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***Ziziphus spina-christi*, a Native Plant from Khuzestan, Iran, as a Potential Source for Discovery New Antimicrobial Agents**

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Abstract: The antibacterial activity of *Ziziphus spina-christi* leaves ethanolic and methanolic extracts were examined using agar disc diffusion method against eight bacteria (*Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Brucella melitensis*, *Bordetella bronchiseptica* and *Pseudomonas aeruginosa*). These extracts had inhibitory effect at various concentrations (0.05, 0.1, 0.2, 0.3 and 0.4 g mL⁻¹) against tested bacteria. The ethanolic extract had the highest activity (20 mm) against *B. bronchiseptica* while the lowest activity (7 mm) was demonstrated by the methanolic extract on *K. pneumoniae*. Studies on the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the methanolic extract on two selected bacteria showed that the *S. dysenteriae* had the highest MIC (18 mg mL⁻¹) and MBC (64 mg mL⁻¹) values.

Key words: Plant extract, *Z. spina-christi*, antibacterial activity, pathogen

INTRODUCTION

Herbal remedies used in the traditional folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help to overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics (Ali *et al.*, 2001). Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects (Doughari, 2006). Infectious diseases account for about half of the death in tropical countries. Many studies indicate that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds (Seyyednejad *et al.*, 2008; Alma *et al.*, 2003; Klausmeyer *et al.*, 2004).

The *Ziziphus* species (Rhamnaceae) are commonly used in folklore medicine for the curing of various diseases. They are wide-spread in the Mediterranean Region, Africa, Australia and tropical America. *Z. spina-christi* has been used in folk medicine as a demulcent, depurative, anodyne, emollient, stomachic, for toothaches, astringents and as a mouth wash (Nazif, 2002). *Ziziphus spina-christi* was shown to contain betulinic and ceanothic acid (Abdelaaty *et al.*, 2001), three cyclopeptide alkaloids as well as four saponin glycosides

(Mahran *et al.*, 1996) and several flavonoids have been isolated from the leaves of *Z. spina-christi* (Amos *et al.*, 2001).

The aim of the present study was to evaluate the antibacterial activity of ethanolic and methanolic extracts obtained from the leaves of *Z. spina-christi* on selected clinical isolates of enteric bacterial pathogens.

MATERIALS AND METHODS

Collection and identification of plant materials: The plants used in this study were collected from Ahwaz in Khuzestan Province of Iran in 2007. The taxonomic identity of this plant was confirmed by our Voucher specimens, deposited at the department of biology, Shahid Chamran University, Iran.

Preparation of extracts: The leaves of *Z. spina-christi* were shade dried at room temperature for 48 h and crushed into powder using electric blender. One gram of this powder was extracted by dissolving in 10 mL of ethanol-distilled water (8:2 w/v), centrifugation (3000 rpm) for 15 min and then collecting the supernatants. This process was repeated three times. Finally, the ethanol was removed through evaporation by incubating at room temperature for 48 h (Seyyednejad *et al.*, 2001; Moazedi *et al.*, 2007). The methanolic extract was prepared following the method described by Okemo *et al.* (2001).

Test isolates: A total of 8 bacterial species were tested. *Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Brucella melitensis*, *Bordetella bronchiseptica* and *Pseudomonas aeruginosa*. These species were originally isolated from clinical materials collected from patients. They were identified using standard biochemical tests.

Determination of antimicrobial activity: Antimicrobial activity of the ethanolic and methanolic extracts of the plant sample was evaluated by the paper disc diffusion method (Maleki *et al.*, 2008). Stock culture of test bacteria were grown in TSB medium at 37°C for 22 h. Final cell concentrations were 10^8 cfu mL⁻¹ with reference to the Mc Farland turbidometry (Burt and Reinders, 2003). One milliliter of this inoculum was added to each plate containing Mueller-Hinton agar plates. (MHA, Oxoid) by sterile cotton swab and allowed to remain in contact for 1 min. Five concentrations of each extract (0.05, 0.1, 0.2, 0.3 and 0.4 g mL⁻¹) were prepared. Sterile 6 mm filter paper discs (Hsieh *et al.*, 2001) were placed on these cultures and immediately 50 µL volumes from each concentration of the two mentioned extracts were added. The plates allowed to remain 1 h at room temperature in order to diffusing the extract across the surface and then were incubated at 37°C for 24 h. The inhibition zone around each disc was measured in (mm) and the assay was carried out three times for each extract. Discs containing different concentrations of three antibiotics (Novobiocin 30 mcg, Nafcillin 1 mcg, Colistin 10 mcg) served as positive controls. Discs impregnated with 80% ethanol were also included to test if it has inhibitory effect on the test bacteria in this study.

Determination of Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) of the extracts was determined for the most sensitive bacterial species for three times. A 16 h culture was diluted with a sterile physiologic saline solution [PS; 0.85% (w/v) sodium chloride] with reference to the 0.5

McFarland standards to achievement of inoculums approximately 10^6 colony forming units (cfu) per milliliter (Burt and Reinders, 2003). A serial dilution was carried out to give final concentrations between 1 and 64 mg mL⁻¹ from crude extract. The tubes were inoculated with 30 µL of the bacterial suspension per milliliter Muller Hinton broth, homogenized and incubated at 37°C. The Minimum Inhibitory Concentration (MIC) value was determined as the lowest concentration of the crude extract in the broth medium that inhibited the visible growth of the test microorganism.

Determination of Minimum Bactericidal Concentration (MBC): To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was harvested from those tubes which didn't show any visible growth and streaked on sterile Muller-Hinton agar. Furthermore, a Muller-Hinton agar was streaked with each of the test organisms, respectively to serve as control. Plates were incubated at 37°C for 18-24 h. After this time the highest dilution that yielded no single bacterial colony on a solid medium was regarded as MBC.

RESULTS

The results showed that these extracts are effective against all of the test organisms. The highest activity (inhibition zone diameter about 20 mm) was demonstrated by the ethanolic extract of *Z. spina-christi* leaves against *B. bronchiseptica* while the lowest activity (inhibition zone diameter about 7 mm) was demonstrated by the methanolic extract against *K. pneumoniae* (Table 1).

On the other hand the ethanolic and methanolic extracts were not active against *E. coli* even in the highest concentration. However, the methanolic extract showed inhibition action at minimal concentration (0.05 g mL⁻¹) against *B. bronchiseptica*, *S. dysenteriae* and *B. melitensis*. These results suggest that antibacterial activity of *Z. spina-christi* ethanolic and methanolic extracts against tested bacteria were increased when used

Table 1: Inhibition zone (mm)* of *Z. spina-christi* ethanolic and methanolic extracts at various concentrations on some bacteria

Bacterial sp.	Various concentrations of extracts										Antibiotic discs		
	Ethanolic					Methanolic							
	0.5	0.1	0.2	0.3	0.4	0.5	0.1	0.2	0.3	0.4	NF	NB	CL
<i>Br. melitensis</i>	9	9	10	11	15	9	11	11	13	15	R	25	R
<i>E. coli</i>	R	R	R	R	R	R	R	R	R	R	R	26	10
<i>S. typhi</i>	R	R	9	R	R	R	R	R	R	R	R	12	R
<i>P. mirabilis</i>	R	R	10	11	13	R	7	R	9	10	R	30	12
<i>B. bronchiseptica</i>	18	20	20	20	15	16	17	17	19	20	R	24	R
<i>P. aeruginosa</i>	R	R	R	R	R	R	R	R	R	10	R	R	14
<i>S. dysenteriae</i>	11	12	13	10	12	11	12	11	12	14	R	15	R
<i>K. pneumoniae</i>	R	8	R	R	R	R	R	7	7	7	R	21	14

Table 2: Antibacterial activity (MIC^a and MBC^b in mg mL⁻¹) of the methanolic extract from *Z. spina-christi* on some tested bacteria

Microorganism	Methanolic extract (mg mL ⁻¹)	
	MIC	MBC
<i>B. bronchiseptica</i>	16	32
<i>S. dysenteriae</i>	18	64

^aMIC: Minimum Inhibitory Concentration, ^bMBC: Minimum Bactericidal Concentration

in higher concentrations. Also the methanolic extract generally showed lower activity against the test organisms compared to the ethanolic extract. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were 16 and 64 mg mL⁻¹, respectively for the two selected bacteria (Table 2).

DISCUSSION

Ethanolic and methanolic extracts of *Z. spina-christi* used in traditional Iran folk medicine, were screened for their antibacterial activity against eight bacterial strains. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Doughari, 2006). The results of this study showed that ethanolic and methanolic extracts from the *Z. spina-christi* inhibited the growth of various species of Gram-negative bacteria. The ethanolic extract showed slightly better killing action than the methanolic extract which means that the ethanolic extract could be used more. *E. coli* was resistant to methanolic and ethanolic extracts. That probably could be due to cell membrane permeability or due to other genetic factors. This result is supported by Nazif (2002) and Awadh Ali (2001). In contrast, Ali-Shtayeh *et al.* (1998) found that ethanolic extract of the *Z. spina-christi* was active against *E. coli* and *P. aeruginosa*. Based on earlier studies on active constituents of *Z. spina-christi*, unsaturated fatty acids represent the major components (83.5%). These unsaturated fatty acids maybe responsible for the broad spectrum antimicrobial activity of this plant (Nazif, 2002). Also the mucilage content amounted to 7.5%. This high content of mucilage makes it promising as a demulcent and emollient in folk medicine (Duke, 1985). In another works it has been reported that several bioactive flavonoids such as furocoumarins and furanocoumarins (Manderfield *et al.*, 1997) and also phenolic compounds have been isolated from parsley leaf and are known to exhibit antibacterial activity (Wong and Kitts, 2006; Maleki *et al.*, 2008). Furocoumarins can inhibit bacterial growth by reacting with DNA and disrupting DNA replication (Seyyednejad *et al.*, 2008), thus explaining the

observed growth inhibition of bacterial species in this study. On the other hand the hydrophobic character of phenolic compounds can potentially impair cellular function and membrane integrity (Raccach, 1984). The capacity of phenolic compounds to chelate transition metals also lowers the reactivity of metal ion by forming an inert metal-ligand complex. Chelation of transition metals, such as iron and copper, reduces bioavailability for bacterial growth (Seyyednejad *et al.*, 2008). The diameters of inhibition zone around the most active extracts were comparable with the standard antibiotics used as a positive control. The whole Gram-negative bacteria were resistant to Nafcillin.

These results were found to be in accordance with the use of a decoction of fresh leaves to promote the healing of fresh wounds, use as a body wash and antiseptic agents in folk medicine.

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REFERENCES

- Abdelaaty, A.S., L. Pieters, S. Apers, N. Nazeif, N. Abdel-Azim, D. Berghe and A. Vlietink, 2001. Chemical and biological investigations on *Zizyphus spina-christi* L. Phytother. Res., 15: 593-597.
- Ali, N.A., W.D. Julich, C. Kusnick and U. Lindequist, 2001. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J. Ethnopharmacol., 74: 173-179.
- Ali-Shtayeh, M.S., R.M.R. Yaghmour, Y.R. Faidi, K. Salem and M.A. Al-Nuri, 1998. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. J. Ethnopharmacol., 60: 265-271.
- Alma, M.H., A. Mavi, A. Yildirim, M. Digrak and T. Hirata, 2003. Screening chemical composition and *in vitro* antioxidant and antimicrobial activities of the essential oils from *Origanum syriacum* L. growing in Turkey. Biol. Pharm. Bull., 26: 1725-1729.
- Amos, B.A.S., C. Wambebe and K. Gamaniel, 2001. Antinociceptive activity of *Zizyphus spina-christi* root bark extract. Fitoterapia, 72: 344-350.
- Burt, A.S. and R.D. Reinders, 2003. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett. Applied Microbiol., 36: 162-167.
- Doughari, J.H., 2006. Antimicrobial activity of *Tamarindus indica* Linn. Trop. J. Pharm. Res., 5: 597-603.

- Duke, J.A., 1985. CRC Handbook of Medicinal Herbs. 1st Edn., CRC Press, Inc., Florida, USA.
- Hsieh, P.C., J.L. Mau and S.H. Huang, 2001. Antimicrobial effect of various combinations of plant extracts. Food Microbiol., 18: 35-43.
- Klausmeyer, P., G.N. Chmurny, T.G. McCloud, K.D. Tucker and R.H. Shoemaker, 2004. A novel antimicrobial indolizinium alkaloid from *Aniba panurensis*. J. Nat. Prod., 67: 1732-1735.
- Mahran, G.H., K.W. Glombitza, Y.W. Mirhom, R. Hartmann and C.G. Michel, 1996. Novel saponins from *Ziziphus spina-christi* growing in Egypt. Planta Med., 62: 163-165.
- Maleki, S., S.M. Seyyednejad, N. Mirzaie Damabi and H. Motamedi 2008. Antibacterial activity of the fruits of Iranian *Torilis leptophylla* against some clinical pathogens. Pak. J. Biol. Sci., 11: 1286-1289.
- Manderfield, M.M., H.W. Schafer, P.M. Davidson and E.A. Zottola, 1997. Isolation and identification of antimicrobial furocoumarins from parsley. J. Food Prot., 60: 72-77.
- Moazedi, A.A., N. Mirzaie Damabi, S.M. Seyyednejad, M.R. Zadkarami and A. Amirzargar, 2007. Spasmolytic effect of *Petroselinum crispum* (Parsley) on rat's ileum at different calcium chloride concentrations. Pak. J. Biol. Sci., 10: 4036-4042.
- Nazif, N.M., 2002. Phytoconstituents of *Ziziphus spina-christi* L. fruits and their antimicrobial activity. Food Chem., 76: 77-81.
- Okemo, P.O., W.E. Mwatha, S.C. Chhabra and W. Fabry, 2001. The kill kinetics of *Azadirachta indica* A. juss. (Meliaceae) extracts on *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Afr. J. Sci. Tech., 2: 113-118.
- Raccach, M., 1984. The antimicrobial activity of phenolic antioxidants in foods: A review. J. Food Safe, 6: 141-170.
- Seyyednejad, M., H. Ebrahimzadeh and A. Talaie, 2001. Carbohydrate content in olive zard c.v. and alternate bearing pattern. J. Int. Sugar, 103: 84-87.
- Seyyednejad, S.M., S. Maleki, N. Mirzaei Damabi and H. Motamedi, 2008. Antibacterial activity of *Prunus mahaleb* and Parsley (*Petroselinum crispum*) against some pathogen. Asian. J. Biol. Sci., 1: 51-55.
- Wong, P.Y.Y. and D.D. Kitts, 2006. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. Food Chem., 97: 505-515.