibacterial effects of prepared extracts, well diffusion method was used [16]. Briefly, bacteria were spread homogeneously on agar plates. After short air dry, 5 wells (6mm in diameter) were made with the help of sterilized cork-borer. Then, each well was loaded with 50µl of 480 mg/ml, 240 mg/ml, 120 mg/ml, 60 mg/ml and 0 mg/ml (control) of test extract. Plates were incubated at 37 °C for 24hrs, 48hrs and 72hrs, photographed and zones of inhibition around

the wells were measured in cm as an indicator of bacterial susceptibility.

Testing bacterial inhibitory concentration (IC₅₀) by broth dilution method

Bacterial inhibitory concentration of test extracts was determined by macro-broth dilution method. Serial dilutions of extract were placed in sample tubes having bacteria and, control tubes having broth only (no bacteria). The total volume per tube was adjusted as 1 ml with broth. Broth cultures were incubated at 37 °C for 24hrs. Later, IC₅₀ value in mg/ml was calculated for each extract against all test bacteria to compare effects in liquid medium.

Statistical analysis

All experiments were performed three times in triplet. Student's t-test was applied to check significant difference using lowest zone of inhibition (except zero) vs each tested zone of inhibition (^ap<0.05; ^bp<0.005, ^cp<0.0001) [17].

Results and discussion

Antibacterial susceptibility

In the present study different *M. oleifera* leaves extracts were prepared using methanol, n-hexane, chloroform and ethyl acetate. Later, their antibacterial effect against *B. cereus*, *E. coli*, *S. enterica*, *S. aureus* and *S. pyogenes* was compared in concentration dependent and incubation dependent manners.

Antibacterial effect of methanolic leaf extract

The tested methanolic (MeOH) leaf extract concentrations i.e. 60 mg/ml, 120 mg/ml, 240 mg/ml and 480 mg/ml showed 0cm, 0cm, 0.09cm, 0.15cm zones of inhibition against *B. cereus*, 0cm, 0cm, 0.13cm, 0.22cm zones of inhibition against *S. aureus*, 0cm, 0cm, 0.09cm, 0.17cm zones of inhibition against *S. pyogenes*, 0cm, 0cm, 0.1cm, 0.15cm zones of inhibition against *S. enterica* and 0.01cm, 0.1cm, 0.18cm, 0.3cm zones of the inhibition against *E. coli*, respectively at 24hr incubation (Table 1). Thus, bacterial susceptibility trend observed at highest tested concentration (480mg/ml) at 24hr incubation was as *E. coli* > *S. aureus* > *S. pyogenes* > *S. enterica* > *B. cereus*. Interestingly, highest zones of inhibition were observed at 24hr than 72hr incubation at all tested concentrations as shown in Table 1.

Antibacterial effect of n-hexane leaf extract

The tested n-hexane (n-Hex) leaf extract concentrations i.e. 60 mg/ml, 120 mg/ml, 240 mg/ml and 480 mg/ml showed 0.45cm, 0.5cm, 0.6cm, 0.61cm zones of inhibition against *B. cereus*, 0.42cm, 0.48cm, 0.5cm, 0.57cm zones of inhibition against *S. aureus*, 0.37cm, 0.44cm, 0.47cm, 0.49cm zones of inhibition against *S. pyogenes*, 0.32cm, 0.36cm, 0.44cm, 0.53cm zones of inhibition against *S. enterica* and 0.31cm, 0.34cm, 0.41cm, 0.47cm zones of the inhibition against *E. coli*, respectively at 24hr incubation (Table 1). Thus, bacterial susceptibility trend observed at highest tested concentration (480mg/ml) at 24hr incubation was as *B. cereus* > *S. aureus* > *S. enterica* > *S. pyogenes* > *E. coli*. Interestingly, highest zones of inhibition were observed at 24hr than 72hr incubation at all tested concentrations as shown in Table 1.

Antibacterial effect of ethyl acetate leaf extract

The tested ethyl acetate (ETAC) leaf extract concentrations i.e. 60mg/ml, 120mg/ml, 240mg/ml and 480mg/ml showed 0.28cm, 0.33cm, 0.38cm, 0.40cm zones of inhibition against *B. cereus*, 0.32cm, 0.38cm, 0.43cm,

0.47cm zones of inhibition against *S. aureus*, 0.37cm, 0.39cm, 0.45cm, 0.50cm zones of inhibition against *S. pyogenes*, 0.27cm, 0.35cm, 0.40cm, 0.46cm zones of inhibition against *S. enterica* and 0.27cm, 0.34cm, 0.40cm, 0.43cm zones of the inhibition against *E. coli*, respectively at 24hr incubation (Table 1). Thus, bacterial susceptibility trend observed at highest tested concentration (480mg/ml) at 24hr incubation was as *S. pyogenes* > *S. aureus* > *S. enterica* > *E. coli* > *B. cereus*. Interestingly, highest zones of inhibition were observed at 24hr than 72hr incubation at all tested concentrations as shown in Table 1.

Antibacterial effect of chloroform leaf extract

The tested chloroform (CHCl₃) leaf extract concentrations i.e. 60 mg/ml, 120 mg/ml, 240 mg/ml and 480 mg/ml showed 0.19cm, 0.21cm, 0.24cm, 0.27cm zones of inhibition against *B. cereus*, 0.10cm, 0.18cm, 0.24cm, 0.28cm zones of inhibition against *S. aureus*, 0.15cm, 0.21cm, 0.26cm, 0.29cm zones of inhibition against *S. pyogenes*, 0.18cm, 0.26cm, 0.31cm, 0.38cm zones of inhibition against *S. enterica* and 0.13cm, 0.16cm, 0.24cm, 0.30cm zones of the inhibition against *E. coli*, respectively at 24hr incubation (Table 1). Thus, bacterial susceptibility trend observed at highest tested concentration (480mg/ml) at 24hr incubation was as *S. enterica* > *E. coli* > *S. pyogenes* > *S. aureus* > *B. cereus*. Interestingly, highest zones of inhibition were observed at 24hr than 72hr incubation at all tested concentrations as shown in Table 1.

M. oliefera, chloroform root bark extract contains aglycon of deoxy-niazimicine (N-benzyl, S-ethyl thioformate) which strongly inhibit *C. albicans*, *P. aeruginosa*, *S. aureus*, *S. dysenteriae*, *S. boydii* and *S. typhi* [18, 14]. Ethyl acetate bark extract of *M. oliefera* is reported to show higher inhibition toward *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Citrobacter freundii* and *Bacillus megaterium* than methanol, chloroform, and aqueous extracts of the same part of the plant [19].

Table 1. Measured zones of bacterial inhibition by various *M. oliefera* leaves extracts.

Test	Extract type	Concentration (mg/ml)				Concentration (mg/ml)				Concentration (mg/ml)				Test bacteria
		60	120	240	480	60	120	240	480	60	120	240	480	
		Zone of inhibition (cm) at 24 hr incubation				Zone of inhibition (cm) at 48hr incubation				Zone of inhibition (cm) at 72hr incubation				
<i>M. olifera</i> leaf	MeOH	0.0±0.00	0.0±0.00	0.09±0.02	0.15±0.03 ^b	0.00±0.00	00.00±0.00	0.08±0.01	0.14±0.04 ^b	0.00±0.00	0.00±0.00	0.08±0.01	0.13±0.03 ^b	<i>B. cereus</i>
	n-Hex	0.45±0.03	0.5±0.02	0.60±0.03	0.61±0.03	0.44±0.01	0.49±0.02	0.56±0.02	0.60±0.01	0.43±0.00	0.48±0.02	0.55±0.03	0.59±0.03	
	ETAC	0.28±0.01	0.33±0.03	0.38±0.01 ^a	0.40±0.02 ^a	0.25±0.03	0.31±0.04	0.38±0.02 ^a	0.39±0.02 ^a	0.22±0.02	0.28±0.02	0.34±0.02 ^a	0.37±0.02 ^a	
	CHCl ₃	0.19±0.09	0.21±0.02	0.24±0.03	0.27±0.02 ^a	0.18±0.02	0.20±0.02	0.23±0.02	0.25±0.02	0.15±0.03	0.17±0.02	0.21±0.02	0.23±0.02	
	MeOH	0.0±0.00	0.0±0.00	0.13±0.01	0.22±0.02 ^a	0.00±0.00	00.0±0.00	0.12±0.02	0.20±0.04 ^a	0.0±0.00	0.0±0.00	0.11±0.02	0.18±0.04 ^a	<i>S. aureus</i>
	n-Hex	0.42±0.04	0.48±0.03	0.50±0.04	0.57±0.03	0.40±0.03	0.46±0.03	0.48±0.03	0.55±0.02	0.39±0.02	0.45±0.02	0.47±0.02	0.55±0.02	
	ETAC	0.32±0.06	0.38±0.03 ^a	0.43±0.03 ^b	0.47±0.08 ^b	0.29±0.03	0.36±0.02 ^a	0.41±0.03 ^b	0.46±0.02 ^c	0.26±0.02	0.33±0.03 ^b	0.40±0.02 ^c	0.45±0.01 ^c	
	CHCl ₃	0.1±0.04	0.18±0.04 ^a	0.24±0.03 ^b	0.28±0.02 ^c	0.08±0.01	0.15±0.03 ^a	0.26±0.01 ^b	0.27±0.01 ^c	0.09±0.01	0.14±0.02 ^a	0.21±0.03 ^b	0.25±0.00 ^c	
	MeOH	0.0±0.00	0.00±0.00	0.09±.00	0.17±0.02 ^c	0.00±0.00	0.00±0.00	0.09±0.02	0.17±0.05 ^c	0.00±0.00	0.00±0.00	0.08±0.02	0.15±0.05 ^c	<i>S. pyogenes</i>
	n-Hex	0.37±0.03	0.44±0.03	0.47±0.04	0.49±0.05 ^a	0.36±0.02	0.42±0.02	0.46±0.02	0.48±0.02 ^a	0.33±0.06	0.40±0.01	0.44±0.03	0.47±0.02 ^a	
	ETAC	0.37±0.02	0.39±0.02	0.45±0.04 ^a	0.50±0.03 ^b	0.32±0.02	0.38±0.02	0.43±0.02 ^a	0.47±0.03 ^b	0.29±0.02	0.34±0.02	0.40±0.04 ^a	0.44±0.04 ^b	
	CHCl ₃	0.15±0.01	0.21±0.02	0.26±0.02 ^a	0.29±0.02 ^b	0.13±0.02	0.20±0.02	0.23±0.03 ^b	0.27±0.02 ^b	0.11±0.02	0.18±0.04 ^a	0.23±0.03 ^b	0.25±0.02 ^c	
	MeOH	0.0±0.00	0.00±0.00	0.1±.00	0.15±0.02 ^a	0.00±0.00	0.00±0.00	0.09±0.01	0.14±0.02 ^a	0.00±0.00	0.00±0.00	0.09±0.01	0.14±0.02 ^c	<i>S. enterica</i>
	n-Hex	0.32±0.00	0.36±0.02	0.44±0.04 ^b	0.53±0.03 ^c	0.3± 0.00	0.33±0.02	0.43±0.03 ^b	0.51±0.03 ^c	0.29±0.01	0.31±0.02	0.41±0.02 ^b	0.49±0.03 ^c	
	ETAC	0.27±0.08	0.35±0.01	0.40±0.03 ^b	0.46±0.02 ^b	0.24±0.03	0.32±0.04	0.38±0.02 ^b	0.43±0.03 ^b	0.21±0.04	0.30±0.04 ^a	0.36±0.04 ^b	0.41±0.03 ^c	
	CHCl ₃	0.18±0.00	0.26±0.03	0.31±0.04 ^a	0.38±0.03 ^b	0.15±0.01	0.25±0.03 ^a	0.29±0.04 ^b	0.35±0.02 ^c	0.13±0.04	0.21±0.02 ^a	0.26±0.03 ^b	0.34±0.02 ^c	
	MeOH	0.01±0.00	0.17±0.01	0.18±0.02 ^b	0.30±0.02 ^c	0.00±0.00	0.09±0.02	0.16±0.04 ^a	0.27±0.05 ^c	0.00±0.00	0.15±0.10	0.13±0.03	0.24±0.04	<i>E. coli</i>
	n-Hex	0.31±0.01	0.34±0.02	0.41±0.03	0.47±0.03	0.29±0.00	0.33±0.02	0.40±0.02	0.46±0.02	0.25±0.00	0.31±0.02	0.39±0.01	0.45±0.02	
	ETAC	0.27±0.01	0.34±0.04 ^a	0.40±0.03 ^b	0.43±0.02 ^c	0.22±0.02	0.32±0.03 ^c	0.37±0.04 ^c	0.42±0.02 ^c	0.19±0.04	0.29±0.02 ^c	0.34±0.05 ^c	0.40±0.03 ^c	
	CHCl ₃	0.13±0.01	0.16±0.08	0.24±0.02 ^b	0.3±0.02 ^c	0.12±0.02	0.15±0.08	0.23±0.01 ^b	0.27±0.02 ^c	0.09±0.01	0.14±0.07	0.18±0.01 ^b	0.25±0.01 ^c	

Table 2. Determination of bacterial inhibitory concentration (IC₅₀) by broth macrodilution method

Extract type	Bacterial inhibition IC ₅₀ ± SEM (mg/ml)				
	<i>B. cereus</i>	<i>S. aureus</i>	<i>S. enterica</i>	<i>S. pyogenes</i>	<i>E. coli</i>
MeOH extract	9.68 ± 0.29	10.91 ± 0.10	9.02 ± 0.00	8.16 ± 0.00	7.07 ± 0.44
n-Hex extract	1.78 ± 0.00	1.70 ± 0.04	2.11 ± 0.11	1.66 ± 0.08	1.74 ± 0.10
ETAC extract	3.84 ± 0.21	2.58 ± 0.13	3.54 ± 0.05	3.50 ± 0.02	2.73 ± 0.27
CHCl ₃ extract	4.44 ± 0.13	4.34 ± 0.97	3.73 ± 0.75	8.36 ± 0.20	7.20 ± 0.17

However, in the present study each leaf extract exhibited different levels of antibacterial effects depending upon test bacteria. Methanol, n-hexane, ethyl acetate and chloroform leaf extracts showed highest zone of inhibition against *E. coli*, *B. cereus*, *S. pyogenes*, *S. aureus* and *S. enterica*, respectively. Interestingly, highest zones of inhibition were observed at lowest incubation (24hr) and lowest zones were observed at 72hr at all tested concentrations which is in accordance to our previous findings [17].

Testing bacterial inhibitory concentration (IC₅₀) by broth dilution method

To evaluate bacterial susceptibility in suspension state, calorimetric method was used. The extract concentration required to achieve 50% bacterial inhibition is expressed as inhibitory concentration (IC₅₀). Methanolic leaves extract showed IC₅₀ values as 9.68 mg/ml, 10.91 mg/ml, 7.07 mg/ml, 8.16 mg/ml and 9.02 mg/ml against *B. cereus*, *S. aureus*, *E. coli*, *S. pyogenes* and *S. enterica*, respectively (Table 2). The IC₅₀ trend for methanolic leaves extract was observed as *E. coli* > *S. pyogenes* > *S. enterica* > *B. cereus* > *S. aureus*. The n-hexane leaves extract showed IC₅₀ values as 1.78 mg/ml, 1.70 mg/ml, 1.74 mg/ml, 1.66 mg/ml and 2.11 mg/ml against *B. cereus*, *S. aureus*, *E. coli*, *S. pyogenes* and *S. enterica*, respectively. The IC₅₀ trend of n-hexane extract was *S. pyogenes* > *S. aureus* > *E. coli* > *B. cereus*

> *S. enterica*. Ethyl acetate leaves extract showed IC₅₀ values as 3.84 mg/ml, 2.58 mg/ml, 2.73 mg/ml, 3.50 mg/ml and 3.54 mg/ml against *B. cereus*, *S. aureus*, *E. coli*, *S. pyogenes* and *S. enterica*, respectively. The IC₅₀ trend of ethyl acetate extract was *S. aureus* > *E. coli* > *S. pyogenes* > *S. enterica* > *B. cereus*. Moreover, chloroform leaves extract showed IC₅₀ values as 4.44 mg/ml, 4.34 mg/ml, 7.20 mg/ml, 8.36 mg/ml and 3.73 mg/ml against *B. cereus*, *S. aureus*, *E. coli*, *S. pyogenes* and *S. enterica*, respectively. The IC₅₀ trend of chloroform leaves was *S. enterica* > *S. aureus* > *B. cereus* > *E. coli* > *S. pyogenes* (Table 2). Kaur *et al* [20] reported that a methanolic leaves extract and 70% ethanolic extract of *M. oliefera* showed antileishmanial activity against *L. donovani* promastigotes. The ethyl acetate fraction of a methanolic extract was reported to show leishmaniasis with an IC₅₀ of 27.5 µg/ml. Moreover, *M. oliefera* extracts are reported to exhibit antiviral, antifungal, antihyperglycemic, antihyperlipidemic, and hypocholesterolemic activities. However, in the present study methanol, n-hexane, ethyl acetate and chloroform leaf extracts showed antibacterial effect against all five tested bacteria. Recently, bacteria including *B. cereus*, *S. aureus*, *S. pyogenes*, *S. enterica* and *E. coli* are reported to show resistance to commonly used antibiotics at alarming rate [1-7]. However, none of the tested bacteria showed resistance against any of the tested extract in well diffusion or

macrodilution method expressing the *M. oleifera* leaves extracts as potent candidates to kill bacteria in semisolid or in liquid medium for bacterial inhibition.

Conclusion

In the present study four extracts from *M. oleifera* leaves were prepared using methanol, n-hexane, chloroform and ethyl acetate. All extracts expressed bacterial inhibition against all tested bacteria i.e. *B. cereus*, *E. coli*, *S. enterica*, *S. aureus* and *S. pyogenes* in both well diffusion (semi solid medium) and macrodilution method (liquid medium) presenting the *M. oleifera* leaves extracts as potent candidates to treat tested bacteria in future.

Authors' contributions

Conceived and designed the experiments: A Ali & N Rafiq, Performed the experiments: N Rafiq, Analyzed the data: H Khurshid, B Akbar, Z H Tarar, Contributed materials/ analysis/ tools: I Ahmed, F Nazir & M Ahmed, Wrote the paper: A Ali, N Rafiq & H Javed.

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