

Full Length Research Paper

Assessment of the antibacterial effects of *Moringa peregrina* extracts

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The antibacterial effect of *Moringa peregrina* (leaves, roots and seeds) ethanolic extracts were investigated. The effect of plant extracts were tested against three bacterial species: *Escherichia coli* (*E. coli* ATCC25922), *Staphylococcus aureus* (*S. aureus* ATCC 43300) and *Klebsiella pneumoniae* (*K. pneumoniae* ATCC 13883). *M. peregrina* ethanolic extracts showed significant antibacterial effect on the three tested bacterial strains using the disc diffusion method. The inhibition zones caused by leaf ethanolic extracts were 14 to 30, 8 to 19 and 9 to 22 mm in diameter against *E. coli*, *K. pneumoniae*, and *S. aureus*, respectively. Root ethanolic extracts showed inhibition zones as 18 to 42, 44 to 59 and 34 to 45 mm in diameter against *E. coli*, *K. pneumoniae*, and *S. aureus*, respectively. Seed extract caused inhibitory zones of 16 to 38, 6 to 32 and 6 to 18 mm in diameter against *E. coli*, *K. pneumoniae* and *S. aureus*, respectively. The results showed that the zones of inhibition for the three bacterial species increased in a dose dependant manner and that the *M. peregrina* root ethanolic extract exhibited more potent inhibition. The other test done to assess the antibacterial effect of *M. peregrina* ethanolic extracts was the minimum inhibitory concentrations (MIC). Such test was conducted on the same three bacterial species, where the MIC for the *M. peregrina* leaf extract for *E. coli*, *K. pneumoniae*, and *S. aureus* were 12.0, 15.0 and 18 mg/ml, respectively. The MIC for seed ethanolic extract was 13.0 and 7.0 mg/ml against *E. coli*, *K. pneumoniae* and *S. aureus* respectively. Also, the MIC for root ethanolic extract of *M. peregrine* against *E. coli*, *K. pneumoniae* and *S. aureus* was 9.0, 3, 2, and 5 mg/ml, respectively. Such low MIC values especially for the root extract represent strong potential for *M. peregrina* as an antibacterial agent.

Key words: *Moringa peregrina*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, minimum inhibitory concentrations (MIC), antibacterial.

INTRODUCTION

Since the early stages for human society's evolution, the usage of medicinal plants in folk medicine is highly a

potential regiment for several disorders therapy. A small percentage of plants is used by humans as food source,

eventually the majority are used for medicinal purposes. Medicinal plants are considered as one of the major constituents for disease therapy in rural areas. Most of the populations in the world depend on herbal medicine for their health care needs (Manandhar, 1994).

Phytochemicals in fruits, vegetables, spices and traditional herbs have been found to play a protective role against human diseases (Schippmann et al., 2002). Different plant extracts as crude, fractionated and sometime whole plant compounds are used for their antibacterial, anti-inflammatory, and antioxidant activities (Lev and Amar, 2002).

In addition, a high sensibility is paid for the usage of medicinal plants in pharmaceutical industries not only due to the toxicity of new synthetic drugs, but also to their high cost. Thus, plants possess medicinal properties play an important role in the discovery of new therapeutic agents (Verma and Singh, 2008). Jordan has Mediterranean like climate and shares different demographical regions as mountains, land scabs and deserts. In Jordan, it is estimated that more than 2000 species belongs to about 700 genera of wild plants grown over the Kingdom. From these species, about 485 (99 families) are used as medicinal plants (Oran and Al-Eisawi, 1998). Ethnopharmacological examination of traditional drugs showed that there are 236 local and imported plants used in Jordan traditionally for treatment of different diseases (Lev and Amar, 2002). The genus *Moringa* which is called miracle tree belongs to "Moringaceae" family with 13 known species (Olson, 2002). *Moringa peregrina* is known in Arabic as "Habb El Yasar, Habb El Pan", the seeds are known as "Habba Ghalia". It is widely grown in dry or semiarid countries neighboring the Red Sea, from Somalia and Yemen to Palestine and to Syria (Somali et al., 1984). The development and increase of antibiotic resistance, as well as the continuously evolving new strains of disease causing bacteria, have pointed to the need for new and safe antibacterial agents discovery. Medicinal plants are an attractive source for new discoveries in antibacterial agents (Cowan, 1999). Various parts of *M. peregrina* possess antibacterial activity (Lockett et al., 2000; Anwar and Rashid, 2007). Majorly, the mechanism of action for these antibacterial compounds is either to confer a death of the microorganism (bactericidal) or by preventing their growth (bacteriostatic). This is just a listing of antibiotic effects in general. More recently, *M. peregrina* aerial parts were fractionated using n-hexane and e - -amyrin - - sitosterol-3-O- -D-glucoside and apigenin were tested against various bacterial and fungal species. Results demonstrated that each of these constituents had significant antibacterial inhibitory effect as compared with standard antibiotics (Abdel-Rahman et al., 2010).

Quinones naturally occurring phenol compounds are found in *Moringa oleifera* (genus *Moringa* "Moringaceae" family with 13 known species: mentioned in the introduction) with laxative effects. *M. oleifera* leaves content such as terpenoids and steroids have antimicrobial effect against *S. aureus*, alkaloids (nitrogen-containing compound) have antimicrobial activity due to their ability to disrupt microorganisms DNA (Bennett et al., 2003). *Moringa* flavonoids, which are many in numbers, have a strong antibacterial activity against many microorganisms by inhibiting the enzyme bound membrane activity (Li-Weber, 2009). *M. peregrina* are taxa of the natural flora in Jordan especially in Wad-Bin-Hammad and Wade-Arabah. This is the first assessment of antibacterial effect of the *M. peregrina* plant ethanolic crude extracts in Jordan.

MATERIALS AND METHODS

Plant

M. peregrina wild trees were collected on June from the Wadi Bin-Hammad Valley in the South of Jordan in Karak. Root, seed and leaf each part were collected separately (exposed root on the surface, seed from large dried pods from under of the tree, and semi dried leaf).

Preparation of ethanolic extract

After collection of the plant material, the leaves, roots and seeds were dried in shade at room temperature (27 to 30°C) to constant weight (to calculate the %yield of the plant extract = dry weight of extract (g)/initial weight of plant sample × 100%). Different parts of the plant were powdered by mortar and pestle and further reduced to a fine powder using blender. Powdered sample were transferred to closed flasks. Each flask sample was extracted with 95% ethanol. Each sample (50 g) mixed with 500 ml of 95% ethanol, kept for shaking at 120 rpm for half an hour and allowed to precipitate at room temperature for 72 h. After 72 h, each extract was filtered by Whatman filter paper. The resulting filtrates were then concentrated in rotary evaporator and left to completely dry.

Bacterial strains and antibacterial activity

Three different bacterial species were used *Escherichia coli* (*E. coli* ATCC25922), *Staphylococcus aureus* (*S. aureus* ATCC 43300) and *Klebsiella pneumonia* (*K. pneumonia* ATCC 13883). These species were kindly provided by Dr. Wael Zereini, Department of Biological Sciences at Mut' ah University, Karak, Jordan.

Antimicrobial activity of *M. peregrina* ethanolic extracts was evaluated by disc diffusion method (Kirby, 1996). Briefly, inoculum containing 106 colony forming unit (CFU)/ml was spread on nutrients agar plates. Sterile filter disc (diameter 6 mm) emerging with 17 µl of ethanolic extract in minimum amount of solvent (17 µl which is the maximum capacity of the disc) at 10, 50 and 100 mg/ml concentrations. One disc paper emerging with 17 µl of ethanol was used as negative control. The previous procedure

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Table 1. Zone of inhibition of ethanolic extracts of *Moringa peregrina* plant at different concentrations on the bacterial growth of *E. coli*.

Tetracycline (10 µg)	Ampicillin (30 µg/disc)	Chloramphenicol (30 µg/disc)	<i>Moringa peregrina</i> extracts (mm)			Concentration (µg/disc)
			Seeds	Roots	Leaves	
			38 ± 2.68	42 ± 1.78	30±0.89	1700
22.5 ± 0.4	7.2 ± 0.17	26 ± 0.49	27 ± 1.7	33 ± 2.25	21 ± 1.4	850
			16 ± 2.7	18 ± 1.55	14 ± 0.81	170

*Mean of three replicates ± SD (Tetracycline, Ampicillin, Chloramphenicol).

Table 2. Zone of inhibition of ethanolic extracts of *M. peregrina* plant at different concentrations on the bacterial growth of *Staphylococcus aureus*.

Tetracycline (10 µg)	Ampicillin (30 µg/disc)	Chloramphenicol (30 µg/disc)	<i>Moringa peregrina</i> extracts (mm)			Concentration (µg/disc)
			Seeds	Roots	Leaves	
			18±2.73	45±1.74	22±2.8	1700
22 ± 0.21	7.5 ± 0.17	25 ± 0.25	13±1.76	40±2.88	16±1.5	850
			6±1.54	34±0.89	9±0.72	170

*Mean of three replicates ± SD.

Table 3. Zone of inhibition of ethanolic extracts of *Moringa peregrina* plant at different concentrations on the bacterial growth of *Klebsiella pneumoniae*.

Tetracycline (10 µg)	Ampicillin (30 µg/disc)	Chloramphenicol (30 µg/disc)	<i>Moringa peregrina</i> extracts (mm)			Concentration (µg/disc)
			Seeds	Roots	Leaves	
			32 ± 1.3	59±2.9	19±1.03	1700
21 ± 0.31	6.4 ± 0.35	27 ± 0.378	14±0.67	50±3.47	13±0.87	850
			6 ± 1.35	44±2.3	8±1.36	170

*Mean of three replicates ± SD.

was repeated with 30 µg/disc of chloramphenicol, tetracycline and with ampicillin at 10 µg/disc. The plates were incubated at 37°C for 24 h. The antibacterial activity was detected by the measurement of the diameter of the zone of inhibitions around each filter disc. Each sample was repeated three times (Romero et al., 2005).

Determination of minimum inhibitory concentration (MIC)

The MICs of the *M. peregrina* ethanolic extracts were identified in triplicates. 0.5 ml of different concentrations of the extracts (from 20 to 0.5 mg/ml) and 2 ml of the nutrient broth were added and first inoculum containing 10⁶ CFU/ml of each organism was added to each tube. A tube without any extracts was used as negative control (media and bacteria), stranded antibiotics (antibiotics disc chloramphenicol has known effect), chloramphenicol was used as positive control. All broth samples were incubated at 37°C for 24 h. The antimicrobial results were measured by observing turbidity at 600 nm wavelength (Bibitha et al., 2002).

RESULTS

Antibacterial effect of *M. peregrina* ethanolic extracts

The antibacterial activities of *M. peregrina* leaves, roots,

and seeds ethanolic extracts against *E. coli*, *S. aureus* and *K. pneumoniae* were tested and compared to known antibiotics (Tables 1, 2, and 3). The results showed that increasing the concentration of the ethanolic extracts increased the zone of inhibition.

In addition, the ethanolic roots' extracts have significantly higher antibacterial activity against *E. coli*, *S. aureus* and *K. pneumoniae* than the leaves or seeds' extracts (Tables 1, 2 and 3).

Furthermore, the antibacterial activity of the *M. peregrina* extracts against gram positive and negative bacteria seemed to be similar. Regarding MIC results, similar results were obtained. The roots' ethanolic extracts yielded lower MIC values, which agreed with the zone of inhibition studies (Table 4). In comparison to known antibiotics, *M. peregrina* ethanolic roots' extracts of 850 µg have higher antibacterial activities than 30 µg/disc of chloramphenicol, tetracycline and ampicillin at 10 µg/disc (Tables 1, 2 and 3).

100 µl of the extracts prepared at three different concentrations (10, 50, and 100 mg/ml) were pipetted onto filtered paper disc and allow to dry overnight at

Table 4. Minimum inhibitory concentration (MIC) of *Moringa peregrina* ethanolic extracts. The antimicrobial results were measured by observing turbidity at 600 nm wavelength.

Bacterial strains	MIC (mg/ml)			Chloramphenicol
	Leaves	Seeds	Roots	
<i>E. coli</i>	12±0.2	13±0.45	3±0.4	0.4± 0.015
<i>S. aureus</i>	18±0.45	9± 0.2	5±0.36	0.88 ±0.032
<i>K. pneumonia</i>	15± 0.15	7±0.4	2± 0.32	0.6 ±0.1

*Mean of three replicates ± SD.

37°C).

DISCUSSION

Traditionally plants with medicinal value have been widely used throughout human history and still are used in many regions around the world (Heinrich, 2000). There is an increasing interest in medicinal plants and homeopathic contemporary medicine especially with the raised awareness of toxic or unwanted side effects of classical drugs (Uprety et al., 2012). The current study, aimed to assess the antibacterial effect of the endemic Jordanian *M. peregrina* plant. There is plethora of studies assessing the role of the *Moringa* plant in folk medicine in many countries (Elbatran et al., 2005; Gupta et al., 2010; Dehshahri et al., 2012). However, there are neither actual uses of *M. peregrina* in Jordan, nor studies done to assess its antibacterial activity of the Jordanian *M. peregrina* ethanolic extracts.

Therefore, the present study examined the effect of short-term application of the ethanolic extracts from different parts of *M. peregrina* on the antibacterial activity against three different bacterial species. Previous studies demonstrated high prevalence of active antibacterial substances in plants. Worldwide, plants and herbs are used differently as remedy for certain diseases (Brantner et al., 1996). In the present study, *M. peregrina* ethanolic extracts exhibited antibacterial activity against broad spectrum of gram negative and gram positive bacteria. The roots ethanolic extract of the plant showed the highest antibacterial effects. Seeds and leaves of the plant also showed antibacterial activity against three bacterial strains, but less than roots extract.

Furthermore, ethanolic extracts showed a dose dependent activity against different bacterial species, but interestingly MIC values were significantly higher against three bacterial species. Afolayan and Meyer (1995) showed low susceptibility of gram negative bacteria toward plant extracts. In this study, *M. peregrina* ethanolic extracts exhibited antibacterial activity against both gram positive and negative bacteria, which indicates that the seed of *M. peregrina* contains compounds with antibacterial activity against both gram negative and positive bacteria; this implicates an activity against

protein structure or even on a transcription level, but not a specific disturbance among cell wall structure.

In conclusion, the activity of *M. peregrina* ethanolic extracts showed antibacterial activity against both gram positive and gram negative bacteria, which indicates that *M. peregrina* contains putative compounds with potential antibacterial activity against protein or DNA structures. The traditional methods of treating bacterial infection are decoction or boiling the plant in water (Nair et al., 2005). In the present study *M. peregrina* ethanolic extracts showed better antibacterial activity than that obtained by Nair et al. (2005).

A further advanced study should be pursued that specifically focuses on the antibacterial agent dosages of these plant parts, which may be used by the pharmaceutical industry.

Conflict of Interests

The authors have not declared any conflict of interests.

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