# Inhibition of human pathogenic bacteria by *Moringa oleifera* cultivated in Jazan (Kingdom of Saudi Arabia) and study of synergy to amoxicillin

Mona Kilany[a,b]

[a]Department of Biology, Faculty of Science, King Khalid University, Abha, Saudi Arabia, [b]Department of Microbiology, National Organization for Drug Control and Research (NODCAR), Cairo, Egypt

Correspondence to Mona Kilany, PhD in Microbiology, Department of Biology, Faculty of Science, King Khalid University, PO Box 9004, Abha, Saudi Arabia, Postal code 61413
Tel: +966 540 793 464;
e-mail: mona.kilany@yahoo.com

**Received** 01 October 2015
**Accepted** 15 November 2015

**Egyptian Pharmaceutical Journal**
2016, 15:38–42

**Background**
The spread of multidrug-resistant strains of bacteria to most antibiotics has led to intensive searches for effective strategies to overcome bacterial infections.
**Aim**
*Moringa oleifera* could be a potentially useful agent for many therapeutic applications, especially antimicrobial.
**Settings and design**
The leaves of *M. oleifera* were collected in October 2014 from Jazan, located in the southern region of the Kingdom of Saudi Arabia.
**Materials and methods**
Different organic solvents were used to extract antimicrobial substances in the plant leaves. The antibacterial activity of each leaf extract was determined *in vitro* and compared with some antibiotics. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of both plant extracts and amoxicillin were investigated against *Bacillus* spp. An evaluation of the synergistic effects of amoxicillin and plant extract was carried out.
**Results**
The results showed that an ethanol extract is the best organic solvent for extracting the antimicrobial substance from *M. oleifera*. In addition, the antibacterial potential of an ethanol extract is comparable to that of some commercial antibiotics. MIC and MBC of the plant extract were 320 and 620 μg/ml, respectively, whereas MIC and MBC of amoxicillin were 25 and 50 μg/ml, respectively. The fractional inhibitory concentration of the plant extract and amoxicillin was determined to be 0.125 and 0.25, respectively. Therefore, ethanolic plant extract can be considered a good synergistic factor to amoxicillin, yielding a fractional inhibitory concentration index $0.375 \leq 0.5$ of the combination.
**Conclusion**
*M. oleifera* leaves may serve as a natural alternative to antibiotics. Moreover, *M. oleifera* can boost the inhibitory effect of amoxicillin, leading to a reduction of administration dose as well as minimizing the side effects of antibiotics.

**Keywords:**
antibacterial, antibiotic, ethanol, *Moringa oleifera*

Egypt Pharm J 15:38–42

1687-4315

## Introduction

Microbial infections are increasing in incidence rapidly worldwide; they are resulting in life-threatening diseases in developing countries [1]. Antimicrobial activities of many plants were investigated, which led to the use of these plants as a therapeutic alternative to antibiotics, particularly considering that is characterized as a natural, safer, and cheaper alternative [2,3]. These antimicrobial agents of plant extracts were produced as secondary metabolites such as alkaloids, phenolic compounds, etc. *Moringa oleifera* is the most frequently cultivated species of a monogeneric family (Moringaceae) that has multiple pharmaceutical effects; therefore, it has been used as a remedy for various diseases, including bacterial and fungal infections, in the traditional medicinal system [4]. It is well known that the leaves of the plant have antimicrobial substances, which are usually found to vary depending on the type of solvent used for extraction. Notably, many antimicrobial substances of Moringa preparations have been reported such as 4-(4´-*O*-acetyl-α-l-rhamnopyranosyloxy)benzyl isothiocyanate, 4-(α-l-rhamnopyranosyloxy)benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-l-rhamnopyranosyloxy) benzyl glucosinolate [5]. The results of monotherapy have improved typically with consecutive treatment for invasive infections; however, combination therapy could be a substitution for monotherapy for invasive infections



 DOI: 10.4103/1687-4315.184029

that are difficult to due to multiresistant species. A combination of antimicrobial compounds might improve the effectiveness of each compound; therefore, a lower dose of each compound can be used. Moreover, some investigations have shown that antimicrobial plant extracts also promoted the effectiveness of some antibiotics. Bantar *et al.* [6] studied the combination of some antibiotics against carbapenem-resistant strains. Obviously, the combination of an aminoglycoside and β-lactams or a fluoroquinolone is still an optimal option for the treatment of invasive infections caused by *Pseudomonas aeruginosa* [7]. Palaniappan and Holley [8] studied the activity of a single natural antimicrobial substance such as carvacrol, thymol, eugenol, allylisothiocyanate, and cinnamaldehyde, as well as the activity when paired with an antibiotic using checkerboard and microdilution methods. In the former assay, the interactions between the inhibitors were characterized by calculating fractional inhibitory concentration (FIC) values. Consequently, the recent appearance of strains with reduced susceptibility to antibiotics and the recurrent spread of multidrug-resistant strains of bacteria have led to research of new infection-combat strategies [9,10]. The current study was carried out specifically to investigate the role of different plant extracts of *M. oleifera* leaves cultivated in Jazan region (Kingdom of Saudi Arabia) against some human pathogenic bacteria. In addition, an attempt was made to investigate the synergy between amoxicillin as an example of a commercial antibiotic and plant extract.

## Materials and methods

### Collection of samples

The leaves of *M. oleifera* were collected in October 2014 from Jazan, located in the southern region of the Kingdom of Saudi Arabia. The fresh plant leaves were cleaned, washed, shade dried, and homogenized to a fine powder and stored in an airtight bottle.

### Preparation of plant extracts

Twenty-five grams of the ground leaves were extracted separately in 500 ml conical flasks with 50 ml of methanol, ethanol, or *n*-hexane. The flasks were shaken at 120 rpm overnight. The extracts were filtered separately using a sterile Whatman no. 1 filter paper. The resulting filtrates were left to air dry and the residues were dissolved in 5% dimethyl sulfoxide to maintain the concentration at 1 g/ml [11]. The plant extracts were sterilized using a syringe filter (0.2 μm pore size).

### Test organisms and antibiotics

Pure overnight-grown culture of bacteria was used for the evaluation of the antimicrobial potential of the leaf extracts including both Gram-negative (*Proteus mirabilis*, *P. aeruginosa*, *Klebsiella* spp., *Shigella* spp.) and Gram-positive bacteria (*Bacillus* spp. and *Staphylococcus aureus*). A standard inoculum of bacterial suspensions was performed to yield optical density 0.5 at 600 nm using a Lambda 25 UV/VIS Spectrometer. All test bacteria were maintained on nutrient agar slants at 4°C. Three antibiotics were used in this study: chloramphenicol (30 μg), streptomycin (10 μg), penicillin (10 μg), and amoxicillin.

### Antimicrobial activity of different plant extracts

The well-diffusion method was used to evaluate the antimicrobial activity of *M. oleifera* leaf extracts [12]. Nutrient agar plates were inoculated with different strains of bacteria separately using sterile swaps. Six millimeter wells were created on the agar surface, onto which the extracts were placed. Afterwards, the plates were incubated at 37°C for 24 h. Antibacterial activity was determined by measurement of the inhibition zone.

### Comparison of the efficacy of different plant extracts and antibiotics

Antimicrobial activities of the ethanolic, methanolic, and *n*-hexane extracts were assayed using the disc diffusion method [3]. Sterile discs (6 mm) were impregnated with each extract separately. The discs were carefully and firmly placed onto the nutrient agar plates seeded previously with standardized bacterial suspensions (optical density 0.5 at 600 nm). Paper discs of chloramphenicol (30 μg), streptomycin (10 μg), and penicillin (10 μg) were used for comparison. Filter paper discs dipped into sterile 5% dimethyl sulfoxide and allowed to dry were used as a negative control. The plates were then incubated at 37°C for 24 h. Antibacterial activity was determined by measurement of the zone of inhibition.

### Determination of minimal inhibitory concentration of ethanol extract

Minimal inhibitory concentration (MIC) of ethanol extract was determined using the two-fold serial dilution method [13]. The dose levels of 20 mg/ml of plant extract were serially diluted in nutrient broth media separately to obtain 0.04, 0.08, 0.16, 0.32, 0.65, 1.25, 2.5, 5, and 10 mg/ml. Each 0.1 ml standardized suspension of *Bacillus* spp. was inoculated into the corresponding tubes. Nutrient broth inoculated with bacteria was used as a positive control and nutrient broth containing the plant extract was used as a negative control. MIC was measured as the lowest concentration of the samples with no visible growth.

### Determination of minimal bactericidal concentration of ethanol extract

Minimal bactericidal concentration (MBC) was detected by preparing different concentrations of plant extract including MIC and three lower concentrations using nutrient agar medium on which aliquots of *Bacillus* spp. were inoculated and then incubated for 24 h at 37°C. The plates were examined for the growth of the challenge microorganism [3,14].

### Determination of minimal inhibitory concentration and minimal bactericidal concentration of amoxicillin

As described above, the two-fold serial dilution method was adopted [13] using different concentrations of antibiotics (0.19, 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, and 100 μg/ml). The lowest concentration of the samples with no visible growth was considered the MIC of amoxicillin.

### Evaluation of synergistic effect of plant extract to amoxicillin

The synergy of the combination of plant extract and amoxicillin (in triplicate) against *Bacillus* spp. was investigated according to the checkerboard method [15]. The concentration gradient of both the plant extract and amoxicillin in combination ranged from 1/32 times the MIC (1/32´MIC) to 4´MIC, inoculated with the initial inoculum of *Bacillus* spp. ($1.5 \times 10^6$ cfu/ml) and incubated at 37°C for 24 h. The concentration of the combination at which the growth was completely inhibited was considered the effective MIC for the combination. To evaluate the effect of the combinations, the FIC was calculated for each antibiotic in each combination. The following formulas were used to calculate the FIC index [16]:

$$\text{FIC of plant extract} = \frac{\text{MIC of combination}}{\text{MIC of plant extract}},$$

FIC index = FIC of amoxicillin + FIC of plant extract.

Synergy was defined as an FIC index of less than or equal to 0.5.

## Results

### Antimicrobial activity of different plant extracts

The results of the antimicrobial screening of different plant extracts of *M. oleifera* are shown in Fig. 1, which show that ethanol extract exerted the highest inhibitory effect toward all test bacteria (11–30 mm inhibition zone). Particularly, *Bacillus* spp., as a model of Gram-positive bacteria, is more sensitive than Gram-negative bacteria as shown in Fig. 2.

### Comparison of the efficacy of ethanol extract and antibiotics

The results indicated that the antimicrobial activity of the plant extracts were comparable to that of antibiotics; most of the test organisms developed resistance to penicillin (10 μg), but they were all susceptible to chloramphenicol and streptomycin in various degrees (Table 1), whereas all of them were sensitive to the leaf extracts, except *Proteus mirabilis*, which was found to be resistant to hexane extract.

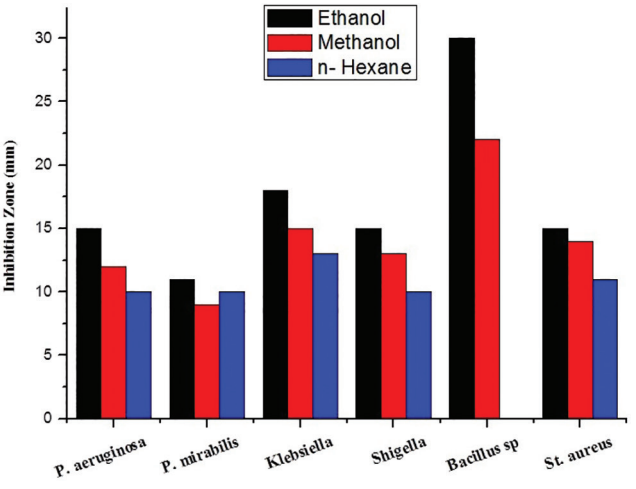
### Determination of minimal inhibitory concentration and minimal bactericidal concentration of ethanol extract

The results indicated that MIC and MBC of *Bacillus subtilis* were found to be at a concentration 0.32 and 0.62 mg/ml, respectively.

### Determination of minimal inhibitory concentration and minimal bactericidal concentration of amoxicillin

The MIC and MBC of amoxicillin against *B. subtilis* were determined to be 25 and 50 μg/ml, respectively.

**Figure 1**

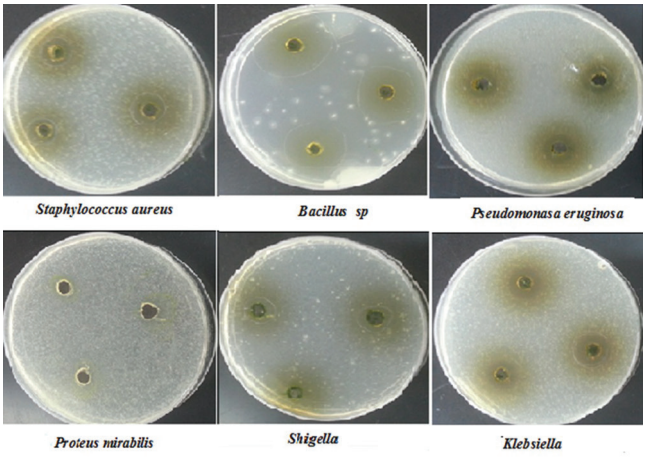



Antimicrobial activity of different plant extracts.

**Table 1 Antimicrobial activity of ethanol extracts and antibiotics**

| Bacterial species | Zone of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Ethanol extract (1g/ml) | Methanol extract (1g/ml) | Hexane extract (1g/ml) | Chloramphenicol (30 μg) | Streptomycin (10 μg) | Penicillin (10 μg) |
| P aeruginosa | 15 | 12 | 10 | 20 | 16 | 0 |
| P mirabilis | 10 | 9 | 0 | 21 | 24 | 0 |
| Klebsiella sp | 18 | 15 | 10 | 20 | 24 | 0 |
| Shigella sp | 15 | 13 | 13 | 20 | 22 | 0 |
| Bacillus sp | 30 | 22 | 11 | 42 | 29 | 25 |
| S aureus | 15 | 14 | 10 | 20 | 20 | 0 |

**Figure 2**




Antimicrobial activity of ethanol extract.

### Evaluation of the synergistic effect of amoxicillin and plant extract

The results also showed that a combination of the plant extract with amoxicillin showed a promising synergistic effect against *B. subtilis*; the FIC of the plant extract and amoxicillin was 0.125 and 0.25, respectively. Consequently, the FIC index of the combination was 0.375≤0.5, meaning that the plant extract is good synergistic to amoxicillin.

## Discussion

Different solvents have different extraction capacities and different spectra of solubility for the phytoconstituents [17]. In the current work, it was shown that an ethanol extract of *M. oleifera* leaves had a broad spectrum of antimicrobial activity against some human pathogenic Gram-positive bacteria more than Gram-negative bacteria at 1 g/ml to different degrees. These results are in agreement with other findings [3,18–20]. The differences in sensitivity between Gram-positive and Gram-negative bacteria may be attributed to their morphological variations; an outer phospholipidic membrane of Gram-negative bacteria carrying the structural lipopolysaccharide components led to cell wall impermeability to lipophilic solutes, whereas porinsporins represent a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da.

However, Gram-positive bacteria are more susceptible because of the presence of an outer peptidoglycan layer, which is not an efficient permeability barrier [21]. The antimicrobial activity of the ethanol plant extract is less than that of antibiotics; this may be because antibiotics are in a purified form, whereas the plant extracts are still in a crude state. Therefore, the antimicrobial activity of the plant extract might be higher after purification. This result is in agreement with Doughari *et al.* [3]. However, it has been found in previous studies that the plant has an effect comparable to that of some antibiotics and is more active than the others, where *M. oleifera* leaf extract showed higher antimicrobial activity than that of tetracycline [22,23]. MIC and MBC are used as the most common tools for the assessment of antimicrobial action [24]. Moreover, MIC is the lowest antibiotic concentration that inhibits growth, but does not kill the organisms. Meanwhile, a higher antibiotic concentration kills the organisms and this is the MBC. Hence, we can determine the extent of success of antibiotic in treatment. Moreover, bacteriostatic antibiotics inhibit the bacterial metabolism or ribosomal protein synthesis, whereas bactericidal antibiotics inhibit cell wall formation. The present study showed that MIC and MBC of the plant extract were higher than those of antibiotics, suggesting that the plant extract acts as a bacteriostatic at lower concentrations and as a bactericidal at higher concentrations. These findings are just in line with the observations of Aliyu *et al.* [25] and Rahman *et al.* [26]. The precise prediction of synergy between the synthetic antibiotic and a natural product relied on the results of in-vitro testing is very crucial. In the current study, the plant extract showed a synergistic effect to amoxicillin against *B. subtilis*. This synergistic action provides scientific bases for the use of concoctions traditionally in the treatment of diseases. This result is in agreement with Tahany *et al.* [27], who assessed a synergistic combination of biologically active components from wild *Moringa peregrine* against different pathogens, indicating the high efficacy of combination therapy over monotherapy. The FIC index of the combination of plant extract and amoxicillin is 0.375≤0.5, indicating that a plant extract is a good synergistic agent to amoxicillin. In addition, Hariharan [28] investigated the synergistic action of a combination of *M. oleifera* with three plant extracts, namely, *Terminalia arjuna*, *Azadira chtaindica*, and *Curcuma longa* against *B. subtilis*, reporting an FIC index of 0.291. In conclusion, *M. oleifera* could be a potent and natural antibiotic for human consumption.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1 Al-Bari MA, Sayeed MA, Rahman MS, Mossadik MA. Characterization and antimicrobial activities of a phenolic acid derivative produced by *Streptomyces bangladeshiensis*, a novel species collected in Bangladesh. Res J Med Med Sci 2006; 1:77–81.

**2** Sharif MDM, Banik GR. Status and utilization of medicinal plants in Rangamati of Bangladesh. Res J Agric Biol Sci 2006; 2:268–273.

**3** Doughari JH, Pukuma M, De N. Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi*. Afr J Biotechnol 2007; 6:2212–2215.

**4** Naqvi1 SAH, Perveen R, Drishak KU, Atif U, Shabbir SG. In-vitro evaluation of against *Xanthomonas compestris* bacterial blight of cotton. Int J Biol Sci 2014; 1:46– 55.

**5** Devendra B, Srinivas N, Prasad Talluri V, Latha PS. Antimicrobial activity of *M. oleifera* Lam. leaf extract, against selected bacterial and fungal strains. Int J Pharma Bio Sci 2011; 2:14–18.

**6** Bantar C, Di Chiara M, Nicola F, Relloso S, Smayevsky J. Comparative *in vitro* bactericidal activity between cefepime and ceftazidime, alone and associated with amikacin, against carbapenem-resistant *Pseudomonas aeruginosa* strains. Diagn Microbiol Infect Dis 2000; 37:41– 44.

**7** Giamarellou H. Prescribing guidelines for severe *Pseudomonas* infections J Antimicrob Chemother 2002; 49:229–233.

**8** Palaniappan K, Holley RA. Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. Int J Food Microbiol 2010; 140:164–168.

**9** Zy EA, Area A, Aam K. Antimicrobial activity of some medicinal plant extracts in Palestine. Pak J Med Sci 2005; 21:187–193.

**10** Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections. BMC Complement Altern Med. 2006; 6:2–10.

**11** Oluduro AO. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-Western Nigeria. Malays J Microbiol 2012; 8:59–67.

**12** Barry AL. Procedure for testing antimicrobial agent in agar media. In: Lorian V, editors. *Antibiotica in laboratory medicines*. Baltimore: Williams & Wilkins Co; 1980. 1–23.

**13** Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. J Ethnopharmacol 2004; 91:105–108.

**14** Suffredini IB, Sader HS, Goncalves AG, Reis AO, Gales AC, Varella AD, *et al.* Screening of antibacterial activity extracts obtained from plants native to Brazilian Amazon rain forest. Braz J Med Ethnopharmacol 2004; 62:183–193.

**15** White RL, Burgess DS, Manduru M, Bosso JA. Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. Am Soc Microbiol 1996; 40:1914–1918.

**16** Ofokansi KC, Attama AA, Uzor PF, Ovri MO. Evaluation of the combined antimicrobial activity of the leaf extract of *Phyllantus muellerianus* with ciprofloxacin. J Pharm Technol Drug Res 2013; 4:1–6.

**17** Srinivasan D, Perumalsamy LP, Nathan S, Sures T. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Ethnopharmacol 2001; 94:217–222.

**18** Bukar A, Uba A, Oyeyi TI. Antimicrobial profile of *Moringa oleifera* Lam. extracts against Mal. J Microbiol 2010; 8:59–67.

**19** Ojiako EN. Phytochemical analysis and antimicrobial screening of *Moringa oleifera* leaves extract. Int J Eng Sci 2014; 3:32–35.

**20** Porte SM. Overview of folk medicine user for typhoid in Indi. J. Res Ayurveda Pharm 2014; 5:219– 224.

**21** Nostro MP, Germano Á, D'Angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol 2000; 30:379–384.

**22** Busani M, Julius MP Voster M. Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. Afr J Biotechnol 2012; 11:2797–2802.

**23** Kalpana S, Moorthi S, kumara S. Antimicrobial activity of different extracts of leaf of *Moringa oleifera* (Lam) against gram positive and gram-negative bacteria. Int J Curr Microbiol App Sci 2013; 2:514–518.

**24** Kiem S, Schentag JJ. Relationship of minimal inhibitory concentration and bactericidal activity to efficacy of antibiotics for treatment of ventilator-associated pneumonia. Semin RespirCrit Care Med 2006; 27:51–67.

**25** Aliyu AB, Musa AM, Abdullahi MS, Oyewale AO, Gwarzo US. Activity of plant extracts used in northern Nigerian traditional medicine against methicillin-resistant *Staphylococcus aureus* (MRSA). Nig J Pharm Sci 2008; 7:1– 8.

**26** Rahman MM, Sheikh MMI, Sharmin SA, Islam MS, Rahman MA, Alam MF. Antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. CMU J Nat Sci. 2009; 8: 219– 227.

**27** Tahany MAAR, Hegazy A, Sayed AM, Kabiel H, El-Alfy T, El-Komy S. Study on combined antimicrobial activity of some biologically active constituents from wild *Moringa peregrine* Forssk. *J Yeast Fungal Res* 2010; 1:15–24.

**28** Hariharan AG. Synergistic antibacterial activity of some medicinal plants against pathogenic bacterial and fungal strains. Pharm Sci 2012; 1:11–14.