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In vitro Antimicrobial and Antioxidant Properties of *Smyrnium cordifolium* Boiss. (Umbelliferae) Extract

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Abstract: The aim of this study was to investigate antioxidant and antibacterial activities of ethanolic extract of *Smyrnium cordifolium* Boiss. the ethanolic extract was subjected to screening for its possible antioxidant activity. Namely DPPH free radical scavenging, FTC system and total phenolic compounds were used. Also, Antibacterial activity of ethanolic extract using Gentamicin and Tetracycline as the reference standards were tested against *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Klebsiella pneumoniae*. The results revealed good antioxidant activity of the extract. The ethanolic extract of *S. cordifolium* exhibited an antibacterial activity at different levels against strains reported as the causal agents of diseases and this extract inhibited Gram positive bacteria significantly higher than Gram negative bacteria. Observed antioxidant and antibacterial properties of ethanolic extract of the *S. cordifolium* in this study showed that this plant might be useful source for the development of new and more potent natural antioxidants and antibacterial.

Key words: *Smyrnium cordifolium* Boiss. (Umbelliferae), antimicrobial, antioxidant, DPPH

INTRODUCTION

Almost all organisms are well protected against free radical damage by either enzymes or compounds, such as ascorbic acid, α-Tocopherol and glutathione. When the mechanism of antioxidant protection becomes unbalanced by the deterioration of different factors, physiological functions can occur which result in diseases or accelerated aging consequently, it is important to find compounds that prevent oxidation. Antioxidants have important preventive roles, not only on undesirable changes in the flavor and nutritional quality of food, but also on tissue damage in various human diseases (Milovanovic *et al*., 2002).

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reaction this situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drug for the treatment of infectious disease for medicinal plants. Antimicrobials with plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of side effects that are often associated with synthetic antimicrobials (Joshi *et al*., 2009).

The safety of synthetic compounds, however, has been cause of concern and has stimulated the evaluation of the effectiveness of natural compounds, or extracts with potent antioxidative and antibacterial activities.

Genus *Smyrnium* belongs to Umbelliferae family (Amiri *et al*., 2006). The essential oils obtained from some species of Umbelliferae family such as *Psammogeton canescens* have antimicrobial activity against Gram-positive and Gram-negative bacteria (Mujeeb-ur-Rahman and Gul, 2002). Other studies revealed that some species extracts of this family (*Anethum graveolens*, *Foeniculum vulgare*, *Trachyspermum ammi*, *Ferula halophila*, *Seseli gummiferum*, *S. resinosum* and *S. hartvigii* have antimicrobial activity against *Staphylococcus aureus*, *Salmonella thyphi*, *Salmonella thyphimurium*, *Shigella flexneri*, *Klebsiella pneumonia*, *Vibrio cholera*, *Pseudomonas aeroginosae*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* (Baldemir *et al*., 2006; Gurinder and Daljit, 2008).

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Smyrnium cordifolium Boiss. is a native of Iran and eaten as a green by some people in west part of Iran. It is often used internally for bladder and kidney swelling.

Only a few reports on the analysis of essential oils of *Smyrnium* species have been published (Bertoli and Pistell, 2004). As the result of these activities, sesquiterpens, monoterpens, lactones, flavonoids and acid folic were reported as major constituents of essential oil of 7 genius of this species.

The essential oils obtained from stem, leaf, root and fruit of *Smyrnium cordifolium* have antibacterial effects that correlate with large amounts of sesquiterpene hydrocarbons such as curzerene, curzerenone and germacrone (Amiri *et al*., 2006).

In this present study, under condition of *in vitro* the extract of Aerial part of *Smyrnium cordifolium* Boiss. (Umbelliferae) was examined as a potential source for active antioxidants and antibacterial.

MATERIALS AND METHODS

Chemicals and instruments: Chemicals and reagents, including 1, 1-diphenyl-2-picrylhydrazyl (DPPH), potassium dihydrogen phosphate buffer, ammonium thiocyanate, ferrous chloride, gallic acid tetracycline, gentamicin and nystatine, Mueller Hinton broth, Sabouraud dextrose broth were purchased from Merck Chemical Co. (Germany). linolenic acid was purchased from Sigms Co. (Mo, USA). All other chemicals and solvents used in this study were of the reagent grade.

The UV-Vis spectrophotometer (model 8453 Hewlett Packard, Agilent Technologies, USA). Rotary evaporator (model 3001 Heidolph, Germany).

Plant material: *Smyrnium cordifolium* Boiss. was collected from Oramanat area, Kermanshah (Western part of Iran) in May 2008 and identified in Kermanshah Research Institute of Forests and Rangelands. The Aerial parts of fresh plants were sliced and air dried with active ventilation at ambient temperature.

Extract preparation: In order to extraction according to conventional procedures (Kumaran and Karunakaran, 2007a, b). About 50 g of powdered plant was treated with 100 mL ethanol at room temperature with stirring. This procedure was repeated five times until the extraction solvent became colorless. The obtained extract was filtered over whatman No.1. Paper filter and the filtrates were collected and then ethanol was removed in vacuum at temperature not exceeding 40°C.

Ferric Thiocyanate (FTC) method: The previously described method was used. A mixture of 4.0 mg of plant extract dissolved in 4 mL of absolute ethanol; of linolenic acid solution (22.5 mg mL^{-1} absolute ethanol); 8 mL of 0.05 M potassium dihydrogen phosphate buffer (pH = 7.0) and 3.9 mL of water was placed in a vial with a screw cap and then placed in a dark oven at 40°C. During incubation (every 24 h) A volume 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate were added to 0.1 mL of this solution. Precisely 3 min after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color measured at 500 nm every 24 h for 96 h. A mixture without a plant extract was used as negative control.

The degree of linoleic acid peroxidation was calculated using the following formula:

$$AA\,(\%) = 100 - \left(\frac{\text{Absorbance of sample t}}{\text{Absorbance of control t}}\right) \times 100$$

DPPH assay: The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 1, 1-Diphenly 2-picryl hydrazyl (DPPH). This spectrophotometeric assay uses the stable radical DPPH as a reagent (Turkoglu *et al*., 2006).

One milliliter of various concentrations of the ethanolic extract (20, 50, 100, 250, 500 μg mL^{-1}) was added to 4 mL of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 512 nm. Inhibition of free radical by DPPH in percent (I%) was calculated with the following equation:

$$I\,(\%) = \left(\frac{\text{A blank} - \text{A sample}}{\text{A blank}}\right) \times 100$$

where, A blank is the absorbance of the control reaction (containing all reagent except the test compound). A sample is the absorbance of the test compound.

The IC_{50} represents the level where 50% of the radicals were scavenged by test samples. The inhibition curve was plotted and IC_{50} values were determined.

Phenolic compounds: Total Phenolic content of the plant extract was determined using folin-ciocaltue reagent. The mixture was shaken thoroughly and made up to 10 mL using distilled water. The amount of total phenolic compounds in the plant extract was determined colorimetrically with the Folin Ciocalteu (FC) reagent and 1.5 mL of 20% sodium carbonate according to earlier

studies (Kumaran and Karunakaran, 2007a, b). The reaction mixture contained 500 μL of 0.1% w/w aqueous dilution of dry extract, 2.5 mL of freshly prepared 0.2 M FC reagent and 1.5 mL of sodium carbonate solution and was kept in the dark under ambient conditions for 2 h to complete the reaction. Then absorbance of the resulting solution was measured at 765 nm in a UV-Vis spectrophotometer. The concentration of total phenolic compounds was expressed as mg of Gallic Acid Equivalents (GAE) per g of Dried Extract (DE), using a standard curve of gallic acid. All measurements were carried out in five replicates.

Statistical analysis: All data were expressed as Mean±SD. Analysis of at least three samples were carried out in triplicates. Student's t-test was carried out to compare the data and results were considered statistically significant at $p<0.05$.

Antimicrobial assay: The antimicrobial activity of *S. cordifolium* Boiss. alcoholic extract was individually tested against a panel of microorganisms, including *Bacillus subtilis* (ATCC 465), *Enterococcus faecalis* (ATCC 29737), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 10031). Bacterial strains were cultured overnight at 37°C in Mueller Hinton broth. For determination of antimicrobial activities disc diffusion method and MIC (minimum inhibitory concentration) measurement were employed. The MIC of ethanolic extract against the test microorganisms was determined by the Micro dilution method (NCCLS (National Committee for Clinical Laboratory Standards, 2000)).

RESULTS

Antioxidant activity of ethanolic extract of *S. cordifolium* Boiss. (Umbelliferae): The ethanolic extract was subjected to evaluation for antioxidant activity using 2-2-diphenyl-1-picrylhydrazyl (DPPH) for measurement of free radical scavenging activity, with Ferric Ammonium Thiocyanate (FTC) method for evaluation of lipid peroxidation properties and total phenolic compounds were used for this purpose. The yield of ethanolic extraction of *S. cordifolium* Boiss. (Umbelliferae) and its DPPH scavenging activity (30.75%), inhibition of linoleic acid peroxidation and IC_{50} (0.2) of the extract are given in Table 1.

The ability of the ethanolic extract of *S. cordifolium* Boiss. (Umbelliferae) to inhibit lipid peroxidation is determined and the results are shown in Fig. 1.

Table 1: Yield of extraction, total amount of plant phenolic compounds and antioxidant capacity of ethanol extract of *S. cordifolium* Boiss. (Umbelliferae)

Factors	Values
Extraction yield	23.43
TPC	111.23
FTC	82.80%
DPPH	30.75%
IC_{50}	0.20

TPC: Total phenolic content, FTC: Ferric thiocyanate method, DPPH: 1-Diphenly 2-picryl hydrazyl, IC_{50}: Inhibitory concentration

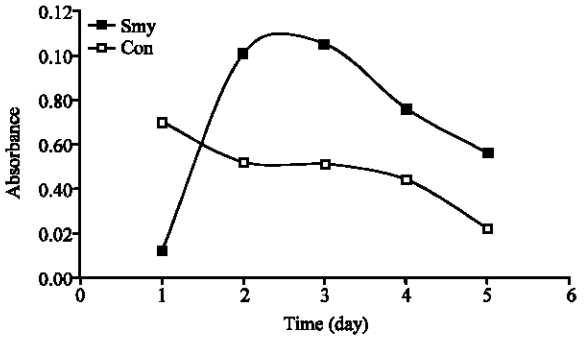

Fig. 1: Antioxidative activity of *S. cordifolium* Boiss. (Umbelliferae) extract in linoleic acid system measured by ferric thiocyanate method. Values represent Mean±SE (n = 3)

Ethanolic extract of *S. cordifolium* Boiss. (Umbelliferae) showed absorbance values greater than the controls (without plant extract) indicating the presence of antioxidant activity.

The DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging capacity of the extract. As antioxidant donates protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Scavenging activity of ethanolic extract of *S. cordifolium* Boiss. (Umbelliferae) against 2, 2-Diphenyl-1-Picrylhydrazyl radical is shown in Fig. 2. The experimental data revealed the effect of free radical scavenger for all concentrations which applied.

The IC_{50} value for plant extract, defined as the concentration of extract, causing 50% inhibition of absorbance, was determined from the concentration response cure plotted for inhibition of the absorbance of DPPH radical at 517 nm.

Since, IC_{50} is the measure of inhibitory concentration, a lower IC_{50} value would reflect greater antioxidant activity of sample. Hence, in the DPPH assay the extract exhibited a notable dose dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 0.2 mg mL^{-1} capacity.

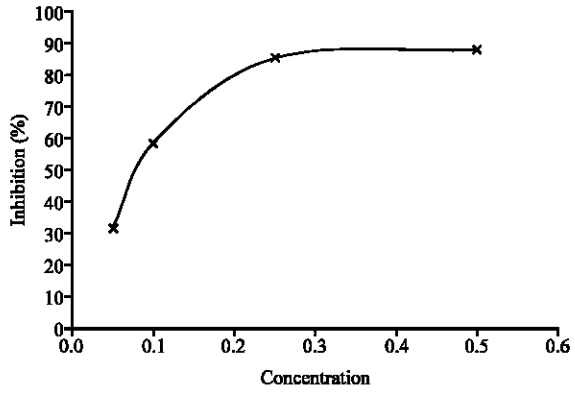

Fig. 2: Scavenging capacity of *S. cordifolium* Boiss. (Umbelliferae) extract on DPPH determined by measuring absorbance of the reaction mixture at 515 nm. Values represent Mean±SE (n = 3)

Table 2: Inhibition zone (mm) and MIC (μg mL^{-1}) values of ethanolic extract of *S. cordifolium* Boiss. (Umbelliferae) in comparison with control

	Ethanolic extract		Tetracycline (30 μg $disc^{-1}$)		Gentamicin (10 μg $disc^{-1}$)	
Microorganism	IZ	MIC	IZ	MIC	IZ	MIC
B. subtilis	14	7.5	21	3.2	-	nt
E. faecalis	13	15	9	6.4	-	nt
S. aureus	15	7.5	20	3.2	-	nt
S. epidermidis	18	3.75	34	1.6	-	nt
E. coli	14	15	-	nt	23	3.2
K. pneumoniae	11	15	-	nt	20	3.2

IZ: Inhibition zone, MIC: Minimum inhibitory zone, nt: Not tested

Antimicrobial activity: The antimicrobial activity of ethanolic extract against six strains of bacteria performed by the disk agar diffusion and micro dilution method (NCCLS, 2000) showed that *S. cordifolium* Boiss. (Umbelliferae) has antibacterial activity which would be attributed to many factors such as the strain of bacteria tested and concentration of active compounds.

The results of sensitivity microorganisms to ethanolic extract of *S. cordifolium* Boiss. (Umbelliferae) are shown in Table 2. The extract show an activity against all bacteria tested.

Generally, these data indicate that Gram-positive bacteria are more sensitive than Gram-negative bacteria to extract with the mean growth inhibition zone (13-18 mm).

Among bacteria tested, *Staphylococcus epidermidis* and *Klebsiella pneumoniae* have most sensitive and resistant, respectively (18 and 11 mm).

DISCUSSION

As far as our literature survey could ascertain, there is no report dealing with antioxidant properties of *Smyrnium* genus. The results of assays agree that the extract of *S. cordifolium* Boiss. (Umbelliferae) displayed high antioxidant.

Present results are in agreement with earlier studies showing a stronger activity of different species of umbelliferae against Gram-positive bacteria (Baldemir *et al*., 2006; Gurinder and Daljit, 2008).

This difference between Gram positive and Gram-negative bacteria sensitivity to ethanolic extract *S. cordifolium* attribute to the absence of outer membrane in Gram-positive bacteria that is almost impermeable to lipophilic compounds and large molecules.

The antibacterial activity of *S. cordifolium* correlates large amounts of sesquiterpene hydrocarbons such as curzerene, curzerenone and germacrone.

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