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Antibacterial Activity of *Prunus mahaleb* and Parsley (*Petroselinum crispum*) Against Some Pathogen

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Abstract: The antibacterial activity of parsley (*Petroselinum crispum*) and *Prunus mahaleb* seed ethanolic extracts were examined using agar disc diffusion method against eleven bacteria (*Bacillus anthracis*, *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Bacillus licheniformis*, *Brucella melitensis*, *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Bordetella bronshiseptica*, *Pseudomonas aeruginosa*). These extracts had inhibitory effect at various concentrations (0.1, 0.2, 0.3 and 0.4 g mL^{-1}) against Gram-positive and Gram-negative bacteria. *Prunus mahaleb* ethanolic extract had antibacterial activity against *P. mirabilis*, *B. anthracis* and *S. aureus*. *B. licheniformis* was the most sensitive organism to the parsley ethanolic extract. Both of the extracts had inhibitory effect against *Br. melitensis*, *E. coli* and *B. licheniformis* in low concentrations (0.1 and 0.2 g mL^{-1}). Based on the results of this study, both plants could be considered as disinfectants or antiseptics, thus confirming their use in folk medicine.

Key words: Parsley (*Petroselinum crispum*), *Prunus mahaleb*, ethanolic extract, antibacterial, pathogen

INTRODUCTION

Antimicrobial activities of various species and their derivatives have been reported by many works (Ozcan and Erkmen, 2001; Sagdic and Ozcan, 2003). The use of alternative medical therapy has increased the interest of pharmacologists and herbalists over the past decade. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made contributions to human health and well being (El-Astal *et al*., 2005). Many studies indicates 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 (Alma *et al*., 2003; Klausmeyer *et al*., 2004). These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or viruses (Holetz *et al*., 2002; Perez, 2003).

The genus *Prunus mahaleb* belongs to the family Rosaceae and comprises more than 400 species. *Prunus mahaleb* commonly known in Europe as santa lucia cherry and in the Arabia as mahleb, has been used in folk medicine as a tonic for sensory organs and the heart, in the treatment of asthma and relief of pains arising from liver, kidney and gastro intestinal troubles (Shams and Schmidt, 2007).

The plant has been used in folk medicine in Iran too. Parsley is culinary herb commonly used to flavour the cuisines of China, Mexico, South America, India and south east Asia (Wong and Kitts, 2006). There is a lack of information about the antimicrobial action of Parsley (*Petroselinum crispum*) and *Prunus mahaleb* seeds, which are medical plants of the Iran flora and widely used in treating certain disease. This study is an attempt to determine the antimicrobial activity of parsley and *Prunus mahaleb* seed ethanolic extracts on selected pathogenic bacteria isolated from patients.

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MATERIALS AND METHODS

Collection and Identification of Plant Materials

The plant materials, parsley and *Prunus mahaleb* seeds (500 g) were obtained from the local market in Khuzestan, Iran in 2007. The taxonomic identities of these plants were confirmed by us. Voucher specimens were deposited at the Botany Department of Agriculture College Shahid Chamran University. The seeds were freed from foreign materials and carefully rubbed between soft cloths to remove dust.

Extraction

The samples were ground to powder. One gram of powder was extracted using 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 by evaporation by incubating at room temperature (Seyyednejad *et al*., 2001; Moazedi *et al*., 2007).

Test Bacteria

A total of eleven bacterial species were tested. The Gram-positive species were *Bacillus anthracis*, *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Bacillus licheniformis* and Gram-negative species were *Brucella melitensis*, *Escherichia coli*, *Salmonella Typhi*, *Proteus mirabilis*, *Bordetella bronshiseptica*, *Pseudomonas aeruginosa*. The species that were not purchased were originally isolated from clinical materials collected from patients. They were identified using standard biochemical tests.

Antibacterial Susceptibility Testing

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 McFarland turbidometry (Burt and Reinders, 2003). One milliliter of this inoculum was added to each plate containing Mueller-Hinton agar (MHA, Oxoid) by sterile cotton swab and allowed to remain in contact for 1 min. Four concentrations of each extract (0.1, 0.2, 0.3, 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 of the each concentration from the two mentioned extracts were added. After 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 millimeter and the assay was carried out three times for each extract. Discs containing different concentrations of six antibiotics (Penicillin 10 mcg, Tetracycline 30 mcg, Novobiocin 30 mcg, Vancomycin 30 mcg, Nafcillin 1 mcg, Colistin 10 mcg) served as positive controls. Discs impregnated with 80% of ethanol were also included to test if it has inhibitory effect on the test bacteria in this study.

RESULTS

The antibacterial activity of the extracts was quantitatively assessed by the presence or absence of inhibition zone and measuring the diameter of the inhibition zone around the discs. Results showed the antibacterial activity of the tested extracts against test bacteria (Table 1). These results suggesting that antibacterial activity of *Prunus mahaleb* ethanolic extract against *P. mirabilis* was decreased when used in lower concentrations, but inhibitory effects of this extract against *B. anthracis* and *S. aureus* observed only in 0.4 g mL^{-1}. Also the results showed that the extract had inhibitory activity against *Br. melitensis*, *E. coli* and *B. licheniformis* in low concentrations, whereas it showed no activity against *S. typhi*, *B. bronshiseptica*, *P. aeruginosa*, *B. subtilis* and *B. pumilus*. However, *P. mirabilis* was the mot susceptible organism to the different concentrations of the ethanolic extract *Prunus mahaleb*.

Table 1: Inhibition zone (mm)* of *P. mahaleb* and parsley ethanolic extracts at various concentrations on some bacteria

	Various concentrations of extracts													
	P. mahaleb				Parsley				Antibiotic discs					
Bacterial spp.	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4	VA	TE	P	NF	NB	CL
Gram-positive bacteria														
B. anthracis	R	R	R	9	R	R	7	7	21	25	R	R	-	-
B. subtilis	R	R	R	R	R	R	R	R	25	18	R	R	-	-
B. pumilus	R	R	R	R	R	R	7	R	18	14	R	R	-	-
B. licheniformis	20	R	R	R	R	30	28	13	16	29	R	R	-	-
S. aureus	R	R	R	9	R	R	R	R	22	R	R	R	-	-
Gram-negative bacteria														
Br. melitensis	11	R	R	R	9	7	R	R	-	-	-	R	25	R
E. coli	13	R	12	10	12	10	10	9	-	-	-	R	26	10
S. typhi	R	R	R	R	R	R	R	9	-	-	-	R	12	R
P. mirabilis	R	7	16	22	R	9	10	13	-	-	-	R	30	12
B. bronshiseptica	R	R	R	R	R	R	R	R	-	-	-	R	24	R
P. aeruginosa	R	R	R	R	R	R	10	11	-	-	-	R	R	14

R: Resistant, VA: Vancomycin 30 mcg; TE: Tetracycline 30 mcg; P: Penicillin 10 mcg; NF: Nafcillin 1 mcg; NB: Novobiocin 30 mcg; CL: Colistin 10 mcg; Diameter of disc (6 mm), -: Not use

The results show in Table 1 indicate that parsley ethanolic extract had inhibitory effect at various concentrations against Gram-positive and Gram-negative bacteria. Also it was effective in lower concentrations on *E. coli*, *Br. melitensis* and *B. licheniformis*. The ethanolic extract of parsley didn't inhibit the growth of *B. subtilis*, *B. bronshiseptica* and *S. aureus*. However *B. licheniformis* was the most sensitive organism to the various concentrations of this extract. Ethanol impregnated discs containing 80% ethanol did not have a zone of inhibition probably due to the volatile nature of ethanol, so it was not considered as a factor that might affect the results.

DISCUSSION

Parallel to increasing the resistance of microorganisms to the currently used antibiotics and the high cost of production of synthetic compounds, pharmaceutical companies are now looking for alternatives. Medicinal plants could be one approach because most of them are safe with little side effects if any, cost less and affect a wide range of antibiotic resistant microorganisms. The results of this study showed that ethanolic extracts from the parsley and *Prunus mahaleb* inhibited the growth of various species of Gram-positive and Gram-negative bacteria. The *Prunus mahaleb* ethanolic extract (0.4 g mL^{-1}) showed significant effect on *P. mirabilis*. In hospitals, *P. mirabilis* is the second most frequently isolated bacteria from *Enterobacteriaceae* after *E. coli* (Champs *et al*., 2000). Also this extract showed inhibitory effect only at 0.4 g mL^{-1} concentration against *B. anthracis* and *S. aureus*. The parsley ethanolic extract inhibited the growth of 8 out of 11 bacterial species. It was effective in lower concentrations on *E. coli*, *Br. melitensis* and *B. licheniformis*. Some researchers have shown that fresh and dried *Parsley* inhibited the growth of *Listeria monocytogenes*, *L. innocua*, *E. coli* O157 H:7, *E. coli* Bs-1 and *E. carotovora* (Manderfield *et al*., 1997). In another work has been reported that Gram-positive bacteria were found to be more susceptible than Gram-negative bacteria. This could be due to the fact that the cell wall of Gram-positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelop (El-Astal *et al*., 2005), but the results obtained in our study were different.

The observed resistance of some bacteria such as *B. subtilis*, *B. bronshiseptica*, *S. typhi*, *P. aeruginosa*, *S. aureus* and *B. pumilus*, probably could be due to cell membrane permeability or other genetic factors. Several bioactive flavonoids such as furocoumarins and furanocoumarins (Manderfield *et al*., 1997) also phenolic compounds have been isolated from parsley leaf and are known to exhibit antibacterial activities (Wong and Kitts, 2006). Furocoumarins can inhibit bacterial growth

by reacting with DNA and disrupting DNA replication (Manderfield *et al*., 1997), 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 (Jay, 1996).

Tannins could be one of the components responsible for the antibacterial activity since it was reported by other studies that tested different plants (Nimri *et al*., 1999). In general, the mechanisms by which microorganisms survive the action of antimicrobial agents are poorly understood and remain debatable (Okemo *et al*., 2001).

The diameters of inhibition zone around the most active extracts were comparable with the standard antibiotics used as a positive control. All of the Gram-positive bacteria were resistant to antibiotics used (Penicillin and Nafcillin), whereas the whole Gram-negative bacteria were resistant to Nafcillin. The biologically active components in the tested plants, especially *Prunus mahaleb*, are not known and needs further analysis. Based on the results of this study we will further investigate the plants that showed broad antibacterial activities *in vivo* to uncover their potential as a source of antibiotics against selected human pathogens. The active plant extracts could also be considered as disinfectants or antiseptics.

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