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Antibacterial Activity of the Fruits of Iranian *Torilis leptophylla* Against Some Clinical Pathogens

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Abstract: The aim of this study was to examine the antimicrobial activity of the methanolic extract of *Torilis leptophylla* was tested on eleven bacteria (*Bacillus anthracis*, *Bacillus subtilis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Bacillus licheniformis*, *Brucella melitensis*, *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Bordetella bronshiseptica* and *Pseudomonas aeruginosa*). Tested extract was effective against all bacteria but not *B. subtilis*. Consequently, the ethanolic extract had antibacterial activity on some pathogens thus confirming their use in folk medicine.

Key words: *Torilis leptophylla*, extract, antibacterial, pathogen

INTRODUCTION

Antimicrobial activities of various species and their derivatives have been reported by many researchers Ozcan and Erkmen (2001) and Sagdic and Ozcan (2003). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Plants are known to produce a variety of compounds to protect themselves against a variety of their own pathogens and therefore can be considered as potential source of different classes of antimicrobial substances (Nimri *et al*., 1999; Grayer and Harborne, 1994). The genus *Torilis* comprises species distributed in Europe, North Africa and southwest Asia. It's represented in Iran by nine species (Bigdeli *et al*., 2004). *Torilis leptophylla* belonging to the Apiaceae family (Baranski *et al*., 2006). This plant has been used in folk medicine for the treatment of gastrointestinal (GI) illnesses in Khuzestan, Iran. To date no pharmacological evidence has been reported to support this claim. The goal of this study was to investigate antibacterial activity of ethanolic extract fruits of *Torilis leptophylla*.

MATERIALS AND METHODS

Collection and identification of plant materials: The plants used in this study were collected from Izeh in Khuzestan province of Iran in 2007. The taxonomic identity of this plant was confirmed by us. Voucher specimens were deposited at the Botany Department of Agriculture College Shahid Chamran University.

Extraction: The samples were ground to powder. One gram of powder was extracted using 10 mL of ethanol-distilled water (8:2 w/v), centrifuging for 15 min and the collecting the supernatants. This process was repeated three times. The ethanol was then removed by evaporation (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* and *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 Mc Farland 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 the harvested extract (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 the culture plated and immediately 50 µL portions of the each concentration of the extract were added. After the plates allowed to remain 1 h at room temperature in order to diffusing the

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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 any effect on the results obtained with the plant extract.

RESULTS

The antibacterial activity of the extract was quantitatively assessed by the presence or absence of inhibition zone and by measuring the diameter of the inhibition zone around the discs. Results showed the antibacterial activity of tested extract against various bacterial species. These results suggesting that antibacterial activity of *T. leptophylla* alcoholic extract against four bacteria including *E. coli*, *S. typhi*, *P. mirabilis* and *B. anthracis* was decreased when used in lower concentrations (Table 1). But inhibitory effects of this extract against *B. bronshiseptica* and *S. aureus* observed only in 0.4 g mL^{-1} concentration. Also, the results showed that the extract had inhibitory activity against *Br. melitensis*, *P. aeruginosa*, *B. licheniformis* and *B. pumilus* at 0.2 g mL^{-1}. On the other hand the ethanolic extract was not active against *B. subtilis* even in the highest concentration used. However, *P. mirabilis* was the most susceptible organism to the different concentration of the ethanolic extracts of *T. leptophylla*. 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 resistance microorganisms. The results of this study showed that ethanolic extract from the *Torilis leptophylla* fruit inhibited the growth of various species of Gram-positive and Gram-negative bacteria. This extract at 0.4 g mL^{-1} concentration showed a high antibacterial activity in such a manner that inhibited the growth of 4 out of 6 tested Gram-negative bacteria. Furthermore, this extract showed inhibitory effect only 0.2 g mL^{-1} concentration against these four species (Table 1). The observed resistant of *B. subtilis* probably could be due to cell membrane permeability or other genetic factors. Some researchers have shown that the alcoholic extract of *T. japonica* had good anti-protozoal activity against *N. caninum* and *T. gondii in vitro* (Youn *et al*., 2004). In another article, 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 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 envelope (El-Astal *et al*., 2005), but the results obtained in this study were different. Coumarins, flavonoids and new bisabolane sesquiterpene ester were isolated from the fruit oil of *T. aroensis* growing in Egypt (Bigdeli *et al*., 2004). Several studies have been conducted on the antimicrobial activity of plant extracts found in folk medicine (Ngwendson *et al*., 2003), essential oils (Alma *et al*., 2003) or isolated

Table 1: Inhibition zone (mm)* of *T. leptophylla* ethanolic extract at various concentration on some bacteria

	Various concentrations of extract				Antibiotic discs					
Bacterial species	0.1	0.2	0.3	0.4	VA	TE	P	NF	NB	CL
Gram-positive bacteria										
B. anthracis	R	8	8	11	21	25	R	R	-	-
B. subtilis	R	R	R	R	25	18	R	R	-	-
B. pumilus	R	10	R	R	18	14	R	R	-	-
B. lichniformis	R	26	R	R	16	29	R	R	-	-
S. aureus	R	R	R	10	22	R	R	R	-	-
Gram-negative bacteria										
Br. meliteusis	R	8	R	R	-	-	-	R	25	R
E. coli	R	R	11	12	-	-	-	R	26	10
S. typhi	R	7	7	8	-	-	-	R	12	R
P. mirabilis	R	8	15	21	-	-	-	R	30	12
B. broushiseptica	R	R	R	8	-	-	-	R	24	R
P. aeruginosa	R	10	R	R	-	-	-	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

compounds such as alkaloids (Klausmeyer *et al*., 2004), flavonoids (Sohn *et al*., 2004), sesquiterpene lactones (Lin *et al*., 2003), diterpenes (El-Seedi *et al*., 2002). Some 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). Some researchers reported that there is relationship between the chemical structures of the most abundant compounds in the tester essential oil and their antimicrobial activity (Sagdic and Ozcan, 2003). 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). The diameter of inhibition zone round the most active extracts were comparable with the standard antibiotics used as a positive control. 4 out of 5 Gram-positive and all of the Gram-positive bacteria were resistance to antibiotics used (Penicillin and Nafcillin), whereas the whole Gram-negative bacteria were resistant to Nafcillin. Antimicrobial assays on plants extracts are valuable in screening and detecting the presence of antimicrobial activities. However such assays do not provide true quantitative measure of the activities of some components present in the extract such as the polar and large molecules which have lower mobility in the water-agar medium (Nimri *et al*., 1999). The biologically active components in the tested plant are not known and needs further analysis. Based on the results of this study we will further investigate the plant that showed broad antibacterial activities *in vivo* to uncover their potential as a source of antibiotics against selected human pathogens. The active plant extract could also be considered as disinfectants or antiseptics.

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