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Antioxidant activity of some edible plants of the Turkmen Sahra region in northern Iran

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ABSTRACT

The antioxidant properties of 10 edible plants of the Turkmen Sahra region in Golestan province in northern Iran, including *Allium paradoxum*, *Allium rubellum*, *Foeniculum vulgare*, *Mentha longifolia*, *Origanum vulgare*, *Prunus divaricata*, *Rubus sanctus*, *Rumex tuberosus*, *Satureja mutica* and *Spinacia turkestanica* were evaluated by four different methods; free radical scavenging using 2,2-diphenyl-1-picrylhydrazyl (DPPH), evaluation of xanthine-oxidase activity, inhibition of lipid peroxidation by the ferric thiocyanate method, and the deoxyribose degradation assay. All species tested except *A. paradoxum* and *P. divaricata* showed antioxidant activity at least in one assay.

The pro-oxidant activities were also assayed for these species and *S. mutica*, *M. longifolia*, and *O. vulgare* showed significant pro-oxidant activity.

1. Introduction

Although the use of wild and traditional edible species has been influenced by the economic attraction of food and non-economic crops, they are important in family food security, since people culturally accept and use them in different ways (Tukan, Takruri, & Al-Eisawi, 1998), but very few ethnopharmacological and phytopharmacological studies have dealt exhaustively with the potential health benefits of such diets.

There is increasing evidence indicating that reactive oxygen species (ROS) and free radical mediated reactions are involved in degenerative or pathological events such as aging, cancer, coronary heart ailments, and Alzheimer's disease (Sun, Wang, Fang, Gao, & Tan, 2004). Reactive oxygen species, including; superoxide anion, hydroxyl radical, and hydrogen peroxide, are generated in specific organelles of the cell under normal physiological conditions (Haraguchi, 2001). Excessive production of these ROS, beyond the antioxidant defense capacity of the body can cause oxidative stress (Aruoma, 1996).

Epidemiological studies have consistently shown that there is a clear significant positive association between the intake of fruits and vegetables and a reduced rate of heart disease, mortality, common cancers and other degenerative diseases as well as aging, and this is attributed to the fact that these foods may provide an optimal mix of phytochemicals such as natural antioxidants, fibres and other biotic compounds (Kaur & Kapoor, 2001). Some antioxidants present in plants are capable of stimulating free radical damage to carbohydrates and DNA in vitro, and may therefore exact pro-oxidant actions in biological systems (Aruoma, 1996), it is therefore important to also examine the pro-oxidant activity of the extracts.

Wild edible plants form an important constituent of traditional diets of Turkmens in the Turkmen Sahra region in Golestan province in northern Iran, who are traditionally an isolated ethnic group (Ghorbani, 2005). The aim of the present study was to evaluate the antioxidant and pro-oxidant activities of 10 edible plants of this region which have not been studied before.

2. Materials and methods

2.1. Materials and reagents

Trichloroacetic acid (TCA) and iron(II)chloride were purchased from Aldrich (Milwaukee, WI, USA). 2-Thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), xanthine, xanthine-oxidase, linoleic acid, ammonium thiocyanate, caffeic acid, allopurinol, ascorbic acid, rutin, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and butylated hydroxytoluene (BHT) were purchased from Sigma (St. Louis, MO, USA). 2-Deoxy-D-ribose was purchased from Fluka (Buchs, Switzerland), and iron(III)chloride, Folin–Ciocalteu, sodium carbonate, absolute ethanol and methanol were purchased from Merck (Darmstadt, Germany). All chemicals and reagents were of analytical grade.

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2.2. Plant samples

Plant samples were collected from the region by trips to the area in March 2004, and in June and July 2005, and were identified by Mr. Ghorbani (Botanist of Traditional Medicine and Materia Medica Research Center). Voucher specimens were deposited in the herbarium of the Traditional Medicine and Materia Medica Research Center (TMRC), Iran. Table 1 includes some information about these plants.

2.3. Preparation of plant extracts

Collected specimens were dried at room temperature and powdered (18–35 mesh in size for each plant powder). A sample (10 g) of the dried powdered was extracted for thrice with 100 ml methanol/water (8:2, v/v), for 24 h on each occasion. The extracts were filtered and the combined filtrates were evaporated under vacuum. The residual solvent of the extracts was evaporated by heating on a water bath. The extract yields are shown in Table 1.

2.4. Free radical scavenging by using DPPH

Experiments were carried out according to the method of Blois (1958) with a slight modification Briefly, a 0.1 mM solution of DPPH radical solution in methanol/water (8:2, v/v) was prepared and then 1 ml of this solution was mixed with sample solution (3 ml) in methanol/water (8:2, v/v). Finally, after 30 min, the absorbance was measured at 517 nm.

Decreasing the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. This activity is given as % DPPH radical scavenging that is calculated by the equation:

$$\%\ \text{DPPH radical scavenging} = \left(\frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}}\right) \times 100.$$

The DPPH solution without sample solution was used as a control. Ascorbic acid and BHT were used as standards.

2.5. Xanthine-oxidase activity

The xanthine-oxidase (XO) activities were measured spectrophotometrically using the procedure of Marcocci, Packer, Droy-Lefaix, Sekaki, and Gardes-Albert (1994). The assay mixture contained, in a final volume of 1 ml, 0.250 ml plant extract solution in 50 mM potassium phosphate buffer, pH 7.4 (final concentration: 100 μg/ml), 0.330 ml xanthine solution (0.15 mM) in water (5 min boiled to completely dissolve the xanthine), and 0.385 ml 50 mM potassium phosphate buffer, pH 7.4. The reaction was started by adding 0.035 ml XO solution in 50 mM potassium phosphate buffer, pH 7.4 (final concentration: 0.2 U/ml) and the change in absorbance recorded at 295 nm for 3 min at room temperature. The results were expressed as a percentage of inhibition enzyme activity:

$$\%\ \text{Inhibition} = \left(1 - \frac{B}{A}\right) \times 100,$$

where A is the change in absorbance of the assay without the plant extract, and B is the change in absorbance of the assay with the plant extract. Allopurinol at a final concentration of 100 μg/ml was used as a standard.

2.6. Total antioxidant activity determination by ferric thiocyanate method (inhibition of lipid peroxidation)

The antioxidant activities of the extracts were determined according to the ferric thiocyanate method as reported by Kikuzaki and Nakatani (1993). A mixture containing the extract (4 ml) in absolute ethanol, final concentration: 200 μg/ml, (extracts were not fully soluble in absolute ethanol, even after treating solutions for 1 h in an ultrasonic bath, so they were filtered and only the soluble parts were further analyzed), 2.51% linoleic acid in absolute ethanol (4.1 ml), 0.05 M phosphate buffer pH 7 (8 ml) and distilled water (3.9 ml), was placed in a vial with a screw cap, and then placed in an oven at 40 °C in the dark. To this solution (0.1 ml) was added 75% ethanol (9.7 ml) and 30% ammonium thiocyanate (0.1 ml). Three min after adding of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid (0.1 ml) to the reaction mixture, the absorbance of red color was measured at 500 nm each 24 h until one day after absorbance of the control (without sample) reached maximum. BHT and caffeic acid were used as standards.

% Inhibition of lipid peroxidation is calculated by equation:

$$\%\ \text{Inhibition} = \frac{A_c - A_s}{A_c} \times 100,$$

where A_s is the absorbance of the sample on the day when the absorbance of the control is maximum, and A_c is the absorbance of the control on the day when the absorbance of the control is maximum.

2.7. Deoxyribose assay

The degradation of deoxyribose by the hydroxyl radical was evaluated by mixing deoxy ribose (2.8 mM), 10 mM KH_2PO_4–KOH buffer (pH 7.4). $FeCl_3$ (100 μM), EDTA (104 μM), H_2O_2 (1 mM), plant extracts in a final concentration of 100 μg/ml, and ascorbate (100 μM), in a final volume of 1 ml (Halliwell, Gutteridge, & Aruoma, 1987).

After incubation at 37 °C for 1 h, deoxyribose damage was measured using the thiobarbituric acid test, 1 ml of 1% (w/v)

Table 1
List of 10 plants consumed by Turkmens in Golestan province, in north of Iran.

Scientific name	Family	Common name	Local name	Plant part used	Extract yield (%)[a]	Voucher no.
Allium paradoxum (M. Bieb.) G.Don.	Lilliaceae	Few flowered garlic	Tareh-soqan	Leaf	49	424
Allium rubellum M. Bieb.	Liliaceae	Assam onion	Ajuvah	Leaf	17	519
Foeniculum vulgare Mill.	Apiaceae	Fennel	Badiyan	Stem–leaf–seed	29	1268
Mentha longifolia (L.) Huds.	Lamiaceae	Horse mint	Agh-bideneh	Leaf	27	727
Origanum vulgare L.	Lamiaceae	Oregano	Kaklic oti	Leaf	38	725
Prunus divaricata Ledeb.	Rosaceae	Cherry plum	Khalchek	Fruit	62	1270
Rubus sanctus Schreber	Rosaceae	Holy bramble	Boursen	Fruit	50	1269
Rumex tuberosus L.	Polygonaceae	Tuberous dock	Ghoiyarfaq	Leaf	26	407
Satureja mutica Fisch & C.A. Mey.	Lamiaceae	Unknown[b]	Kamar oti	Aerial parts	21	864
Spinacia turkestanica Iljin	Chenopodiaceae	Unknown[b]	Smnagh	Leaf	29	410

[a] w/w yield in terms of initial dried material.
[b] There was no available English common name.

thiobarbituric acid (TBA) in 50 mM NaOH and 1 ml of 2.8% (w/v) trichloroacetic acid were added to the reaction mixture and were incubated at 100 °C for 20 min to develop color due to the malondialdehyde-like product of deoxyribose damage. After cooling, the absorbance was measured at 532 nm. Thiourea was used as a standard.

The % inhibition of degradation of deoxyribose was calculated by equation:

$$\% \text{ Inhibition} = \left(\frac{A_o - A_I}{A_o}\right) \times 100,$$

where A_o is the absorbance of the assay (without sample) and A_I is the absorbance of the assay (with sample).

In addition, the pro-oxidant effect of extracts was evaluated by the deoxyribose assay in which ascorbate was omitted from the deoxyribose reaction mixture (Aruoma, 1996). The pro-oxidant effects of extracts were expressed as relative percentages (%) compared to the control, and were calculated as follows:

$$\text{Pro-oxidant effect} = [A_{532} \text{ control} - A_{532} \text{ sample}/A_{532} \text{ control}] \times 100.$$

The control contained 10 mM phosphate buffer (pH 7.4) instead of the extracts.

2.8. The amount of flavonoids and phenolic compounds

The content of total phenolic compounds in plant methanolic extracts was determined by Folin–Ciocalteu method (1927). One millilitre of 0.01 g/ml methanolic plant extract was mixed with 5 ml Folin–Ciocalteu reagent (diluted tenfold with distilled water) and 4 ml (7.5 g/100 ml) sodium carbonate. After 1 h at room temperature the absorption of clear solutions was read at 765 nm. For the preparation of calibration curve, different concentrations of ethanolic gallic acid solutions were mixed with the same reagents as described above, and after 30 min the absorption of clear solutions was measured. The amount of total phenolic compounds was expressed as gallic acid equivalents (GAE) in milligrams per gram dry plant extract. The experiment was repeated thrice and the mean value was reported.

The content of flavonoids was determined using the aluminium chloride colorimetric method. One millilitre of 0.01 g/ml methanolic plant extract was mixed with 1 ml of 2% AlCl3 ethanol solution. After 1 h at room temperature, the absorbance was measured at 420 nm. The results were expressed in mg rutin in g dry matter by comparison with standard rutin treated in the same conditions (Kumazawa, Hamasaka, & Nakayama, 2004).

2.9. Statistical analysis

Data are presented as the mean ± standard deviation (SD) of each triplicate or more tests; except that total antioxidant activities were performed twice.

Correlation coefficients (R) to determine the relationship between two variables were calculated using MS Excel software (CORREL statistical function).

3. Results

3.1. Free radical scavenging by using DPPH

Ten edible plants were assayed for DPPH radical scavenging activity at a concentration of 1 mg crude extract/ml and most of the extracts showed acceptable activity (>50%) (Table 2). Vitamin C and BHT were used as standards at the final concentration of 1 mg/ml.

The highest DPPH radical scavenging activity (%) was shown by *Mentha longifolia* (93.68 ± 0.41) and *Satureja mutica* (93.39 ± 2.55), which were at the same level with the standards. *Prunus divaricata* (21.52 ± 1.54), and *Allium paradoxum* (19.56 ± 4.10) showed the lowest activity.

3.2. Xanthine-oxidase inhibitory activity

Xanthine-oxidase inhibitory activity of each plant was measured at 100 μg crude extract/ml. Of the extracts assayed, seven demonstrated xanthine-oxidase inhibitory activity, among these, the highest activity (%) was seen by *S. mutica* (55.96 ± 1.28). Two plant extracts considered to have minimal or no xanthine-oxidase inhibitory activity (<3%) included, *Rumex tuberosus* (−0.52 ± 0.86) and *P. divaricata* (−5.41 ± 1.07) (Table 2).

3.3. Inhibition of lipid peroxidation

Fig. 1 displays the inhibitory activity of extracts against linoleic acid peroxidation. Except that *A. paradoxum* and *Rubus sanctus*, the other plant extracts showed inhibition activity against linoleic acid peroxidation, especially *M. longifolia* and *Foeniculum vulgare* which significantly decreased the absorbance.

Table 2
Radical scavenging activity in the DPPH assay, xanthine-oxidase activity inhibition, and the amount of flavonoids and phenolic compounds.

Plant extracts and standards	DPPH activity[a] (%)	Xanthine-oxidase activity inhibition[b] (%)	Total phenolic compounds[c]	Total flavonoids[d]
Satureja mutica	93.39 ± 2.55	55.96 ± 0.28	51.19 ± 1.07	31.11 ± 0.35
Mentha longifolia	93.68 ± 0.41	26.12 ± 2.79	37.86 ± 0.27	29.42 ± 0.25
Origanum vulgare	90.46 ± 2.07	41.30 ± 3.84	59.03 ± 2.05	44.25 ± 0.17
Rubus sanctus	83.27 ± 1.07	5.38 ± 0.49	4.52 ± 0.27	4.66 ± 0.15
Foeniculum vulgare	69.65 ± 0.70	14.36 ± 1.35	7.74 ± 0.58	16.49 ± 0.06
Spinacia turkestanica	67.41 ± 2.13	10.38 ± 2.54	5.23 ± 0.36	19.01 ± 0.21
Allium rubellum	66.12 ± 3.32	16.63 ± 1.02	2.48 ± 0.62	33.00 ± 0.46
Rumex tuberosus	63.46 ± 1.06	−0.52 ± 0.86	12.92 ± 0.35	7.42 ± 0.37
Prunus divaricata	21.25 ± 1.54	−5.41 ± 1.07	11.35 ± 0.48	0.87 ± 0.41
Allium paradoxum	19.56 ± 4.10	11.75 ± 3.04	3.98 ± 0.13	9.42 ± 0.26
Vitamin C	97.15 ± 2.01	–	–	–
BHT	96.47 ± 1.61	–	–	–
Alluporinol	–	97.79 ± 0.32	–	–

[a] Radical scavenging activity in the DPPH assay (n = 3 experiments); plant extracts and standards 1 mg/ml, final conc.
[b] Xanthine-oxidase inhibition (%) at 100 μg/ml. The test was performed in triplicate for each plant extract.
[c] mg/g plant extract in gallic acid equivalent. The experiment was carried out in triplicate.
[d] mg/g plant extract in rutin equivalent. The test was performed thrice.

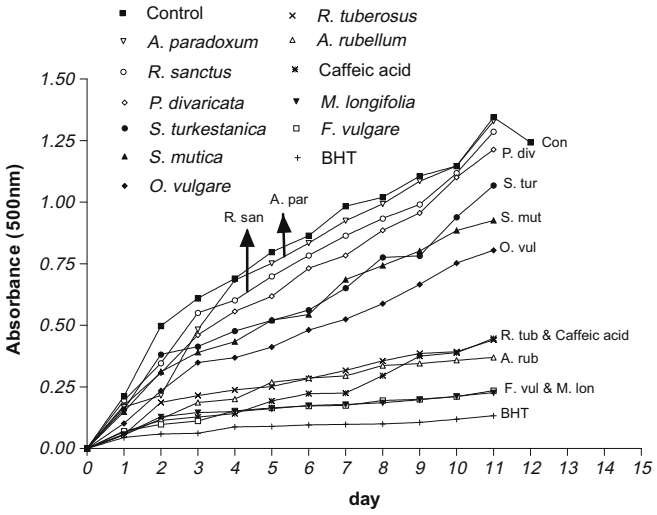

Fig. 1. Total antioxidant activity. Final conc. 200 μg/ml. Standard; BHT and caffeic acid. A low absorbance value represents a high level of antioxidant activity.

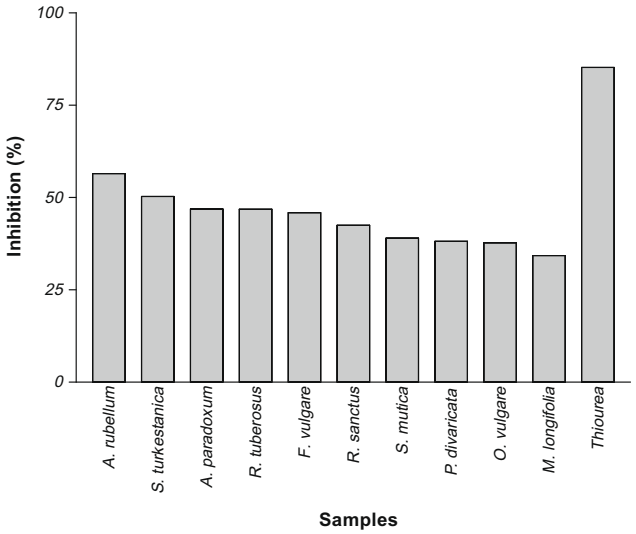

Fig. 2. Deoxyribose degradation inhibitory effect (%). Final conc. 100 μg/ml. Standard; thiourea. All extracts showed activity lower than thiourea.

Table 3
Lipid peroxidation inhibitory effects (%) of extracts in comparison with the standards (BHT and caffeic acid) on the 11th day. The tests were performed in duplicate.

Sample	Inhibition (%)
Mentha longifolia	83.12 ± 1.07
Foeniculum vulgare	82.52 ± 0.79
Allium rubellum	72.72 ± 1.35
Rumex tuberosus	67.25 ± 1.74
Origanum vulgare	40.14 ± 3.08
Satureja mutica	31.15 ± 0.39
Spinacia turkestanica	20.59 ± 1.48
Prunus divaricata	9.73 ± 1.81
Rubus sanctus	4.38 ± 0.96
Allium paradoxum	1.18 ± 0.24
BHT	90.11 ± 1.45
Caffeic acid	66.76 ± 0.73

Table 4
Pro-oxidant effects (%) of plant extracts.

	Optical density (A 532 nm)	Pro-oxidant effects (%)
Control	0.125	–
Origanum vulgare	0.296	136.8
Satureja mutica	0.196	56.8
Mentha longifolia	0.165	32
Prunus divaricata	0.117	0
Rumex tuberosus	0.107	0
Foeniculum vulgare	0.106	0
Rubus sanctus	0.098	0
Spinacia turkestanica	0.091	0
Allium paradoxum	0.089	0
Allium rubellum	0.082	0
Thiourea	0.023	0

Although *M. longifolia*, *F. vulgare*, *Allium rubellum* and *R. tuberosus* exhibited higher inhibitory effect than caffeic acid, none of the extracts were better than BHT (Table 3).

3.4. Deoxyribose degradation

All of the extracts showed moderate inhibitory activity against deoxyribose (DR) damage as shown in Fig. 2. Among the 10 plants, *A. rubellum* showed the highest DR degradation inhibitory activity (56.45 ± 1.56%) and *M. longifolia* showed the lowest (34.28 ± 0.72%).

In the evaluation of the pro-oxidant activity of extracts, three plants including, *Origanum vulgare*, *S. mutica* and *M. longifolia*, showed significant pro-oxidant activities; results are shown in Table 4.

3.5. The amount of flavonoids and phenolic compounds

The content of phenolic compounds (mg/g) in plant extracts, determined from regression equation of calibration curve ($y = 0.425x + 0.032$, $R^2 = 0.99$) and expressed in gallic acid equivalents (GAE), varied between 2.48 and 59.03 (Table 2). The highest amounts were found in extracts of *O. vulgare* (59.03 ± 2.05), *S. mutica* (51.19 ± 1.07) and *M. longifolia* (37.86 ± 0.27).

The content of flavonoids in plant extracts (mg/g), expressed in rutin equivalents (regression equation of calibration curve, $y = 0.966x + 0.030$, $R^2 = 0.99$). The highest amounts were found in extracts of *O. vulgare* (44.25 ± 0.17), *A. rubellum* (33.00 ± 0.46) and *S. mutica* (31.11 ± 0.35) (Table 2).

4. Discussion

It has been proposed that xanthine-oxidase is an important source of oxygen-derived free radicals in reperfused tissue. Reperfusion introduces molecular oxygen into tissues and initiates the xanthine-oxidase reaction, resulting in the production of superoxide anion and other toxic oxygen species which attack cellular constituents including cell-membrane lipids (Haraguchi, 2001). Xanthine-oxidase catalyses the oxidation of hypoxanthine to xanthine and to uric acid in humans (Nakanishi et al., 1990). Therefore the determination of xanthine-oxidase-inhibiting activity in plant extracts can be useful both for studying their antioxidant activity and for studying their potential anti-gout action.

S. mutica and *O. vulgare*, which have acceptable inhibitory activity against xanthine-oxidase, belong to the Lamiaceae family. Although the xanthine-oxidase inhibitory effect of *M. longifolia* (Lamiaceae) was not as high as *S. mutica* and *O. vulgare*, it showed 26% inhibitory activity which was higher than the other species. These three plants showed the highest DPPH inhibition percentages as well; 93%, 93% and 90% for *M. longifolia*, *S. mutica*, and *O. vulgare*, respectively.

The Lamiaceae family is a rich source of plant species containing large amounts of phenolic acids (Zegorka & Glowniak, 2001). Flavonoids also occur in the Lamiaceae family in a variety of structural forms including flavones, flavonols, flavonones, dihydroflavonols and chalcones (Tomas-Barberan & Gil, 1992). These phytochemical components are known to be responsible for the antioxidant capacity of fruits and vegetables (Pellati, Benvenuti, Magro, Melegari, & Sorgani, 2004). Flavonoids are described as hydrogen-donating antioxidants by virtue of the reducing properties of the multiple hydroxyl groups attached to their aromatic ring system, along with their ability to delocalize the resulting phenoxyl radical within the structure. It is recognized that polyphenolic flavonoids are able to scavenge different reactive oxygen radicals such as the hydroxyl and superoxide radicals (Teixeira et al., 2005). It has been reported that flavonoids (Cos et al., 1998) and polyphenols (Costantino, Albasini, Rastelli, & Benvenuti, 1992) possess potent inhibition on xanthine-oxidase activity.

There are some reports concerning the presence of flavonoids and phenolic compounds in *M. longifolia* (Ghoulami, Il-Idrissi, & Tetouani, 2001) and *O. vulgare* (Skerget et al., 2005), which might be responsible for their DPPH radical scavenging and xanthine-oxidase inhibitory activity. Although no reports on the phenolic constituents of *S. mutica* could be found, there are reports for other species of *Satureja* (Sunches de Rojas, Somoza, Ortega, & Villar, 1996).

Our investigation for the content of flavonoids and phenolic compounds determined that *O. vulgare, S. mutica* and *M. longifolia,* have considerable amounts of flavonoids and phenolic compounds (Table 2).

Correlation coefficients between data of xanthine-oxidase activity inhibition and total flavonoids and phenolic compounds were 0.82 and 0.84, respectively, confirming that, phenolic compounds and flavonoids are likely to contribute to the xanthine-oxidase inhibitory activity of these plant extracts.

Correlation coefficient between data of DPPH scavenging activity of the plant extracts and total flavonoids ($R = 0.64$) and phenolic compounds ($R = 0.59$) was medium. Although the three plants of the Lamiaceae family, which showed the best results in the DPPH assay, had considerable amounts of flavonoids and phenolic compounds, *R. sanctus*, which had lower content of flavonoids and phenolic compounds, possessed remarkable DPPH scavenging activity, therefore detailed determination of phenolic metabolites, or even other compounds, in the plant extracts is required for the comprehensive assessment of individual compounds showing DPPH scavenging activity.

Cellular damage, due to lipid peroxidation, causes serious derangements, such as ischemia-reperfusion injury, coronary arteriosclerosis, diabetes mellitus and neurodegenerative diseases. It is also associated with aging and carcinogenesis (Haraguchi, 2001).

In the ferric thiocyanate method, *F. vulgare* (Apiaceae), *M. longifolia* (Lamiaceae) and *A. rubellum* (Liliaceae) showed the most inhibitory activity against lipid peroxidation. Unlike the results in the DPPH assay and xanthine-oxidase inhibition assay, *S. mutica* (Lamiaceae) and *O. vulgare* (Lamiaceae) showed moderate activity against lipid peroxidation, suggesting that different compounds were involved in showing activity.

Although flavonoids can inhibit lipid peroxidation (Haraguchi, 2001), there are some reports that terpenoids have more potent activity in lipid peroxidation inhibition (Olatunde Farombi, Ogundipe, Samuel Uhunwangho, Adeyanju, & Olarenwaju Moody, 2003; Pietri, Maurelli, Drieu, & Culcasi, 1997). For the lipid peroxidation inhibitory effects, besides the structural effects, other factors, such as the hydrophobicity of the compounds should be taken into account (Silva, Ferreres, Malva, & Dias, 2005).There are various types of phytochemicals, including flavonoids, coumarins, xanthones, phenylpropanoids and terpenoids, which are effective in preventing of lipid peroxidation (Haraguchi, 2001). Synergistic effects of different compounds, existing in the extracts of *F. Vulgare*, *M. longifolia* and *A. rubellum*, might be responsible for their significant activities against lipid peroxidation.

In the deoxyribose assay, hydroxyl radicals are generated by a mixture of Fe^{3+}, ascorbate, and H_2O_2 in the presence of a slight molar excess of EDTA over the Fe^{3+} salt (Halliwell et al., 1987). The sugar deoxyribose is degraded on exposure to the hydroxyl radical. The OH radicals attack the deoxyribose and set off a series of reactions that eventually result in the formation of MDA (malondialdhyde). MDA may be detected by its ability to react with thiobarbituric acid in acid conditions. Generation of $OH^{\cdot}$ by reaction of H_2O_2 with the transition metal ions already bound onto the DNA would lead to strand breakage, base modification and deoxyribose fragmentation (Aruoma, 1996). Any other molecule added to the reaction mixture that is capable of reacting with $OH^{\cdot}$, should compete with DR for $OH^{\cdot}$; so, it will decrease the deoxyribose degradation (Halliwell et al., 1987). Consequently the DR assay has become a useful experimental tool for investigating the ability of molecules to react with $OH^{\cdot}$ (Aruoma, 1996).

When ascorbate is omitted from the DR reaction mixture, the ability of added compounds to reduce the Fe^{3+}–EDTA complex can be tested; this has been developed into an index for pro-oxidant molecules in nonlipid systems (Aruoma, 1996). In the evaluation of pro-oxidant action of the extracts, three species; *O. vulgare*, *S. mutica* and *M. longifolia* (all belonging to the Lamiaceae family) showed significant pro-oxidant activity, which might be attributed to their high content of phenolic compounds (correlation coefficient between the optical density of plant extracts at 532 nm and the total content of phenolic compounds was 0.95).

Although the ability of polyphenols to protect cells from oxidative stress has been demonstrated, there is increasing evidence for their pro-oxidant cytotoxicity (Galati & O'Bbrien, 2004). The same polyphenol compounds could behave as both antioxidants and pro-oxidants, depending on their concentration and free radical source (Cao, Sofic, & Prior, 1997).

It has been also demonstrated that some plant-derived phenolics (gossypol, quercetin and myricetin) have the effect of accelerating the generation of hydroxyl radicals (Laughton, Halliwell, Evans, Robin, & Hoult, 1989), which can damage DNA, lipids and other biological molecules. Antioxidants and transition metal-chelating agents can prevent the toxic effects of pro-oxidants in mammalian cells (Nemeikaite-Ceniene, Imbrasaite, Sergediene, & Cenas, 2005).

It has been proposed that the pro-oxidant action of polyphenolics may be an important mechanism of their anticancer and apoptosis-inducing properties (Hadi, Asad, Singh, & Ahmad, 2000). Therefore, *O. vulgare*, *S. mutica* and *M. longifolia* should be further investigated to determine their cytotoxicity and anticancer activities, especially which, these species showed no pro-oxidant activity in the ferric thiocyanate assay (none of them accelerated lipid peroxidation more than the control).

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