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Evaluation of antiradical properties of methanolic, ethanolic, acetonic and hexan extracts of *Rusamarinus officianalis L.* and *Ranunculus bulbosus* from Jiroft city

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Abstract

In this research Methanolic, Ethanolic, Acetonic and Hexan extracts of *Rusamarinus officianalisL* and *Ranunculus bulbosus* from plants grown in Jiroft city were evaluated for their antiradical properties. Two plants of *Rusamarinus officianalisL* and *Ranunculus bulbosus* were collected from Jiroft Azad university research farm. After drying the plant materials in shade, extracts were obtained by methanol 80%, ethanol 50%, acetone and hexane solvents and antioxidant activity was measured by DPPH method. In both of plant methanolic extracts had highest amount of antiradical activity and hexane extracts had the lowest. Results of this study showed the *Rusamarinus officianalisL* extracts contained higher amount of antiradical properties than extracts of *Ranunculus bulbosus*. The obtained results show that extracts of two plants of *Rusamarinus officianalisL* and *Ranunculus bulbosus* possess antiradical activity and could be used as a natural antioxidant ingredient in food and drug industries.

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Introduction

Botanical extracts are one of the richest sources of antioxidants that counteract both free radicals and oxygen reactive species. The solvent and extraction conditions used in the extraction may influence the quantity, antioxidant composition and biological activity.

Rosmarinus officinalis L. (Lamiaceae) is a plant widely distributed in Europe, Asia and Africa and one of its growing areas is the Mediterranean basin. Rosemary is widely known for its numerous applications in the field of food but also for an increasing interest in its health promoting properties. There are three groups of compounds in the rosemary extracts: phenolic diterpenes, flavonoids and phenolic acids. The major antioxidant compounds are carnosic acid and carnosol, abietane-type diterpenes, rosmarinic acid, a hydroxycinnamic acid ester (Ozlem *et al.*, 2007). Naturally occurring compounds in rosemary extracts (Wu *et al.*, 1982; Ho *et al.*, 1983) have been reported to exhibit antioxidant properties greater than BHA and equal BHT. The extracts rosemary (Chang *et al.*, 1977; Economou *et al.*, 1991; Banlas *et al.*, 1992), were examined in order to determine their antioxidative activity against autoxidation in different substrates, mostly in lard. Their antioxidative activity depends on the solvent used, but the structure activity relationship of them have not been completely investigated (Chang *et al.*, 1997; Banlas *et al.*, 1992).

The genus *Ranunculus* belongs to the family Ranunculaceae, which comprise 50 genera and 2000 species, distributed throughout the northern hemisphere. It is also found in southern temperate regions, in the tropic where they are usually confined to higher altitude. The most common use of Ranunculus species is for the treatment of antirheumatism, rubifacient and intermittent fever. For this use, the plant is commonly prepared as decoction. It is also indicated as a remedy for antihemorrhagic (*Ranunculus repens*) (David *et al.*, 2000), neuralgia pains, anti-spasmodic, diaphoretic (Ranunculus bulbosus) (Maria *et al.*, 2009), vermifacient, anthelmintic (*Ranunculus hirtellus*) (Sanjay *et al.*, 2006), tympany, conjunctivitis of an eye (*Ranunculus laetus*) (Pande *et al.*, 2007). Some compounds isolated from *Ranunculus* have shown strong antimicrobial, antibacterial and antiradical activities (Mares *et al.*, 1987; Misra and Dixit, 1978; Tocan and Baron, 1969).

Several methods have been applied to extract antioxidant compounds from plants; solvent extraction is one of the most common methods for this means. This is a method for separating a substance from one or more others by using a solvent. Commonly used solvents for extracting various substances from plant material are water, aqueous mixtures of ethanol, methanol and acetone (Sun and Ho, 2005).

Most of the reported results associated functional properties of rosemary with the composition of polyphenolic compounds. Therefore, the objective of this study was to evaluate the antiradical activity of Methanolic, Ethanolic, Acetonic and Hexan extracts of *Rusamarinus officianalis L* and *Ranunculus bulbosus* from Jiroft city.

Materials and methods

Chemicals and reagents

DPPH, methanol, ethanol, acetone and hexane were purchased from Merck (Darmstadt, Germany); all chemicals were of reagent grade.

plant material

The plants, *Rusamarinus officianalis L* and *Ranunculus bulbosus* were collected from Jiroft local farms in spring and summer.

plants material extraction

The aerial parts of *Rusamarinus officianalis L* and *Ranunculus bulbosus* plants were collected, shade dried for seven days and ground. The dried powder of plants (1g) was soaked in 10 ml methanol–water (80:20, v/v), ethanol-water (50:50), acetone and hot hexane. Extraction carried out at ambient temperature for 24 h. The ratio of methanol and

ethanol with water used yielded the highest yield of phenolic compounds and flavonoids during preliminary trials. Similar ratio of methanol to water was used by biglari *et al.* (2008).). Each extract was filtered with Whatman No. 1 filter paper. The filtrate obtained from methanol evaporated to dryness at 40 °C in a rotary evaporator (BuchiLaborator). Then all of extracts were dried by a freeze dryer. Dried samples stored at 4 °C until use. (Arab Shahi and Urooj, 2006)

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the Blios (1958) method. Briefly, 1 ml of a 1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (at concentrations of 100, 200, 500 and 1000 ppm). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The quality of the radical scavenging property of two plants was determined by calculating the IC50. The IC50 value is the concentration of each plant extract required to scavenge the DPPH radical to 50% of the control.

Statistical analysis

All these experiments were replicated three times, and the average values are reported. The effect of different solvents on antioxidant activity of two plants were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at 5% significant level using the SAS software (2001) program.

Results and discussion

Radical scavenging activity (DPPH˙) The scavenging activity of DPPH˙ radicals has been widely used to determine the free radical-scavenging activity. DPPH˙ is a stable free radical that is dissolved in methanol and its color shows a characteristic absorption at 517 nm. Antioxidant molecules scavenge the free radical by hydrogen donation and the color from the DPPH˙ assay solution becomes light yellow resulting in a decrease in absorbance. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation (Dvorakova *et al.*, 2008).

Table 1. Comparison of extraction capability of methanol, ethanol, acetone and hexane on *Rusamarinus officianalis L.* and *Ranunculus bulbosus* as percentage of DPPH at various concentrations.

solvents	concentrations (ppm)	*Rusamarinus officianalis L*	*Ranunculus bulbosus*
	100	53.35^{c}	21.8^{g}
Methanol/water	200	56.67^{b}	32.45^{f}
	500	66.71^{a}	37.42^{e}
	1000	69.92^{a}	48.8^{c}
	100	47.28^{c}	16.6^{h}
Ethanol/water	200	50.64^{c}	25.45^{g}
	500	58.64^{b}	31.8^{f}
	1000	64.71^{ab}	37.55^{e}
	100	40.82^{e}	10.7^{j}
acetone	200	46.56^{d}	16.33^{h}
	500	52.71^{c}	22.45^{g}
	1000	58.11^{b}	30.61^{f}
	100	34.61^{f}	6.54^{j}
hexane	200	40.12^{e}	12.47^{h}
	500	46.39^{d}	18.52^{h}
	1000	48.55^{c}	24.27^{g}

(Means with same superscripts had no significant difference with each other (P >0.05)

Our data suggests an inverse correlation between the amount of DPPH and the value of IC50.

Results of two plants analyzes showed that solvent significantly ($p < 0.05$) influence DPPH˙ scavenging activity (table 1). For *Rusamarinus officianalis L*, DPPH˙ determined by four solvents of methanol–water (80:20, v/v), ethanol-water (50:50), acetone and hexane at various concentration of extracts (100, 200, 500 and 1000 ppm) ranged from 53.35 to 69.92%, from 47.28 to 64.71%, from 40.82 to 58.19 and from 34.61 to 48.55%, respectively. In the case of *Ranunculus bulbosus*, DPPH˙ scavenging activity of methanol–water (80:20, v/v), ethanol-water (50:50), acetone and hexane at various concentration of extracts ranged from 21.8 to 48.8%, from 16.6 to 37.55%, from 10.7 to 30.67 and from 6.54 to 24.27%, respectively.

Table 2. IC50 of methanolic, ethanolic, acetone and hexane extracts of *Rusamarinus officianalis L.* and *Ranunculus bulbosus.*

solvents	*Rusamarinus officianalis L*	*Ranunculus bulbosus*
Methanol/water	89.91 ppm	1107.6 ppm
Ethanol/water	198.2 ppm	1349.1 ppm
acetone	491.23 ppm	1558.45 ppm
hexane	1028.41 ppm	1941.37 ppm

Literature data showed that DPPH˙ scavenging activity differs depending on used solvent and plant matrix. Antiradical activity of two plants differed significantly depending on solvents used and the highest activity was determined in metanolic extracts of *Rusamarinus officianalis L.* Among solvent studied, methanolic extracts exhibited the highest antiradical activity, and hexane extracts, the lowest (methanolic > ethanolic > acetonic> hexane) (table 1).

The results are consistent with the literature Perez-Jimenez and Saura-Calixto (2006) measured the antioxidant properties of catechin and gallic acid mixture extracted with solvents such as methanol water and a mixture of acetone/water and methanol/water. A factor with a key influence on the determination of antioxidant activity seems to be the level of solvent polarity. In the study with date palm fruit, it has been reported that with increasing polarity of the solvent an increase of antiradical properties was observed (shahdadi, 2010). The presence of water, also during extraction, may affect the distribution of antioxidants between the polar and a polar fraction.

Price and Sipro (1985) and Price and Spitzer (1993) reported that the highest extraction of phenolic compounds from plant material with methanol-water (80:20, v/v) was achieved during the first stage of extraction.

Chavan and Amarowicz (2013) used methanol-water, ethanol-water and acetone-water solvent systems (80:20, v/v) for extraction of phenolics, tannins and sugars from beach pea (Lathyrus maritimus L.). Against our results. They showed that acetone-water system extracted considerably higher amounts of phenolic compounds and condensed tannins than the ethanol-water or methanol-water systems.

Conclusions

Analysis of the TPC and free radical scavenging activity of horseradish extracts showed differences depending on solvent and extracts concentration. As the best solvent methanol / water solutions can be chosen. *Rusamarinus officianalis L.* at all concentrations and solvents had higher antiradical activity than *Ranunculus bulbosus*. These results confirm that active moistening is effective and can increase the amount of extractive substances from herbal materials.

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