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Identification and Quantification of Phenolic Compounds of Tunisian *Rosmarinus officinalis* L.

K. HCINI[1,*], J.A. SOTOMAYOR[2], M.J. JORDAN[2] and S. BOUZID[1]

[1]Département des Sciences Biologiques, Faculté des Sciences de Tunis, Campus universitaire, 2092 El-Manar II Tunis, Tunisia
[2]Laboratory of Aromatic and Medicinal Plants of the Departamento de Recursos Naturales y Desarrollo Rural, Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), C/Mayor, s/n La Alberca, 30150 Murcia, Spain

*Corresponding author: Tel: +216 94 491 686, E-mail: hcinikheiria@yahoo.fr

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The total phenolic contents in extracts of rosemary plants was determined. High-performance liquid chromatography (HPLC) was used to identify and quantify the phenolic compounds of *Rosmarinus officinalis* L. Eleven different compounds (gallic acid, caffeic acid, ferulic acid, rosmarinic acid, coumaric acid, carnosol, carnosic acid, hesperidin, luteolin, apigenin and genkwanin) were identified and quantified. HPLC analysis led to the identification of rosmarinic acid, carnosol and carnosic acid as the major compounds of *R. officinalis* L. methanolic extracts. Therefore, supplementing a balanced diet with herbs may have beneficial health effects. Rosemary extracts obtained by HPLC analysis were shown to be promising with regard to their incorporation into various foods, cosmetics and pharmaceutical products.

Key Words: *Rosmarinus officinalis* L., HPLC analysis, Phenolic compounds.

INTRODUCTION

In the search of plants as a source of natural antioxidants, some medicinal plants and fruits have been extensively studied for their antioxidant activity and radical scavenging in the last several years[1-3]. Herbs and aromatic plants, which are highly widespread in the Mediterranean region, are of commercial interest for their essential oils[4]. Some of them, including sage and Rosemary[5,6], thyme[7,8] and Rosemary[2,9] have already been studied for their antioxidant activity.

Phenolic substances are widely distributed in the plant kingdom and have been reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilatory actions[3,10]. The antioxidant effect of herbs phenolics compounds has also been studied in relation to the prevention of coronary diseases and cancer, as well as age-related degenerative brain disorders[11,12] .

On the other hand, the search for natural antioxidants in aromatic plant origin is also being explored as an alternative to the synthetic antioxidants used in food and pharmaceutical industries[10,13-15]. The use of synthetic antioxidants in the food is severely restricted as to both application and level. Among the natural antioxidants, rosemary has been widely accepted as one of the spices, along with sage, with the highest antioxidant activity[16-18]. It is well known that the activity of rosemary extracts in food industry and medicine due to the presence of some important antioxidant oil and phenolic components[2,6]. Many compounds have been isolated from rosemary, including flavones, diterpenes, steroids and triterpenes. Rosemary extracts contain active antioxidative factors such as phenolic diterpenes, flavonoids and phenolic acids[19]. The main antioxidant activity of *R. officinalis* was attributed to rosmarinic acid and the diterpene phenolics carnosol and carnosic acid[6]. The antioxidant activity of rosemary extracts depends on their composition. The influence of environmental growing conditions can modulate the contents of rosmarinic and carnosic acids and thus the antioxidant potential of rosemary plant extracts[6, 9, 20].

The purpose of this study was to identify and quantify major phenolic compounds of methanolic extracts of rosemary by HPLC and to evaluate antioxidant activity to find new potential sources of natural antioxidants.

EXPERIMENTAL

Aerial parts plants of *R. officinalis* L. were randomly collected from three geographic origins (Beja, Sidi Bouzid and Gabes) in Tunisia. Details of collection sites are given in Table-1. Fresh aerial parts of plants were dried in a forced-air drier at 35 ºC for 48 h, until it reached a constant weight. Stem and leaves of *R. officinalis*, were collected at the flowering stage from different localities and identified by Dr. Sotomayor, botanist at the IMIDA institution and one of the authors of the present publication. Voucher specimens of the species are

TABLE-1
DESCRIPTION OF THE ORIGINAL LOCATIONS OF THE POPULATIONS OF *Rosmarinus* officinalis L.

Population	Code	Position	Altitude (m asl)	Mean temperature (ºC)		Annual precipitation (mm)
				Max. in hottest month	Min. in coldest month	
Gabes	ROG	33º54'N, 10º06'E	4	32.5	5.8	193
Sidi Bouzid	ROSB	35º04'N, 09º37'E	354	37.8	5.0	237
Tunis	ROB	36º51'N, 10º19'E	3	32.5	5.6	473

deposited at the herbarium_of the Laboratory of vegetable Biotechnology and morphogenesis at the Faculty of Sciences of Tunis under the numbers ROB 2008-117, ROSB 2008-118 and ROG 2008-119), respectively, for the (Beja, Sidi Bouzid and Gabes) sites.

Extraction of phenolic compounds: Distilled plant material was dried in a forced-air drier at 35 ºC for 48 h, (until it reached a constant weight) and ground to pass a 2-mm sieve. For the extraction dried samples (0.5 g) were firstly extracted with 20 mL of petroleum ether under stirring and taken to dryness at room temperature. Secondly, they were extracted using 150 mL of methanol in a Soxhlet extractor (B-811) (Buchi, Flawil, Switzerland) for 2 h and under nitrogen atmosphere. Methanolic extracts were taken to dryness at 40 ºC under vacuum conditions in an evaporator system (Syncore Polyvap R-96) (Buchi, Flawil, Switzerland). The residue was redissolved in methanol and made up to 5 mL. The concentration of the extracts was expressed in terms of dry weight per mL of solvent. The extracts were kept in vials at -80 ºC until their corresponding analysis[21]. Two extracts were prepared for each sample.

HPLC analysis: For the HPLC analysis, a method adapted from Zheng and Wang[9] was performed on a reverse phase ZORBAX SB-C_{18} column (4.6 mm × 250 mm, 5 µm pore size, Hewlett Packard, USA) using a guard column (ZORBAX SB-C_{18} 4.6 mm × 125 mm, 5 µm pore size, Hewlett Packard, USA) at ambient temperature. Extracts were passed through a 0.45 µm filter (Millipore SAS, Molsheim, France) and 20 µL were injected in a Hewlett Packard (Germany) system equipped with a G1311A quaternary pump and G1315A photodiode array UV/visible detector. The mobile phase was acetonitrile (A) and acidified water containing 5 % formic acid (B). The gradient was as follows: 0 min, 5 % A; 10 min, 15 % A; 30 min, 25 % A; 35 min, 30 % A; 50 min, 55 % A; 55 min, 90 % A; 57 min, 100 % A and then held for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/min and the wavelengths of detection were set at 280 and 330 nm. The identification of the phenolic components was made by comparison of retention times with those of commercially available standard compounds. Phenolic compound contents were expressed in µg/g of dry plant material weight.

RESULTS AND DISCUSSION

Eleven phenolic compounds were identified in the methanolic extracts of *R. officinalis* L., including five phenolic acids (caffeic acid, ferulic acid, rosmarinic acid, coumaric and gallic acid), two phenolic diterpenes (carnosic acid and carnosol) and four flavonoids (luteolin, apigenin, genkwanin and hespiridin). The results are shown in Table-2. The extracts of *R. officinalis* L. were the first marketed natural antioxidants. Several phenolic compounds of rosemary determined in this study were similar in content and concentration to those in previous reports[2,6,9]. Among the mentioned phenolic compounds, rosmarinic acid was present in the largest amounts ranging from 5286.19 to 11138.69 µg/g followed by carnosol and carnosic acid. Much lower contents were detected for hesperidin, coumaric and genkwanin, whereas the lowest rates were obtained for gallic acid (47.12-10.56 ± 0.703 µg/g). These phenolic compounds in rosemary extracts are very potent antioxidants and are utilized in many food products[9]. The identified compounds were previously reported in *R. officinalis* L. extracts: rosmarinic acid and carnosic acid[2,22]. Differences among phenolic compound levels, compared with our results, can be related to the distillation process, because the drying and/or distillation treatments of *R. officinalis* L. strongly affected the content of the two compounds of higher antioxidant

TABLE-2
CONTENT OF PHENOLIC COMPOUNDS METHANOLIC EXTRACTS OF *Rosmarinus officinalis* L.

Identified compound	Content (µg/g of dry plant material weight)		
	ROB	ROG	ROSB
Phenolic acids			
Gallic acid	33,54 ± 1,442	10,56 ± 0,703	47,12 ± 0,453
Caffeic acid	212,43 ± 8,892	136,55 ± 6,478	283,19 ± 6,934
Ferulic acid	63,00 ± 4,486	34,95 ± 3,655	75,33 ± 4,72
Rosmarinic acid	5286,19 ± 19,222	6262,36 ± 157,969	11138,69 ± 348,609
Coumaric acid	472,44 ± 7,443	240,55 ± 9,982	544,02 ± 4,38
Phenolic diterpenes			
Carnosol	5334,81 ± 26,913	5309,04 ± 86,348	5420,25 ± 1,401
Carnosic acid	1229,14 ± 18,737	788,25 ± 25,943	972,11 ± 27,673
Flavonoids			
Hesperidin	963,90 ± 10,47	618,66 ± 15,895	1171,79 ± 37,542
Luteolin	174,36 ± 1,394	132,02 ± 1,959	166,25 ± 1,178
Apigenin	68,77 ± 3,529	82,25 ± 4,721	93,04 ± 5,763
Genkwanin	571,25 ± 4,128	632,03 ± 4,59	463,91 ± 13,285
Total	***	****	****

activity: rosmarinic and carnosic acid[2]. However, present samples seem to have higher concentrations of rosmarinic acid compared with previous studies[2,9]. These results indicated that the phenolic compounds had a major contribution to the antioxidant capacity of herbs. Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids[1].

Rosemary samples collected in Sidi Bouzid regions showed the higher levels of phenolic compounds with largest proportions of rosmarinic acid, carnosol and carnosic acid and hespiridin. Previous studies also reported that the phenolic composition of the natural extracts and their antioxidative performance vary widely depending on environmental conditions[6,20,22]. In agreement with these findings, our plants cultivated in different habitats showed significant differences in the quantitative composition of some phenolic compounds. The relationships between total phenolic content and antioxidant properties of many plants have been investigated in previous studies[9].

Conclusion

Rosemary extracts obtained by HPLC extraction were shown to be promising with regard to their incorporation into various foods, cosmetics and pharmaceutical products. There was a positive linear correlation between the phenolic content and antioxidant capacity of the herbs. This study revealed that Rosemary is an effective potential source of natural antioxidants. Therefore, supplementing a balanced diet with herbs may have beneficial health effects. Further studies are necessary to evaluate antioxidant activity to find new potential sources of natural antioxidants and evaluate the relationship between phenolic compounds and antioxidant activity to confirm that phenolic constituents are responsible for antioxidant activity of rosemary.

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