Comparative study of rosmarinic acid content in some plants of Labiatae family

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ABSTRACT

Background: Plants of Labiatae are used in traditional medicine and phytotherapy. Rosmarinic acid (RA) is a phenolic compound which is found in many genus of Labiatae and exhibits important biological activities. **Materials and Methods:** In this investigation, RA contents of 29 species of Labiatae named *Salvia officinalis, Salvia limbata, Salvia virgata, Salvia hypoleuca, Salvia macrosiphon, Salvia choloroleuca, Melissa officinalis, Origanum vulgare, Lavandula angustifolia, Rosmarinus officinalis, Thymus daenensis, Thymus citriodorous, Thymus pubescens, Thymus vulgaris, Zataria multiflora, Mentha piperita, Mentha pulegium, Mentha longifolia, Mentha spicata, Mentha aquatica, Mentha crispa, Perovskia artemisoides, Zhumeria majdae, Satureja hortensis, Satureja khuzistanica, Satureja bachtiarica, Satureja atropatana, Satureja mutica* and *Satureja macrantha* were determined by using high-performance liquid chromatographic method. **Results:** The results showed that RA content in different species of Labiatae was 0.0-58.5 mg g^{-1} of dried plants. The highest amount of RA was found in *Mentha* species especially *M. spicata.* **Conclusion:** *M. spicata* can be considered as a new source of rosmarinic acid.

Key words: High-performance liquid chromatographic, Labiatae, *Mentha spicata*, rosmarinic acid

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INTRODUCTION

Plants of Labiatae family have been used in traditional medicine for exhaustion, weakness, depression, memory enhancement, circulation improvement, strengthening of fragile blood vessels,[1] inflammation, infection,[2] indigestion and gastritis.[3] Researchers have proved that these plants are source of compounds with antioxidant,[4] anti-inflammatory,[5] anti-allergic,[6] anti-depression,[7] anti-hyperglycemic[8] and antimicrobial[9-11] properties. These activities are mostly related to their phenolic compounds content especially rosmarinic acid (RA), an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid [Figure 1][1] which was isolated for the first time from *Rosmarinus officinalis* L. leaves and later found in other species of Labiatae and Boraginaceae. RA has interesting properties which has led to a broad range of applications from food preservatives to cosmetics.[12] Different studies have shown that antioxidant activity of RA is more than vitamine E[13] or Trolox.[14] RA has been reported to have some biological activities *in vitro* such as antiviral properties[15] including anti-HIV-1,[16] antibacterial, antioxidant, anti-carcinogenic,[17] and anti-

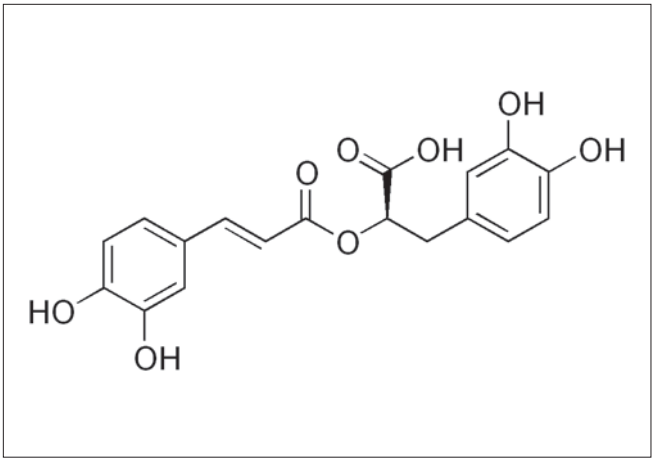

Figure 1: The structure of rosmarinic acid

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allergic activities.[18] *In vivo* studies have shown that RA exhibit anti-allergic,[19,20] anti-thrombotic,[21] and anti-carcinogenic[22,23] properties as well. This compound is also efficient against peroxidative damage to biomembranes.[24] Nowadays, many products have been prepared from RA in pharmaceutical, cosmetic, and food industries. RA can be found in many plants but usually rosemary plant is used as the major source. This matter has caused to increase of demand and price of the plant.[25] Therefore, finding other plants containing high amount of RA is very important to introduce as new sources. This compound has been reported to occur in several taxonomically non-related families of the plant kingdom, but it is found abundantly in Labiatae.[26] In this investigation, in order to mark the best source of RA in Labiatae plants which grow in Iran, RA contents of 29 plants have been determined by using High-performance liquid chromatographic (HPLC) method. All of the plants are used as medicinal herbs or in food industries in different pats of Iran.

MATERIALS AND METHODS

Plant material

Aerial parts of *Salvia officinalis* L., *Salvia limbata* C. A. Mey., *Salvia virgata* Jacq., *Salvia hypoleuca* Benth., *Salvia macrosiphon* Boiss., *Salvia choloroleuca* Rech. f. and Aell., *Melissa officinalis* L., *Origanum vulgare* L., *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L., *Thymus daenensis* Celak, *Thymus citriodorous* (Pers.) Schreb., *Thymus pubescens* Boiss. and Kotschy ex Celak, *Thymus vulgaris* L., *Zataria multiflora* Boiss., *Mentha piperita* L., *Mentha pulegium* L., *Mentha longifolia* (L.) Huds., *Mentha spicata* L., *Mentha aquatica* L., *Mentha crispa* L., *Perovskia artemisoides* Boiss, *Zhumeria majdae* Rech., *Satureja hortensis* L., *Satureja khuzistanica* Jamzad, *Satureja bachtiarica* Bunge, *Satureja atropatana* Bunge, *Satureja mutica* Fisch. and C. A. Mey., and *Satureja macrantha* C. A. Mey., were collected from their growing area of Iran [Table 1] during flowering stage in summer 2008. Herbarium specimens were kept at the Herbarium of the Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences.

Chemicals

Methanol (HPLC grade), 2-propanol (analytical grade), *O*-phosphoric acid (analytical grade) were purchased from Merck (Germany). The standard of rosmarinic acid was prepared from Aldrich (Germany). The water used in HPLC and for sample preparation was produced with a Purelab UHQ (ELGA) with a resistivity over 18 MΩ·cm.

Table 1: Gradient time program for analysis of rosmarinic acid in Labiatae plants

C (%)	B (%)	A (%)	Time (min)
10	10	80	0
15	15	70	10
20	20	60	15
20	20	60	20

A: *O*-phosphoric acid in water, B: *O*-phosphoric acid in methanol, C: *O*-phosphoric acid in 2-propanol

Instrumentation

A Waters high performance liquid chromatograph system comprising vacuum degasser, quaternary pump, auto sampler and a waters 2996 diode array detector was used. UV spectra were collected across the range of 200--900 nm extracting 330 nm for chromatograms. The column, an ACE 5 C_{18}, (250 × 4.6 mm) was maintained at 30 °C. Mobile phase used for separation was the mixture of 0.085% *O*-phosphoric acid in water (A), 0.085% *O*-phosphoric acid in methanol (B), and 0.085% *O*-phosphoric acid in 2-propanol (C) in gradient mode [Table 1]. The flow rate, detection wavelength and sample injection volume were 1.0 ml min^{-1}, 330 nm, and 20 µl, respectively.[27] The chromatographic peak of rosmarinic acid was confirmed by comparing the retention time and UV spectra with that of related to the reference standard. Quantization was performed by using calibration curve of rosmarinic acid.

Preparation of standard solutions

Stock standard solution was prepared accurately by weighing 10 mg of rosmarinic acid reference standard into 10 ml volumetric flask and dissolving in water: methanol: 2-propanol (each one contained 0.085% *O*-phosphoric acid) (80:10:10) with the aid of ultrasonic. Serial dilutions (1-150 µg/ml) were made from stock solution.

Sample preparation

Milled and powdered samples (200 mg) were accurately weighed into a 25-ml tube, and extracted with 25 ml of the same solvent system for preparing standard solutions, during 30 min by ultrasonic. The resulting mixture was centrifuged at 4500 r/min for 5 min, and the supernatant transferred to a 100-ml volumetric flask. The residual solid was extracted for two more times with 25 ml of the same solvent mixture by ultrasonic, and centrifuged as above. The supernatants were combined, and diluted to 100 ml with the same solvent mixture. Each sample was extracted three times and injected (three times) to HPLC for analysis.

RESULTS AND DISCUSSION

Several mobile phases including methanol, water, acetonitrile, 2-propanol, THF and TFA in different combinations were tested. Finally, it was found that a 0.085% *O*-phosphoric acid in water: 0.085% *O*-phosphoric acid in methanol: 0.085% *O*-phosphoric acid in 2-propanol in gradient mode in 20 min [Table 1] gave the best

separation.[27] After comparison between C_8 and C_{18} columns, the best separation efficacy was obtained by using C_{18} column. HPLC chromatogram of *Mentha spicata* sample and UV spectrum of RA in 11.16 min obtained from PDA detector have been shown in Figures 2 and 3, respectively. Comparison between purity threshold and purity angle reported in em-power software showed that the method is specific for rosmarinic acid and reported peak is completely separated from other interfering compounds. The linear relationship between detector response and different concentrations of rosmarinic acid (eight levels) was confirmed in range of 1-150 μg/ml with correlation coefficient of 0.9983 and equation of $y = 45337x$-19410. In order to obtain the best recovery and peak shape of rosmarinic acid, different solvents and extraction methods were examined. Methanol, methanol followed by CCl_4, methanol:water, methanol followed by hexane and water:methanol:2-propanol (each one contained 0.085% *O*-phosphoric acid) were used to investigate the effect of solvents on the RA extraction. Moreover, the effect of extraction time on the content of RA was studied (data was not shown). Finally, water:methanol:2-propanol (each one contained 0.085% *O*-phosphoric acid) (80:10:10) and ultrasonic for 30 min in three repeats were selected as the best parameters for RA extraction method.

The results showed that among analyzed plants belong to different genus of Labiatae family, the most RA contents were found in *Mentha* species. As it is observed in Table 2, all *Mentha* species contain RA in considerable concentration (19.3--58.5 mg g^{-1}) and *M. spicata* showed the highest amount of RA. Rosemary has been considered as a main source of RA in many countries[25] but the results demonstrated that the plant growing in Iran contains low RA concentration (7.2 mg g^{-1}) compare to other investigated plants. Therefore, other plants such as *Salvia officinalis, Melissa officinalis, Thymus citriodorous, Perovskia artemisoides* and especially *Mentha spicata* which is widespread in Iran and very easy to access can be used as a source of RA in pharmaceutical, food and cosmetic industries. As it has been shown in Table 2, no rosmarinic acid was detected in *Thymus pubescens*, *Salvia choloroleuca* and *Zataria multiflora*. Several investigations have been carried out in order to find new RA resources among plants. Achamlale *et al*.,[25] showed that RA contents in *Zostera noltii* and *Z. marina* samples varied from 2.2 to 18.0 mg g^{-1} and 1.3--11.2 mg g^{-1}, respectively. They believed that the high RA content of these two sea-grasses is of interest for both cosmetic and herbal industries. Similar study has been performed on *Melissa officinalis* during different harvesting time. It has been shown that *M. officinalis* contained 39.1 mg g^{-1} of RA during full flowering stage[28] which is almost similar to RA content of *M. officinalis* from Iran (36.5 mg g^{-1}). Another investigation on rosemary, sage, thyme, spearmint and lavender has proved that the plants contained 10.3, 10.4, 6.6, 10.7, and 2.0 mg g^{-1} of RA, respectively.[1] RA contents of rosemary and lavender obtained in our study were almost similar to the previous study. Therefore, rosemary can not be considered as RA source in all countries. Since, RA content of a plant is known to depend considerably on extrinsic and intrinsic factors including soil and climatic conditions, plant ontogenesis phases, harvest and plant storage,[29-32] therefore, it is necessary to analyze the plants which are growing in each country for finding the best source of rosmarinic acid.

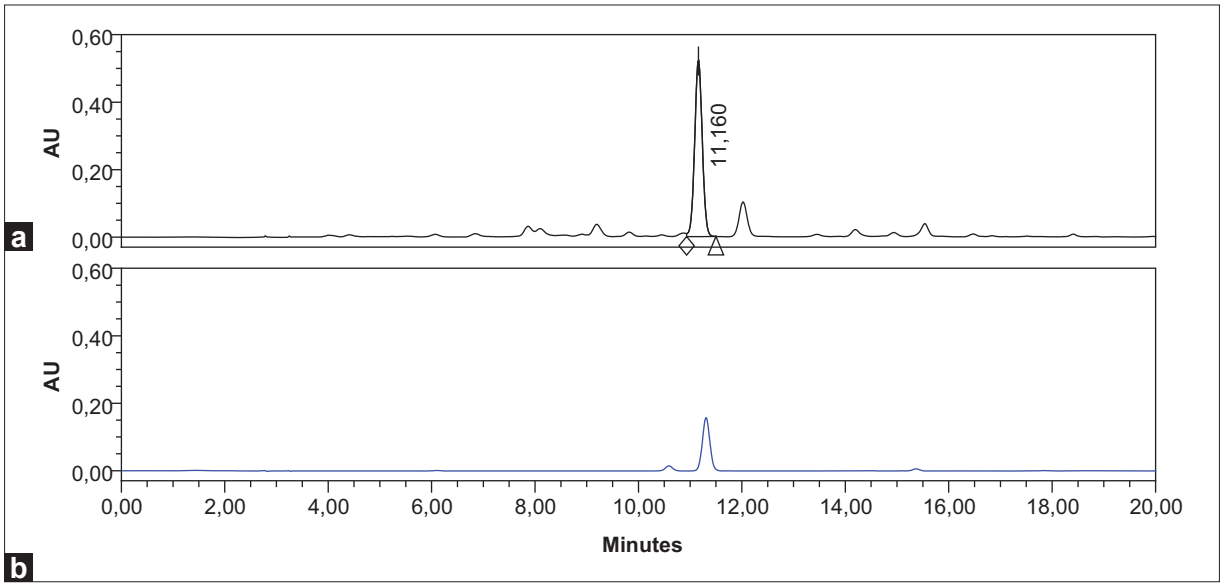

Figure 2: High-performance liquid chromatographic Chromatograms of (a) *Mentha spicata* extract and (b) rosmarinic acid standard solution

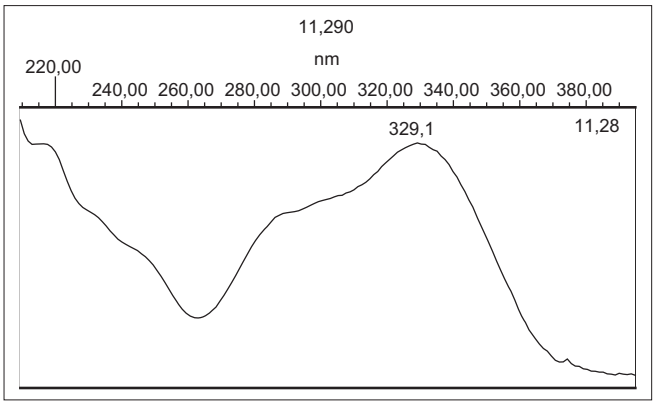

Figure 3: UV spectrum of rosmarinic acid obtained from PDA detector at 11.16 min

Table 2: Collection areas and concentration of rosmarinic acid in plants of Labiatae family

Plant name	Collection area	RA Concentration (mg g^{-1})*
Lavandula angustifolia	Tehran, Abali road	1.7 ± 0.2
Mellisa officinalis	Mazandaran, Salmanshahr	36.5 ± 0.8
Mentha aquatica	Golestan, Gorgan	24.6 ± 0.2
Mentha crispa	Golestan, Gorgan	19.3 ± 0.2
Mentha longifolia	Golestan, Gorgan	26.6 ± 0.3
Mentha piperita	Golestan, Gorgan	28.2 ± 0.3
Mentha pulegium	Golestan, Gorgan	23.4 ± 0.3
Mentha spicata	Golestan, Gorgan	58.5 ± 1.4
Origanum vulgare	Mazandaran, Salmanshahr	25.0 ± 0.1
Perovskia artemisoides	Khorasan	31.3 ± 0.2
Rosmarinus officinalis	Tehran, Abali road	7.2 ± 0.1
Salvia hypoleuka	Tehran, Damavand	4.3 ± 0.03
Salvia limbata	Semnan, Ahovan	7.5 ± 0.1
Salvia macrosiphon	Tehran, Delichaee	6.4 ± 0.1
Salvia officinalis	Mazandaran, Salmanshahr	39.3 ± 0.9
Salvia virgata	Mazandaran, Ghaemshahr	16.4 ± 0.1
Salvia choloroleuca	North Khorasan, Bojnoord	0.0
Satureja atropa	Easth Azarbayjan, Ahar road	2.8 ± 0.04
Satureja bachtiarica	Chaharmahal and Bakhtiari	5.7 ± 0.1
Satureja hortensis	Mazandaran, Salmanshahr	16.3 ± 0.5
Satureja khuzistanica	Lorestan, Khorramabad	1.2 ± 0.02
Satureja macrantha	West Azarbayjan, Oshnaviyeh road	4.2 ± 0.1
Satureja mutica	Gilan, Manjil	19.0 ± 0.6
Thymus citriodorus	Tehran, Zardband	31.5 ± 0.1
Thymus daenensis	Golestan, Gorgan	14.3 ± 0.4
Thymus pubescens	Tehran, Polour	0.0
Thymus vulgaris	Golestan, Gorgan	23.5 ± 0.5
Zataria multiflora	Fars, Shiraz	0.0
Zhumeria majdae	Hormozgan	2.4 ± 0.1

*Data are mean of three sample ± SD

CONCLUSION

RA is found in most of Labiatae plants growing in Iran and its concentration in some of species such as *Salvia officinalis, Melissa officinalis, Thymus citriodorous, Perovskia artemisoides* and *Mentha spicata* is considerable. These plants especially *Mentha spicata* can be used as RA resources in food, cosmetic and pharmaceutical industries instead of rosemary which contains low concentration of RA compare to other studied plants.

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