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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (21): 3895-3899, 2007
ISSN 1028-8880

Comparison of Antioxidant and Free Radical Scavenging Activities of the Essential Oils from Flowers and Fruits of *Otostegia persica* Boiss.

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Abstract: The aerial parts of the endemic plant of *Otostegia persica* Boiss. in two different stages of flowering and fruiting were hydro-distilled to extract oils in the yields of 0.3 and 0.15% (v/w), respectively. The oils were analyzed by GC and GC/MS. Twenty-eight and thirty-one components were identified, representing 97.59 and 94.61% of the oils, respectively. The main compounds of the essential oil flowers (EOFL) were alpha-pinene (17.21%), 1-octen,3-ol (13.44%) and cubenol (7.27%), whereas diisooctyl phthalate (45%) and hexadecanoic acid (11.07%) were the major constituents of the essential oil of the fruits (EOFR). The oils were screened for their possible antioxidant activities by two complementary test systems, namely DPPH free radical-scavenging and ammonium thiocyanate. In both tested systems, EOFL exerted greater antioxidant and radical scavenging activity. In the first case, EOFL exerted antioxidant activity with an IC_{50} 19.8±1.8 μg mL^{-1} almost similar to BHA and ascorbic acid (15.2±1.1 and 17.4±1.3), respectively. In the ammonium thiocyanate system, the inhibition rate of oxidation of linoleic acid for EOFL was estimated 93.5±2.8. The higher activity of this oil in comparison to EOFR may be attributed to its high content of monoterpenes, especially oxygenated ones in the oil of the flowers.

Key words: *Otostegia persica*, essential oil, antioxidant activity, GC-MS, free radical, lamiaceae

INTRODUCTION

Antioxidant activity is a complex process that can occur through several mechanisms. Recently, interest has increased in naturally-occurring antioxidants that can be used to protect human beings from oxidative stress damage (Scalbert *et al*., 2005). Oxygen radicals are most responsible for the development of many diseases including carcinogenesis, atherosclerosis and heart diseases (Al-Dabbas *et al*., 2006). Aromatic herbs and spices have been used for a long time, not only to improve or modify the flavour of foods, but also to avoid its deterioration (Mata *et al*., 2007). Actually, however, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multi-purpose functional use (Ormancey *et al*., 2001; Sawamura, 2000).

The genus of *Otostegia* (Lamiaceae) comprises 20 species that are distributed over the east of Asia, of which *Otostegia persica* Boiss is endemic to Iran, Pakistan and Afghanistan (Mozafarrian, 1998). The flowers of the plant are widely used as an additive to yoghurt, buttermilk and meat. It also has been used in Iranian traditional medicine as analgesic in toothache and arthritis (Zargari, 1989). Hydro alcoholic extract of *O. persica* alleviate morphine withdrawal syndrome (Hajhashemi *et al*., 2004). However we have previously reported some antioxidant flavonoids from methanol extract of this plant (Sharififar *et al*., 2005; Yassa *et al*., 2005). As far as our literature survey could ascertain, we could reach no report on the chemical composition and antioxidant activity of the essential oils of *O. persica*. So the aims of this study were: (i) to determine the chemical composition of the essential oils of the flowers and fruits separately, (ii) to compare the *in vitro* antioxidant properties of these essential oils to find if there is any relation between their chemical composition and antioxidant activities.

MATERIALS AND METHODS

Plant material: The aerial parts of the *Otostegia persica* Boiss. at its flowering and fruiting seasons (late June and August, 2003, respectively) were collected from Dehbakri, Kerman, in southeastern of Iran. The plant was authorized

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with Dr. Mozaffarian. A voucher specimen has been deposited at the Herbarium of the pharmacognosy Department of Faculty of Pharmacy in Kerman University of Medical Science. The air-dried and ground parts of the plant were submitted to hydro distillation for 3 h using a Clevenger-type apparatus.

Gas Chromatography (GC): The essential oils were analyzed using a Shimadzu QP 5000 gas chromatograph equipped with a FID detector and HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Helium was the carrier gas, at a flow rate of 1 mL min^{-1}. Diluted samples (1/100 in acetone, v/v) of 1.0 μL were injected manually and in the split less mode.

Gas Chromatography/Mass Spectrometry (GC/MS): GC-MS analysis of the essential oils were performed under the same conditions with GC (column, oven temperature, flow rate of the carrier gas) using a Shimadzu QP 5000 gas chromatograph equipped with a Shimadzu QP 5050 mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley 2001 library data of the GC-MS system and literature data (Adams, 1996).

DPPH assay: This spectrophotometric assay uses stable 2,2'-diphenylpicrylhydrazyl (DPPH) radical as a reagent (Burits and Bucar, 2000). Fifty microliter of various concentrations of the samples in methanol were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition of DPPH free radical in percent (I%) was calculated in following way:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Where:

A_{blank} = The absorbance of the control reaction (containing all reagents except the test compound),

A_{sample} = The absorbance of the test compound.

Sample concentration providing 50% inhibition (IC_{50}) was calculated form the graph plotting inhibition percentage against sample concentration. Tests were carried out in triplicate.

Ammonium thiocyanate method: The antioxidant activity of the essential oils of the *O. persica* was determined using ammonium thiocyanate method (Masude *et al*., 1992). Each sample (500 μL) in 0.5 mL of deionized water was mixed with linoleic acid emulsion (2.5 mL, 0.02 M, pH = 7.0) and phosphate buffer (2 mL, 0.2 M, pH = 7.0). The linoleic acid emulsion was prepared by mixing 0.284 g of linoleic acid, 0.284 g of tween 20 as emulsifier and 50 mL phosphate buffer and then the mixture was homogenized. The reaction mixture was incubated at 37°C. Aliquots of 0.1 mL were taken at different intervals during incubation. The degree of oxidation was measured by sequentially adding ethanol (4.7 mL, 75%), an ammonium thiocyanate (0.1 mL, 30%) and ferrous chloride (0.1 mL, 0.02 M in 3.5% HCl). After 3 min the peroxide values was determined by reading the absorbance at 500 nm using a spectrophotometer. A control was performed with linoleic acid but without the oils. BHA and ascorbic acid were used as positive controls. Tests were carried out in triplicate.

RESULTS AND DISCUSSION

GC/MS analysis of the essential oils: Aerial parts of *O. persica* in flowering and fruiting stages were subjected to hydro distillation using a Clevenger apparatus and the yellow-colored essential oils were obtained. In the first case, twenty-eight compounds were identified, representing 97.59% of the total oil. Oil yield of the plant was determined as 0.3% (v/w). As a result, the oil profile obtained from GC/MS analysis exhibited the presence of the major compounds of alpha-pinene (17.21%), 1-octen, 3-ol (13.44%), cubenol (7.27%) and thujanol (5.45%). Of the hydrocarbon sesquiterpenes, cubenol (7.27%) and alpha-neo-clovene (5.12%) are outclassed. GC and GC/MS analysis revealed that monoterpene derivatives are represented in high amount (49.6%, approximately) in EOFL which 13.87% of them are oxygenated, followed by sesquiterpenes (20.75%). In the oil of fruits, 31 compounds were identified representing the 94.61% of the oil. Diisooctyl phthalate (45%) and hexadecanoic acid (11.07%) were the major constituents of oil of the fruits. This oil is characterized by the high percent of unsaturated fatty acids and diterpenes (88.3%) and low monoterpene and sesquiterpene derivatives (2.39 and 5.85%, respectively) (Table 1).

Antioxidant activities: Antioxidant activity is a complex process that can occur through several mechanisms. Due to its complexity more than one test must be carried out when evaluating the antioxidant activity of pure compounds or extracts (Aruoma, 2003). In this study, two classical antioxidant tests were carried out, the DPPH and ammonium thiocyanate tests. The first gives information about the ability of the tested compounds to scavenge

Table 1: Chemical composition of oils of flowering and fruiting tops from *O. persica* (diluted 1/100 in acetone v/v)[3]

	Flowering tops (A)			Fruiting tops (B)		
Peak No.	Compound	KI[4]	Composition (%)	Compound	KI	Composition (%)
1	Hexenol-z-3	857	00.98	α-pinene	9380	00.83
2	α-pinene	938	17.21	furan,2-pentyl	1003	00.81
3	1-octen,3-ol	982	13.44	meta-xylene,4-ethyl	1034	00.17
4	ρ-cymene	1028	10.68	trans verbenol	1149	00.97
5	Limonene	1031	20.10	para-menth-1,5 dienol	1170	00.29
6	Linalool	1098	40.19	α-terpineol	1191	00.50
7	Dihydrolinalool	1134	40.94	γ-terpinyl acetate	1354	00.63
8	Trans verbenol	1144	30.49	β-damacenone	1357	10.29
9	Thujanol	1146	50.45	tetrahydronaphtalene,trimethyl	1383	00.15
10	Pinocampheol	1170	20.96	β-calarene	1445	00.13
11	n-nonanol	1171	10.34	cuparene	1499	00.16
12	4-terpineol	1177	20.00	myristicine	1511	20.11
13	Myrtenol	1194	20.57	δ-cadinene	1530	10.89
14	n-decanal	1204	30.17	8,9-dehydro-neo,isolongifolene	1558	00.34
15	Isodihydrocarveol	1212	10.15	caryophylene oxide	1570	10.40
16	n-decanol	1272	10.27	cis-a-copaene,8-ol	1595	20.06
17	2-undecanone	1291	10.34	dillapiol	1610	00.27
18	4-nerol acetate	1341	10.52	phytan	1665	00.18
19	α-cis-bergamotene	1415	10.47	octadecane	1791	00.67
20	α-neo-clovene	1447	50.12	hexadehydro-farensyl acetate	1850	00.70
21	Decalactone	1493	20.39	n-nonadecane	1889	10.65
22	Betabisabolene	1509	10.76	10-demethylsqualene	1932	00.43
23	Geranyl butyrate	1562	20.12	hexadecanoic acid methyl ester	1939	10.25
24	Caryophylene oxide	1581	10.50	betaisoamylionone	1954	10.47
25	Dehydroapo farensol	1587	10.53	hexadecanoic acid	1960	11.07
26	Guaiol	1595	20.36	n-eicosane	1980	00.37
27	Cubenol	1627	70.27	di- (2-ethylhexyl phthalate)	2042	70.66
28	5-neocedranol	1676	10.27	9,12-octadecadienoic acid, -methyl ester	2064	10.90
29				phytol propionate	2079	70.56
30				octadecanoic acid methyl ester	2090	00.70
31				diisooctylphtalate	2187	45.00
32						
Total			97.59			94.61

[3]: Relative percentages of the compounds were obtained electronically from FID area percent data, [4]: Kovats index on non-polar DB-5 ms column in reference to n-alkanes

free radicals and the second about the ability of tested samples to delay lipid per oxidation by reacting with chain-propagating peroxyl radicals faster than these radicals can react with proteins or fatty acid side-chains. Plant extracts or oils are complex mixtures and reports of antioxidant activities evaluated by different tests are not always concordant (Sachetti *et al*., 2005; Tepe *et al*., 2005; Trouilla *et al*., 2003). Therefore, antioxidant activity of the plant oils studied here was determined by two complementary test systems namely DPPH and ammonium thiocyanate systems. By using these systems, it is possible to make a better conclusion on the antioxidant nature of these two oils.

DPPH assay: The free radical-scavenging activity was determined by the DPPH test. This test aims to measure the capacity of the oils to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) formed in solution by donation of hydrogen atom or an electron (Tepe *et al*., 2005). IC_{50} value of the EOFL and EOFR were determined as 19.8±1.8 and 29.2±2.0 μg mL^{-1}, respectively (Table 2). The best results were obtained with the EOFL of the plant (IC_{50} = 19.8±1.8 mL^{-1}) in comparison to EOFR (IC_{50} = 29.2±2.0 μg mL^{-1}). These values are lower than those found with the positive controls of BHA and ascorbic acid. EOFL is composed mostly of terpenoids and the antioxidant activity of numerous terpenes has already been evaluated (Ruberto and Baratta, 2000).

Table 2: Antioxidative capacities of the essential oils of *O. persica*

	Test system	
Sample	DPPH.IC_{50} (μg mL^{-1})	Ammonium thiocyanate (% of inhibition)
Flowering tops oil	19.8±1.8	93.8±2.8
Fruiting tops oil	29.2±2.0	63.0±3.1
BHA	15.2±1.1	97.9±2.9
Ascorbic acid	17.4±1.3	90.2±2.1
Negative control	0.0	0.0

Ammonium thiocyanate method: This test simulates the oxidation of the membrane lipid components in the

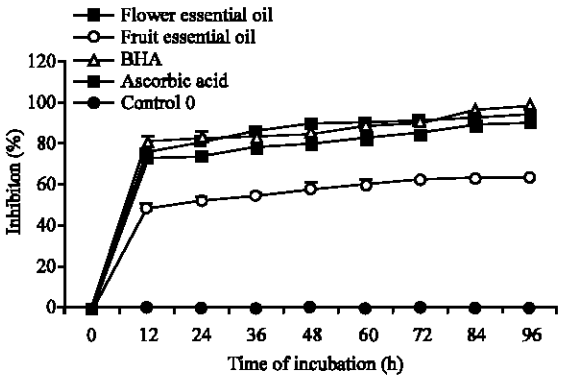

Fig. 1: Antioxidant activity of flowering and fruiting tops of *O. persica* by ammonium thiocyanate method

presence of antioxidants inside the cells. It also measures the capacity to inhibit the formation of conjugated diene hydro peroxide arising from linoleic acid oxidation (Tepe *et al*., 2005). In this test, EOFL and EOFR of *O. persica* exhibited 93.5±2.8 and 63.0±3.1% inhibition against linoleic acid oxidation, respectively. The antioxidant activity of the EOFL was determined close to BHA and ascorbic acid whereas the EOFR exhibited weak activity (Table 2). This difference could be attributed to high percent of monoterpenes of EOFL (49.6%). Hydrocarbon monoterpenes present antioxidant activity due to the presence of strongly activated methylene groups and this is clearer in ammonium thiocyanate system where a competition with the activated methylene in C-11 of linoleic acid may be hypothesized. This can explain the strong activity of the EOFL in linoleic acid oxidation. It is obvious from the Fig. 1, the activity of the EOFL in inhibition of oxidation of linoleic acid in the most times of incubation is close to BHA activity. In some intervals the activity of essential oil of flowers is more than BHA. This activity is obviously more than ascorbic acid (Fig. 1). In this study, EOFL of *O. persica*, was found to possess remarkable radical-scavenging and especially strong antioxidant activities. The bioactive components of this oil can act as primary and secondary antioxidants, scavenging free radicals and can therefore inhibit the lipid per oxidation. There are no reports in the literature concerning the antioxidant activities of these compounds and the activity of EOFL could be attributed to the contribution of other compounds of the oil. Further experiments are necessary to verify the relation between chemical composition and antioxidant activity.

CONCLUSIONS

The lamiaceae plants have many medicinal uses especially due to their high content of essential oils. There are poor reports in the literature concerning the genus of *Otostegia* especially for *O. persica*. In present study chemical composition and antioxidant activity of the oil of the flowers (EOFL) and fruits (EOFR) of *O. persica* have been studied. The obtained results showed major compounds of EOFL of *O. persica* were determined to be from monoterpene derivatives (49.6%, approximately) (Table 1). Sesquiterpene hydrocarbons exert a low, if any, antioxidant effect (Ruberto and Baratta, 2000). The percent of the both hydrocarbons and oxygenated monoterpenes are much higher in EOFL of *O. persica* and antioxidant capacity of this oil could be attributed to them As can be seen in the (Table 1). The flowering effect on the monoterpene content of plant oils have been reported elsewhere (Dudai *et al*., 1992). In conclusion, it seems that the quantity and quality of the EOFL of *O. persica* is an important and determining factor for its activity. This oil has no phenolic compounds and this shows that the presence of the phenolic compounds is not obligatory for the antioxidant activity. At last by regarding the antioxidant activity of EOFL of *O. persica*, this oil may be an alternative to more toxic synthetic antioxidants as additives in food, pharmaceutical and cosmetic preparations.

REFERENCES

Adams, R.P., 1996. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corpoartion, Carol Stream, IL.

Al-Dabbas, M.M., T. Suganuma, K. Kitahara, D.X. Hou and M. Fujii, 2006. Cytotoxic, antioxidant and antibacterial activities of *Varthemia iphionoides* Boiss. extracts. J. Ethnopharmacol., 108: 287-293.

Aruoma, O.I., 2003. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat. Res., pp: 523-524, 9-20.

Burits, M. and F. Bucar, 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res., 14: 323-328.

Dudai, N., E. Putievsky, U. Ravid, D. Palevitch and A.H. Halevy, 1992. Monoterpene content in *Origanum syriacum* as effected by environmental conditions and flowering. Phys. Plant, 84: 453-459.

Hajhashemi, V., M. Rabbani, G.R. Asghari and Z. Karami-Saravi, 2004. Effects of *Otostegia persica* (Burm) boiss on morphine withdrawal syndrome in mice. Iranian J. Pharmaceut. Res., 3: 30-35.

Masude, T., J. Isobe, A. Jitoe and N. Nakamati, 1992. Antioxidant curcuminoids from rhizomes of *Curcuma zanthorrhiza*. Phytochemistry, 31: 3645-3647.

Mata, A.T., C. Proença, A.R. Ferreira, M.L.M. Serralheiro, J.M.F. Nogueira and M.E.M. Araújo, 2007. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. Food Chem., 103: 778-786.

Mozafarrian, V., 1998. Dictionary of the Names of Iranian Plants. Farhange Moaser Publisher. Tehran.

Ormancey, X., S. Sisalli and P. Coutiere, 2001. Formulation of essential oils in functional parfumery. Parfums, Cosmetiques Actualites, 157: 30-40.

Ruberto, G. and M.T. Baratta, 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem., 69: 167-174.

Sachetti, G., S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini and M. Radice, 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem., 91: 621-632.

Sawamura, M., 2000. Aroma and functional properties of Japanese yuzu (*Citrus junos* Tanaka) essential oil. Aroma Res., 1: 14-19.

Scalbert, A., C. Manach, C. Morand and C. Remesy, 2005. Dietary of polyphenols and the prevention of diseases. Crit. Rev. Food Sci. Nutr., 45: 287-306.

Sharififar, F., N. Yassa and A. Shafiee, 2005. Antioxidant activity of *Otostegia persica* (labiateae and its constituents. Iranian J. Pharmaceut. Res., pp: 235-239.

Tepe, B., D. Daferera, A. Sokmen, M. Sokmen and M. Polissiou, 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem., 90: 333-340.

Trouilla, P., C.A. Calliste, D.P. Allais, A. Simon, A. Marfak and C. Delage, 2003. Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the limousine countryside as herbal teas. Food Chem., 80: 399-407.

Yassa, N., F. Sharififar and A. Shafiee, 2005. *Otostegia persica* as a source of natural antioxidants. Pharmaceut. Biol., 43: 33-38.

Zargari, A., 1989. Medicinal Plants. Vol. 2, Tehran University Publisher, Tehran.