African Journal of Pharmacy and Pharmacology Vol. 6(13), pp. 968-972, 8 April, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.079
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Proximate composition, antimicrobial and antioxidant activities of six wild edible celeries (*Smyrnium* L.)

Ersin Minareci[1]*, Bülent Ergönül[2] and Fatih Kalyoncu[1]

[1]Department of Biology, Celal Bayar University, Muradiye, Manisa, Turkey.
[2]Department of Food Engineering, Celal Bayar University, Manisa, Turkey.

Accepted 20 March, 2012

In this study, proximate composition, antimicrobial and antioxidant activities of six *Smyrnium* taxa, including, *Smyrnium olusatrum*, *Smyrnium perfoliatum*, *Smyrnium rotundifolium*, *Smyrnium cordifolium*, *Smyrnium connatum* and *Smyrnium creticum* (Umbelliferae) were determined. Antioxidant properties of methanol extracts were studied by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. Among all the *Smyrnium* taxa, *S. olusatrum* showed the most potent radical scavenging activities. Antimicrobial activities of these taxa were studied using agar well diffusion method and *S. perfoliatum* showed broad-spectrum antibacterial activity with inhibition zones ranging from 13 to 25 mm.

Key words: *Smyrnium*, antimicrobial, antioxidant, umbelliferae.

INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis and atherosclerosis as well as in degenerative processes associated with ageing (Turkoglu et al., 2007). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherols and glutathione (Elmastas et al., 2005). When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing. However, antioxidant supplements or antioxidant-containing foods may be used to help the human body to reduce oxidative damage (Cazzi et al., 1997).

Many species of fruits, vegetables, herbs, cereals, sprouts and seeds have been investigated for antioxidant activity during the past decade (Halliwell and Gutteridge, 2003). Natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress, the latter being considered a cause of ageing and degenerative diseases (Niki et al., 1994).

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, problems 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. Antimicrobials with plant origin have enormous therapeutic potential (Khanahmadi et al., 2010).

Turkey is one of the richest areas in the middle latitudes in terms of plant diversity. The main reasons for this are: Climates varieties, geomorphological and soil diversities, and the situation of the area at the junction of three flora region. The flora of Turkey is relatively rich (about 12000 taxa) and still a great number of new species are being described (Avcı, 2005). The genus *Smyrnium* as a member of family Umbelliferae Juss., is a biennial plant growing in western and southern Europe, especially in coastal areas of the British Islands and the Mediterranean region (Bertoli et al., 2004). *Smyrnium* L.

*Corresponding author. E-mail: ersinminareci@gmail.com.

includes 6 taxa in the Turkish flora: *Smyrnium olusatrum* L., *Smyrnium perfoliatum* L., *Smyrnium rotundifolium* Miller, *Smyrnium cordifolium* Boiss., *Smyrnium connatum* Boiss. and Kotschy and *Smyrnium creticum* Miller.

Smyrnium taxa are constantly considered as a plant with diuretic, depurative and aperient properties, particularly through its root. However, their most outstanding quality is perhaps as an antiscorbutic because of its high vitamin C content. The fruit has carminative and stomachic properties (Bermejo and Leon, 1994). Their commonest use has been as a fresh vegetable, with a preference being shown for its leaves, young shoots and leaf stalks, which impart a pleasant flavor similar to celery, although somewhat sharper (Bermejo and Leon, 1994).

Despite the medicinal potential of plants in Turkey being considerable, knowledge of this area and studies on these plants is scarce (Kalyoncu et al., 2006). To the best of our knowledge, no information is available on the antimicrobial and antioxidant nature of these plants. Our objective was to evaluate the proximate chemical content, antimicrobial and antioxidant activities of six *Smyrnium* taxa.

MATERIALS AND METHODS

Plant materials

In this study, six *Smyrnium* taxa, including, *S. olusatrum* L.; *S. perfoliatum*; *S. rotundifolium* (Miller) Hartwig; *S. cordifolium* Boiss.; *S. connatum* Boiss. and Kotschy and *S. creticum* Miller collected from Anatolia were analyzed for their proximate chemical content, antimicrobial and antioxidant activities. Origins of these herbs are given in Table 1. Voucher specimens were deposited in the Herbarium of Botany, Department of Biology, Celal Bayar University. The aerial parts of these plants were used in the present study.

Antimicrobial activity assay

The dried and powdered aerial parts were reduced to coarse powder. 5 g of each taxon was extracted with 40 ml of methanol at room temperature with stirring for 3 days (125 cycles/min). The methanol was evaporated to dryness after extraction progress. Sample solutions were prepared by dissolving the extracts in dimethyl sulfoxide (DMSO) (1 ml). *In vitro* antimicrobial studies were carried out by the agar well diffusion method against test microorganisms. Bacterial strains (*Escherichia coli* ATCC 39628, *Enterobacter cloacae* ATCC 13047D, *Salmonella typhimurium* CCM 5445, *Sarcina lutea* ATCC 9341NA) grown on nutrient agar at 37℃ for 24 h and a yeast (*Candida albicans* ATCC 10231) grown on potato dextrose agar at 27℃ for 48 h were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standards [10^6 Colony Forming Units (CFU)/ml]. Briefly, 50 µl inoculum (containing approximately 10^5 bacteria per ml and 10^4 yeast) was added to 25 ml melted Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium cooled at 45℃. This was then poured into 90 mm diameter Petri dishes and maintained for 1 h at room temperature. Small wells (6 mm diameter) were cut in the agar plate using a cork borer; 50 µl of extract concentration with a negative control (DMSO, 50 µl) was loaded in the wells. The dishes were preincubated at 4℃ for 2 h to allow uniform diff usion into the agar. After pre-incubation for bacteria, the plates were incubated at 37℃ for 24 h and 30℃ for 48 h for yeast (Oskay and Sari, 2007). The antimicrobial activity was evaluated by measuring the inhibition zone diameter observed. In addition, commercial antibiotics [penicillin G (10 IU), nalidixic acid (30 µg), novobiocin (30 µg), and nystatin (10 µg)] were used as positive control to determine the sensitivity of the strains (Kalyoncu and Oskay, 2008). All experiments were performed in triplicate.

Antioxidant activity assay

The capacity to scavenge the "stable" free radical DPPH was monitored according to the method of Barros et al. (2007). Various concentrations of methanolic extracts from plants (2 ml) were mixed with 2 ml of methanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: $\% RSA = [(A_{DPPH} - A_S) / A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The assays were carried out in triplicate and the results expressed as mean values ± standard deviations. BHT and BHA were used as standard.

Chemical composition assay

The water amount and total carbohydrates of plant samples were determined according to AOAC (2006). Total protein was determined by the Kjeldahl method. Protein was calculated using the general factor of 6.25. The weight of fat extracted from 5 g of plant sample was determined to calculate the lipid content (AOAC, 2006). Diethyl ether was used as an extraction solvent where the extraction was performed for 4 h. 2 g of the sample, in a porcelain container, was ignited and incinerated in the muffle furnace at about 550℃ for 8 h until a greyish white ash was obtained (AOAC, 2006).

Statistical analysis

The data presented are the averages of the results of three replicates with a standard error of less than 5%.

RESULTS AND DISCUSSION

Extraction yields

The yields of methanol extracts of six *Smyrnium* taxa are given in Table 3.

Antimicrobial activity assay

Antimicrobial activity of six *Smyrnium* taxa has been evaluated *in vitro* against four bacterial species and one yeast, which are known to cause dermic and mucosal infections besides other infections in humans. All *Smyrnium* taxa studied in this work showed antimicrobial activity against at least one of the test microorganisms

Table 1. The location and habitats of studied specimens in *Smyrnium*.

Taxa	Location and habitat
S. olusatrum	Izmir: Central exit of Izmir-Cesme highway, inside fence area, 50 m, 38°16'366"N, 26°22'298"E, 12.03.2010
S. perfoliatum	Bolu: Bolu towards Abant Lake, Akcaalan village, 1000 m, 40°39'335"N, 31°24'028"E, 22.04.2010
S. rotundifolium	Izmir: Kemalpasa, Bağyurdu town, 190 m, 38°24'588"N, 27°38'472"E, 11.03. 2010
S. cordifolium	Ankara: Northeast of Hasanoğlan district, Hasanoğlan stream,1350 m, 36°36'039"N, 33°03'195"E, 15.06.2010
S. connatum	Denizli: Honaz district towards Honaz Mountain, 1000 m, 37°44'331"N, 29°14'487"E, 09.04.2010
S. creticum	Manisa: Between Muradiye and Emiralem (Menemen), 70 m, 38°36'561"N, 27°09'069"E, 21.05.2010

with inhibition zones ranging from 9 to 25 mm (Table 2). The most active taxon was *S. perfoliatum* which showed broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, whereas the least active taxon was *S. olusatrum*.

Maximum inhibition was shown by methanol extract of *S. perfoliatum* against *Enterobacter cloacae* as 25 mm (Table 2). Also, the methanol extract of *S. perfoliatum was* determined to be similarity effective to that of some comparison antibiotics (Table 2). All studied taxa have no anti-yeast activity against *C. albicans*.

Khanahmadi et al. (2010) reported that ethanol extract of *S. cordifolium* has inhibition activity (11 to 18 mm zone diameter) against six bacterial strains. Its ethanol extract showed antibacterial activity against *E. coli* with 14 mm zone diameter; but in our study, methanol extract of this plant species has 10 mm zone diameter against *E. coli*.

Free-radical scavenging activity

The methanol extracts of plant samples were subjected to screening for possible antioxidant activity by the DPPH free radical scavenging method. The model of scavenging the stable DPPH radical is widely used to evaluate antioxidant activities over a relatively short time compared to other methods. DPPH is a stable free radical with a characteristic absorption at 517 nm and as antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging values of *Smyrnium* extracts as percentage are shown in Table 3.

Methanol extracts of *S. olusatrum* showed the strongest radical scavenging effect (96.75%) at 1.08 mg/ml. This activity was followed by *S. creticum* (96.68%) and *S. cordifolium* (96.22%), respectively (Table 3). The lowest scavenging activity was exhibited by *S. connatum* (92.51%). However, the scavenging effect for BHT was 98.41% and BHA was 98.21% at 1.0 mg/ml.

The amounts of monoterpenes and sesquiterpenes in leaf essential oil of *S. olusatrum* were found as 27.0 and 71.0%, respectively. Leaf essential oil of *S. olusatrum* showed β-myrcene (14.0%) and β-phellandene (9.0%) among the main components. Furanodiene was found as another typical component in the leaf (19%) (Bertoli et al., 2004).

Khanahmadi et al. (2010) reported that ethanol extract of *S. cordifolium* has 30.75% free radical scavenging activity. In our study, radical scavenging value of methanol extract of *S. cordifolium* higher than previous study (Table 3). In previous studies, the antioxidant activities of methanolic extracts of several plants have been reported (Assimopoulou et al., 2004; Al-Fatimi et al., 2007).

Özgen et al. (2003) found that methanolic extracts of *Onosma argentatum* Hub.-Mor. and *Rubia peregrina* L. scavenged 98.00 and 94.20% of DPPH radicals, respectively. At 1.0 mg/ml, the methanolic extracts of *Acacia nilotica* (L.) Delile, *Aerva javanica* (Burm.f.) Juss. ex Schult., *Solanum nigrum* L. and *Tamarindus indica* L. scavenged 94.62, 91.80, 95.30 and 93.86%, respectively (Al-Fatimi et al., 2007).

Proximate analysis

Proximate analysis was carried out on six *Smyrnium* taxa. Results of proximate composition are presented in Table 4. *S. connatum* had the highest concentration of

Table 2. Antimicrobial activities of six *Smyrnium* taxa (inhibition zones / mm).

Microorganism	Taxa									
	Scn*	Sco	Scr	Sol	Spr	Srt	P	Na	No	Nys
Escherichia coli	12.0	10.0	10.0	-	13.0	10.0	-	26.0	-	-
Enterobacter cloacae	12.0	12.0	14.0	11.0	25.0	11.0	12.0	12.0	22.0	-
Sarcina lutea	11.0	9.0	11.0	9.0	22.0	10.0	20.0	10.0	28.0	-
Salmonella typhimurium	10.0	-	10.0	-	24.0	9.0	-	-	40.0	-
Candida albicans	-	-	-	-	-	-	-	-	-	22.00

*Scn, *S. connatum*; Sco, *S. cordifolium*; Scr, *S. creticum*; Sol, *S. olusatrum*; Spr, *S. perfoliatum*; Srt, *S. rotundifolium*; P, Penicillin G; Na, Nalidixic acid; No, Novobiocin; Nys, Nystatin; -: no activity.

Table 3. Extraction yields and antioxidant activity values of *Smyrnium* taxa.

Taxa	Extraction yields (%)	RSA (%)	Conc. (mg/ml)
S. connatum	9.55	92.51 ± 0.09	1.09
S. cordifolium	19.99	96.22 ± 0.09	1.67
S. creticum	11.29	96.68 ± 0.00	1.01
S. olusatrum	14.40	96.75 ± 0.47	1.08
S. perfoliatum	11.37	94.63 ± 0.09	1.60
S. rotundifolium	17.96	96.15 ± 0.00	0.99
BHT*		98.41	1.00
BHA**		98.21	1.00

*, Butylated hydroxytoluene; **, Butylated hydroxyanisole.

Table 4. Proximate chemical composition (%, dry weight) of six *Smyrnium* taxa.

Taxa	Ash	Fat	Moisture	Protein	Carbohydrate
S. connatum	14.69 ± 0.91	5.44 ± 0.04	12.27 ± 0.38	18.20 ± 0.86	49.41 ± 2.18
S. cordifolium	10.58 ± 1.34	9.09 ± 0.04	12.22 ± 0.03	12.43 ± 0.78	55.69 ± 2.19
S. creticum	13.69 ± 0.35	5.63 ± 1.06	11.40 ± 0.33	13.11 ± 0.00	56.18 ± 0.39
S. olusatrum	10.41 ± 0.30	5.66 ± 0.08	11.57 ± 0.18	11.46 ± 1.04	60.92 ± 0.47
S. perfoliatum	10.13 ± 0.25	4.93 ± 0.97	12.61 ± 0.00	11.93 ± 0.21	60.41 ± 0.51
S. rotundifolium	11.63 ± 0.91	6.66 ± 0.05	12.21 ± 0.10	15.18 ± 1.08	54.33 ± 1.84

protein (18.20%) followed by *S. rotundifolium* and *S. creticum*, while *S. olusatrum* had the least (11.46%). With respect to moisture content, *S. perfoliatum* had the highest value (12.61%) and *S. creticum* the least value (11.40%). *S. olusatrum* had the highest carbohydrate (60.92%) and ash was highest in *S. connatum* (14.69%). The lipid values are between 4.93% (*S. perfoliatum*) and 9.09% (*S. cordifolium*) (Table 4).

The protein contents of the plants analyzed in this study were similar to those obtained in the previous studies; Madibela et al. (2002) reported that protein contents of *Tapinanthus lugardii* (N. E. Br.) Danser, *Viscum verrucosum* Harv. and *V. rotundifolium* L. f. are 11.90, 7.90 and 12.80%, respectively. Also, protein contents of *Centella asiatica* (L.) Urb., *Erythrina crista-galli* L. and *Lasia spinosa* (L.) Thwaites were reported to be 12.70, 24.20 and 17.90%, respectively (Maisuthisakul et al., 2008). In the same study, ash contents of those plants are 12.60, 7.70 and 1.30%; fat contents are 6.20, 5.10 and 3.80%, and carbohydrate contents are 53.10, 40.30 and 45.50%, respectively.

Conclusions

Antioxidant properties of plants are usually related to low-molecular weight compounds, in particular to the phenolic fractions. Therefore, a wide range of these potentially beneficial phenolic compounds could be natural substrates of oxidative enzymes, such as peroxidases or

polyphenol oxidases, which are present in high levels in some plants (Barros et al., 2010).

On the basis of the results, it is suggested that the extract of *Smyrnium* taxa evaluated here could be of use as an easily accessible source of natural antimicrobial and antioxidant for the nourishment. However, at present, the active component in the extract responsible for the observed biological activity is unknown. Therefore, further work could be done on the isolation and purification of the active components from the crude extracts of *Smyrnium* taxa for showing the mode of action of them. As far as our literature survey could ascertain, there is no information about the antioxidant, antimicrobial activities and chemical compositions of *Smyrnium* taxa collected from different regions of Anatolia.

ACKNOWLEDGEMENT

The authors wish to thank Celal Bayar University Scientific Research Projects Commission (BAP) (Project No: FEF-2009-004) for the financial support of this study.

REFERENCES

Al-Fatimi M, Wurster M, Schröder G, Lindequist U (2007). Antioxidant, antimicrobial and cytotoxic activities of selected medicinal plants from Yemen. J. Etnopharmacol., 111: 657-666.

AOAC (2006). Official methods of analysis. (16th Ed. ed.), Association of Official Analytical Chemists, Arlington VA, USA. p. 1200.

Assimopoulou AN, Boskou D, Papageorgiou VP (2004). Antioxidant activities of alkannin, shikonin and *Alkanna tinctoria* root extracts in oil substrates. Food Chem., 87: 433-438.

Avci M (2005). Çeşitlilik ve endemizm açısından Türkiye'nin bitki örtüsü. İstanbul Üniversitesi Edebiyat Fakültesi Coğrafya Bölümü Coğrafya Dergisi, 13: 27-55 (In Turkish).

Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem., 103: 413-419.

Barros L, Oliveira S, Carvalho AM, Ferreira ICFR (2010). In vitro antioxidant properties and characterization in nutrients and phytochemicals of six medicinal plants from the Portuguese folk medicine. Industrial Crops Prod., 32: 572-579.

Bermejo JE, Leon J (1994). Neglected Crops: 1492 From a Different Perspective. Plant Production and Protection Series, No: 26, FAO, Rome, Italy, p. 341.

Bertoli A, Pistelli L, Morelli I, Fraternale D, Giamperi L, Ricci D (2004). Volatile constituents of different parts (roots, stems, and leaves) of *Smyrnium olusatrum* L. Flavour Fragrance J., 19: 522-525.

Cazzi R, Ricardy R, Aglitti T, Gatta V, Petricone P, De Salvia R (1997). Ascorbic acid and β-carotene as modulators of oxidative damage. Carcinogenesis, 18: 223 - 228.

Elmastas M, Gulcin I, Ozturk L, Gokce I (2005). Investigation of antioxidant properties of spearmint (*Mentha spicata* L.). Asian J. Chem., 17: 137 - 148.

Halliwell B, Gutteridge JMC (2003). Free radicals in biology and medicine. Oxford University Press, Oxford, U.K., p. 704.

Kalyoncu F, Cetin B, Saglam H (2006). Antimicrobial activity of common madder (*Rubia tinctorum* L.). Phytother. Res., 20: 490 - 492

Kalyoncu F, Oskay M (2008). Antimicrobial activities of four wild mushroom species collected from Turkey. Proc. 6th Int. Conference on Mushroom Biology and Mushroom Products, Bonn, pp. 31-35.

Khanahmadi M, Rezazadeh SH, Taran M (2010). *In vitro* antimicrobial and antioxidant properties of *Smyrnium cordifolium* Boiss. (Umbelliferae) extract. Asian J. Plant Sci., 9: 99-103.

Madibela OR, Letso M, Boitumelo WS, Masedi M, Alton K (2002). Chemical composition of four parasitic plants harvested over a period of 6 months from two sites in Botswana. Animal Feed Sci. Technol., 95: 159-167.

Maisuthisakul P, Pasuk S, Ritthiruangdej P (2008). Relationship between antioxidant properties and chemical composition of some Thai plants. J. Food Comp. Anal., 21: 229-240.

Niki E, Shimaski H, Mino M (1994). Antioxidantism-free radical and biological defence. Gakkai Syuppan Center, Tokyo.

Oskay M, Sari D (2007). Antimicrobial screening of some Turkish medicinal plants. Pharm. Biol., 45:176 - 181.

Özgen U, Houghton PJ, Ogundipe Y, Coşkun M (2003). Antioxidant and antimicrobial activities of *Onosma argentatum* and *Rubia peregrina*. Fitoterapia., 74: 682-685.

Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. Food Chem., 101:267 - 273.