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Full Length Research Paper

Antioxidant activity of the methanolic extracts of some species of *Phlomis* and *Stachys* on sunflower oil

Katayoun Morteza-Semnani[1]*, Majid Saeedi[2] and Somayeh Shahani[1]

[1]Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.
[2]Department of Pharmaceutics, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

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Antioxidant effects of the methanolic extract of *Phlomis bruguieri*, *P. herba-venti, P. olivieri*, *Stachys byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa* were tested in sunflower oil stored at 70ºC, by measuring peroxide values after regular intervals and compared with rosemary-, green tea- and BHA-containing samples. The methanolic extracts of *P. bruguieri* and *S. laxa* were found to be most effective in stabilizing sunflower oil.

Key words: Antioxidant activity, extracts, sunflower oil, peroxide value, *Phlomis*, *Stachys*.

INTRODUCTION

Oils are an important part of the human diet. More than 90% of the world oil production from vegetable, animal and marine sources is used as food or as an ingredient in food products. Sunflower oil is used widely for deep frying. One of the most reactions leading to quality loss is rancidity of the food products. Rancidity is the development of an off-flavour by oxidation and hydrolysis which makes the food unacceptable (Tawfik and Huyghebaert, 1999). Synthetic antioxidants are widely used to retard undesirable changes as a result of oxidation in many foods. Excessively oxidized fats and oils are not suitable for nutritive purposes, because the oxidation products of oils have toxic effects. Many synthetic substances such as butylated hydroxyanisole (BHA), propyl gallate and citric acid are commonly used in lipids to prevent oxidation. Recently, these synthetic substances have been shown to cause such as enlarge the liver size and increase microsomal enzyme activity. Therefore, there is need for other compounds to effect as antioxidants and to render food products safer for mankind.

Plant originated antioxidants have been used in oils or lipid containing foods in order to prevent oxidative deterioration (Aruoma et al., 1996; Ozcan, 1999). The antioxidant activity displayed by spices or other anti-oxidants depends on several factors such as the concentration, the temperature, the hydrophobic, hydrophylic or amphiphatic character, the presence of synergists and the chemical nature of the food or medium to which they are added (Ozcan, 1999). The most important natural antioxidants being exploited commercially are tocopherols, but unfortunately they are much less effective than BHA or BHT (butylated hydroxytoluene). The search for and development of other antioxidants of natural origin are, therefore, highly desirable. Plants used worldwide for culinary purposes have gained the interest of many research groups. Ground material or various extracts from such sources have been assessed so for as potent antioxidants in lipid systems (Ozcan and Akgul, 1995). The antioxidant activity of the plants is attributed to some components. The chemical composition of plants, differ due to several factors e.g. plant variety, climate, soil, cultivation practices, harvesting time, processing method (Ozcan and Akgul, 1995). Some plant extracts of the family Labiatae have already been tested, but they showed sometimes contradictory antioxidant activity when used under different conditions (Econonou et al., 1991).

Therefore, and also for the exploitation of indigenous sources, this preliminary research work was conducted to study the effects of the methanolic extracts from some Iranian plants as antioxidants in sunflower oil. *Phlomis*

*Corresponding Author's E-mail: semnani_k@yahoo.co.uk
Tel/Fax +98-151-3543082.

bruguieri Desf., *P. herba-venti* L., *P. olivieri* Benth., *Stachys byzantina* C. Koch. (syn. *S. lanata* Jacq.), *S. inflata* Benth., *S. lavandulifolia* Vahl and *S. laxa* Boiss. & Buhse, (Labiatae) are known plants (Mozaffarian, 1996) and were selected for this test. 17 species from the genus *Phlomis* are distributed in Azerbaijan, Fars, Gilan, Hamadan, Isfahan, Kurdestan and Mazandaran Provinces of Iran (Mozaffarian, 1996; Rechinger, 1982). 34 species from the genus *Stachys* have been found in Iran (Mozaffarian, 1996); *S. byzantina*, *S. inflata*., *S. lavandulifolia* and *S. laxa* are aromatic plants, growing wild in Azerbaijan, Golestan, Khorasan, Mazandaran and Theran Provinces of Iran (Rechinger, 1982). We have already reported the chemical composition of the essential oils of *P. bruguieri*, *P. herba-venti, S. byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa* (Morteza-Semnani et al., 2004 and 2006; Morteza-Semnani and Saeedi, 2005). A literature survey has shown that there is no report on the antioxidant activity of these plants from Iran.

MATERIALS AND METHODS

Plant material

In June 2005, the floweing aerial parts of *P. bruguieri* and *P. herba-venti* were collected from the suburb of Sari and Larijan (Mazandaran province, North of Iran), respectively and *P. olivieri*, *S. byzantina*, *S. inflata*, *S. lavandulifolia*, *S. laxa* were collected from the suburb of Behshahr (Mazandaran province, North of Iran). *Camellia sinensis* L. (green tea) and *Rosmarinus officinalis* L. (Rosemary) were also collected from the suburb of Lahijan and Rasht (Gilan Province, North of Iran), respectively, in the same year. The plants were identified by Department of Botany, Research Center of Natural Resources of Mazandaran. Voucher specimens (herbarium No. 175, 118, 176, 151, 152, 154, 155, 122 and 123, respectively) were deposited at the Herbarium of the Research Center of Natural Resources of Mazandaran.

Preparation of extracts

The floweing aerial parts of plants were dried in the shade and powdered so that all the material could be passed through a mesh not larger than 0.5 mm. Powdered materials of each plant (100 g) were soaked in 1 L of methanol (Merck Co., Germany) for 1 day, and the steps were repeated twice, followed by filtration through filter paper. The filtrates were evaporated to dryness under reduced pressure and weighed.

Sunflower oil

Refined oil without adding any antioxidant was kindly supplied by Ghoncheh Company in Sari city. Its peroxide number was 1.8 meq kg^{-1}. The oil was selected for being the most widely used as edible oil in Iran.

Antioxidant activity measurement

The antioxidant activity of the methanolic extracts was tested on sunflower oil and expressed as decrease in the rate of peroxide formation. A calculated quantity of the extract was added into sunflower oil, and the mixture was stirred. BHA-containing and control samples (without adding any antioxidant) were also prepared under the same condition. Since rosemary and green tea have known antioxidant activity (Ozcan, 1999; Ozcan and Akgul, 1995; Morteza-Semnani et al., 2003; Hras et al., 2000), the other extracts were also compared with rosemary and green tea. All samples of 20 g each were incubated in 10 × 100 mm open beakers at 70ºC in the dark. The peroxide values of the samples were determined at definite time intervals according to the Method Cd 8-53 of the American oil Chemists' Society (A.O.C.S., 1997) (Ozcan and Akgul, 1995).

Statistical analysis

ANOVA followed Tukey test was used to determine significant differences between groups and $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

The yield of methanolic extracts of *P. bruguieri*, *P. herba-venti, P. olivieri*, *S. byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa* was10.6, 10.1, 9.2, 14.0, 14.3, 10.1 and 10.6%, respectively. Table 1 presents the antioxidant activity of the methanol extracts, compared with that of the rosemary-, green tea-, BHA-containing and the control samples. Of the extracts, the most effective ones are also shown in Figure 1. All extracts (0.2, 0.5 and 1%) showed more antioxidant activity in comparison with control group after 7, 14, 21 and 28 days ($P<0.001$). The extracts of *P. bruguieri* (0.2, 0.5 and 1%) after 7, 14, 21 and 28 days, *P. herba-venti* (0.2 and 0.5%) after 21 days, *P. Olivieri* (0.2 and 0.5%) after 14, 21 and 28 days, *P. Olivieri* (1%) after 7 days, 21 and 28 days, *S. byzantina* (0.5 and 1%) and *S. laxa* (0.2, 0.5 and 1%) after 7 days, *S. byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa* (0.2 and 0.5%) after 14, 21 and 28 days and *S. byzantina*, *S. inflata*, *S .lavandulifolia* and *S. laxa* (1%) after 21 and 28 days showed more antioxidant activity than rosemary at the same concentrations.

The peroxide values of the oils containing *P. bruguieri*, *P. olivieri*, *S. byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa* (0.2, 0.5 and 1%) and *P. herba-venti* (0.2%) after 7, 14, 21 and 28 days and *P. herba-venti* (0.5%) after 7, 21 and 28 days and *P. herba-venti* (1%) after 7 days were less than green tea at the same concentration ($P<0.001$). The difference between peroxide values of the oils containing *P. herba-venti* (0.2%) after 14 days, *S. byzantina* (0.2%), *S. inflata* (1%) and *S. lavandulifolia* (0.2, 0.5 and 1%) after 7 days and rosemary at the same concentrations was no significant ($P>0.05$). The peroxide values of the oils containing *P. bruguieri* (0.2, 0.5 and 1%) and *P. olivieri* (1%) after 7, 14, 21 and 28 days, *P. herba-venti* (1%) after 21 days, *P. olivieri* (0.2 and 0.5%) after 14, 21 and 28 days, *S. byzantina* (0.5 and 1%) after 7, 14, 21 and 28 days, *S. byzantina* (0.2%) after 14, 21 and 28 days, *S. inflata* (0.2, 0.5 and 1%) after 14 and 28 days, *S. inflata* (1%) after 21 days, *S. lavandulifolia* (0.2%) after 14 and 28 days, *S. lavandulifolia* (0.5 and 1%) after 7, 14 and 28 days, *S. laxa* (0.2%) after 21 and 28 days, *S. laxa* (0.5%) after 7, 21 and 28 days and *S. laxa* (1%) after 7, 14, 21 and 28 days were less than BHA ($P<0.001$).

Table 1. Antioxidant activity of extracts added to sunflower oil[a] stored in the dark at 70ºC.

Extract	% (w/w)	Peroxide value[b] (meq kg^{-1}) ± S.D. after days			
		7	14	21	28
Phlomis bruguieri	0.2	27.46±0.623	51.90±0.583	59.72±0.543	82.63±0.566
	0.5	25.29±0.629	38.08±0.583	51.55±0.543	70.92±0.566
	1	12.28±0.629	32.01±0.583	40.86±0.543	66.04±0.566
Phlomis herba-venti	0.2	45.80±0.289	83.17±0.289	107.83±0.289	179.67±0.289
	0.5	45.73±0.764	80.67±0.289	100.17±0.289	165.57±0.603
	1	44.00±0.500	76.67±0.283	87.67±0.289	155.12±0.289
Phlomis olivieri	0.2	56.73±0.623	64.03±0.583	76.7±0.548	83.29±0.566
	0.5	41.80±0.629	62.34±0.583	72.30±0.543	79.38±0.560
	1	26.38±0.589	55.94±0.583	53.95±0.543	61.81±0.560
Stachys byzantina	0.2	36.13±0.629	55.90±0.589	63.49±0.543	75.48±0.566
	0.5	29.99±0.623	51.90±0.583	59.72±0.543	71.57±0.560
	1	26.74±0.623	43.81±0.583	57.84±0.548	61.81±0.560
Stachys inflata	0.2	49.50±0.623	60.32±1.166	117.24±0.543	126.23±0.566
	0.5	46.97±0.629	55.94±0.589	105.30±0.545	112.57±0.566
	1	34.33±0.629	52.91±0.583	61.61±0.548	73.53±0.566
Stachys lavandulifolia	0.2	36.13±0.629	64.03±0.583	123.85±0.548	145.75±0.566
	0.5	33.96±0.629	59.99±0.583	121.96±0.543	137.29±0.566
	1	31.80±0.623	57.97±0.583	118.19±0.548	134.04±0.566
Stachy laxa	0.2	35.05±0.623	78.19±0.583	82.67±0.543	91.09±0.560
	0.5	29.63±0.623	66.05±0.589	78.90±0.543	87.19±0.566
	1	20.96±0.623	52.57±1.010	67.27±0.548	76.78±0.560
Camellia sinensis	0.2	62.15±0.623	94.36±0.583	157.79±0.543	184.79±0.566
	0.5	51.31±0.623	76.17±0.583	131.39±0.543	180.89±0.566
	1	49.14±0.623	70.10±0.583	78.58±0.543	124.28±0.566
Rosmarinus officinalis	0.2	38.30±0.623	84.92±1.010	135.16±0.543	156.81±0.566
	0.5	36.13±0.629	74.14±0.583	114.42±0.548	149.98±0.560
	1	33.96±0.629	38.08±0.583	72.92±0.543	106.71±0.566
BHA	0.02	36.13±0.629	68.08±0.583	95.57±0.548	150.63±0.566
Control		90.35±0.625	146.25±0.583	221.92±0.548	312.97±0.566

a Initial (zero day) peroxide value of the oil was 1.8 meq kg-1.
b Data are the average of three separate experiments.

The difference between peroxide values of the oils containing *S. byzantina* (0.2%), *S. inflata* (1%), *S. lavan-dulifolia* (0.2%) and *S. laxa* (0.2%) after 7 days, *S. laxa* (0.5 %) after 14 days and BHA was no significant ($P>0.05$).

The methanolic extracts of *P. bruguieri* and *S. laxa* were found to be most effective in stabilizing sunflower oil. Plants belonging to the genus *Phlomis* have been shown to contain iridoid glucosides, flavonoid glycosides, phenylethanoid glycosides, diterpene glycosyl esters and nortriterpenes (Harput, et al., 2006; Morteza-Semnani and Saeedi, 2005). Phytochemical investigation of *Stachys* species has shown the occurrence of flavonoids, diterpenes, phenyl ethanoid glycosides and saponins (Khanavi et al., 2005). The antioxidant activities of flavornoids are well known and can exert their antioxidant activity by various mechanisms, for example, by scavenging radicals, which initiate lipid peroxidation and lipid peroxide radicals, by binding metal ions, and by inhibiting enzymatic systems responsible for free radical generation (Lebeau et al., 2000). The antioxidant properties of many herbs and spices are reported to be effective in retarding the development of rancidity in oils and fatty foods. It is known that a number of natural extracts from herbs, spices and some vegetables are stable to autoxidation due to the presence of some natural components e.g. phenollic compounds. The antioxidant activity of extracts depends on the type and polarity of extraction solvent, the isolation procedures, purity and identity of antioxidant active components from the raw materials. The use of synergistic mixtures of antioxidants allows a reduction in

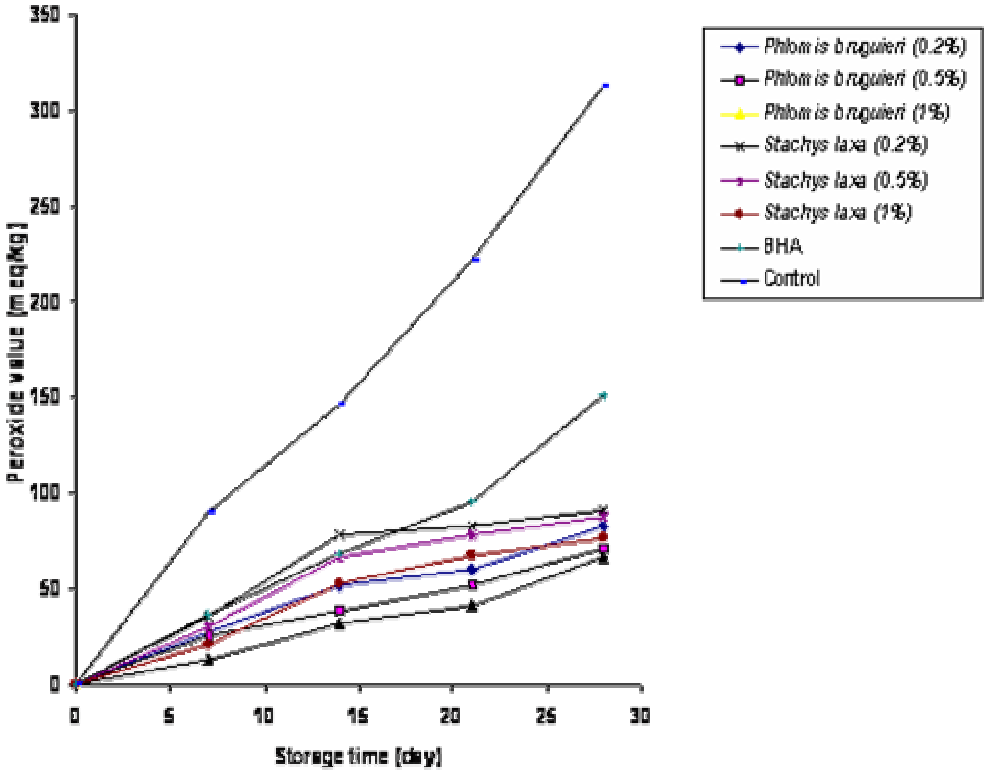

Figure 1. Antioxidant activity of the methanolic extracts of *Phlomis bruguieri* and *Stachys laxa* (0.2, 0.5 and 1%) in comparison with BHA and control groups.

the concentration of each substrate and also increases the antioxidative effectiveness as compared with the activity of each separate compound (Abdalla and Roozen, 1999). It is possible that natural antioxidants exhibit complex interfacial affinities between air-oil interfaces that significantly affect their relative activities in different lipid systems (Frankel, 1996).

In 2003, the in vitro antioxidant activity of the ethanol extracts obtained from 21 aromatic plants belonging to the Lamiaceae family was investigated; the extracts of *S. spruneri* and *P. lanata* exhibited the same activity as alpha-tocopherol (Couladis et al., 2003). Antioxidant and radical scavenging activities were found to be predominant in highly polar extracts of some Turkish plants; the water-solubles of *P. leucophracta* P.H.Davis & Hub.-Mor., *P. kurdica* Rech. fil and *P. russeliana* (Sims) Bentham presented the most significant activity (Tasdemir et al., 2004). Methanol extracts of aerial flowering parts of four endemic *Stachys* taxa: *S. anisochila* Vis. et Pancic, *S. beckeana* Dorfler & Hayek, *S. plumosa* Griseb. and *S. alpina* L. ssp. *dinarica* Murb. were investigated on their antioxidant activity; all *Stachys* extracts, with the exception of *S. plumosa*, exhibited high anti-DPPH activity (Kukic et al., 2006). Erdemoglu et al. (2006) determined the antioxidant activities of four Lamiaceae plants by using 1,1-diphenyl-2-picrylhydrazyl (DPPH); all extracts were shown to possess a significant scavenger activity against DPPH free radical; the extracts scavenged 50% of DPPH radical ranging in the following descending order: *Salvia viridis* > *Stachys byzantina* > *Salvia multicaulis* > *Eremostachys laciniata*.

Our results supported the previously reported antioxidant activity of the genus *Phlomis* and *Stachys*. The results concluded that the methanolic extracts of *P. bruguieri* and *S. laxa* have a potential source of antioxidants of natural origin. Further studies are necessary to elucidate the mechanism(s) of antioxidant activity of these pants.

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