

# Spectrophotometric Analysis of Rosmarinic Acid Extracted from Raw Material of Rosmarin (*Rosmarinus officinalis* L.) from Tunisia

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## ABSTRACT

The aim of our study is the qualitative and quantitative determination of hydroxycinnamic acids in terms of rosmarinic acid from the herb of *Rosmarinus officinalis* from Tunisia. Analysis by thin layer chromatography was carried out in a solvent system formed of formic acid P, acetone anhydride P and methylene chloride P (with the following volumes: 8.5: 25: 85). The identification of the phenolic derivatives was carried out in UV light (365 nm) as a function of the specific fluorescence of caffeic acid and rosmarinic acid (blue fluorescence). Spectrophotometric analysis was used to quantify the amount of hydroxycinnamic acids in terms of rosmarinic acid. The content of the sum of hydroxycinnamic acids in terms of rosmarinic acid was:  $5.88 \pm 0.25\%$ .

**Keywords:** *Rosmarinus officinalis* L., phytochemical analysis, rosmarinic acid, caffeic acid, thin layer chromatography, spectrophotometry.

## INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is a medicinal plant with pronounced antioxidant, antibacterial and fungicidal action [1-4]. It has a fairly wide range of natural distribution, covering the Mediterranean region, Italy, Spain, southern France, Tunisia, Morocco, Algeria. This species is known in the culture of many countries, and it grew in the territory of the Russian Federation only under conditions of introduction into the territory of botanical gardens, such as in the Nikitsky Botanical Garden and the Botanical Garden of the Pyatigorsk Medical and Pharmaceutical Institute.

This work is a fragment of complex pharmacological studies, including comparative morphological-anatomical and ecological-geographical research, as well as phytochemical research of medicinal rosemary samples taken under various ecological conditions on the territory of the Republic of Tunisia. The aim of our study was the qualitative and quantitative determination of hydroxycinnamic acids in the herb of *Rosmarinus officinalis* (*Rosmarinus officinalis* L.), from Tunisia.

## MATERIALS AND METHODS

The object of the study was the aerial part of officinal rosemary (stem and leaf), harvested during the vegetative phase on July 29, 2016 (Fig. 1), growing under natural conditions (Nador forest in the

region of Bizerte, forest Rimel in the region of Bizerte and on the site of Djebel Bargou, Siliana) on the territory of the governor of Bizerte, located in the north of Tunisia as well as on the governor of Siliana located in the north west of Tunisia (Figure 1).



**Figure 1:** Rosemary (stem and leaf), harvested during the vegetative phase on July 29, 2016.

## RESULTS AND DISCUSSION

The quality indices of the medicinal raw material of rosemary growing under natural conditions in Tunisian according to the methods of Pharmacopoe of Russia GF XIII [5] were determined.

To identify and determine the quantitative content of

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hydroxycinnamic acids in vegetable raw materials (leaves and stems of rosemary), extractions with 70% ethyl alcohol were prepared with a raw material-extractant ratio of 1:40.

The extraction was subjected to chromatographic analysis using paper and thin layer chromatography (Filtrak paper of various numbers, chromatographic plates of Sylwoff, Sorbfil and Merck grades).

On the chromatogram was applied with a micropipette 0.01 ml of a hydro-alcoholic extract of vegetable raw materials of the sample.

The assay was carried out in the following solvent systems: chloroform-methanol water (24: 14: 3), toluene - ethyl formate - formic acid (50:40:10), acetic acid in the presence of butanol - water (4: 1: 2) acetic acid 2% P anhydrous formic acid - acetone P - F methylene chloride (8.5: 25: 85). The chromatogram was examined in UV light before and after treatment with specific reagents. Hydroxycinnamic acid was detected in specific fluorescent UV light (365 nm) using appropriate reagents [9,10]. The analysis results are shown in the the chromatograms (Figure 2 and 3).

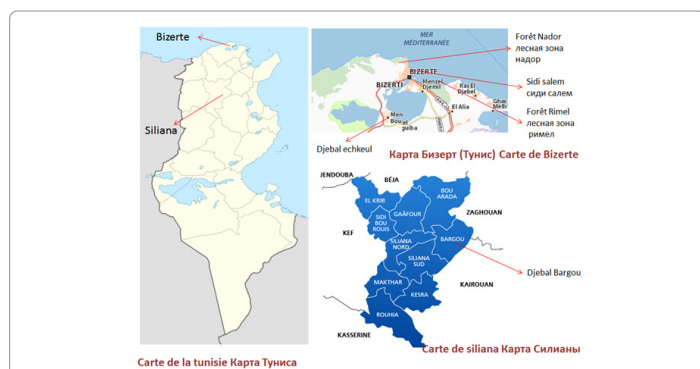


Figure 2: Rosemary (*Rosmarinus officinalis* L.) sampling locations in Tunisia.

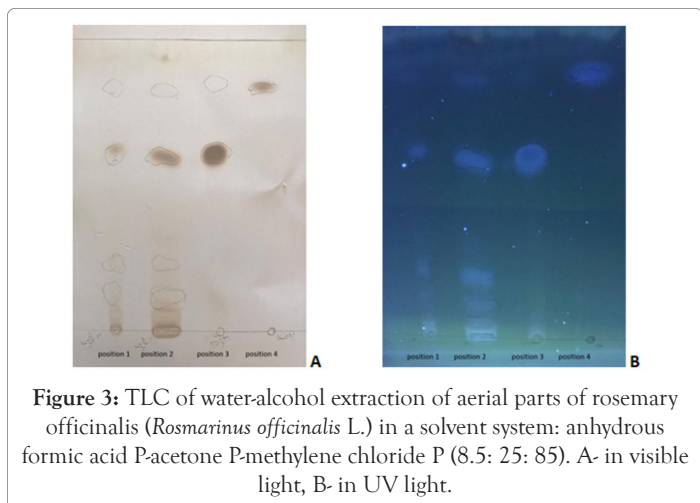


Figure 3: TLC of water-alcohol extraction of aerial parts of rosemary officinalis (*Rosmarinus officinalis* L.) in a solvent system: anhydrous formic acid P-acetone P-methylene chloride P (8.5: 25: 85). A- in visible light, B- in UV light.

Spectrophotometric analysis was used to quantify the amount of hydroxycinnamic acids in terms of rosmarinic acid [7]. The UV spectra of the water-alcohol extracts of rosemary in comparison with the UV spectrum of rosmarinic acid were analyzed. In a 100 ml flask (Erlenmeyer), 1.00 g of raw materials crushed into powder of rosemary were added to which 40 ml of 70% ethanol were added, the mixture was heated on a steam bath with a cooling system at reflux for 30 min. Subsequently the flask was cooled and the solution was filtered.

1 ml of the resulting extract was placed in a volumetric flask

and the volume was adjusted to the 25 ml mark with 70% ethyl alcohol (solution A). 5ml of solution A was placed in a volumetric flask and the volume was adjusted to the 25ml mark with 95% ethanol (solution B). Optical density was measured on an SF-2000 spectrophotometer over wavelength.  $\lambda = 328 \pm 2$  nm. 95% alcohol was used as a compensating solution. The analysis of the UV spectra (Figures 4 and 5) shows that the absorption maxima  $\lambda_{max} = 328 \pm 2$  nm for the solutions studied coincide, which allows a direct spectrophotometric determination of the content of the sum of hydroxycinnamic acids at  $\lambda = 328 \pm 2$  nm in terms of rosmarinic acid.

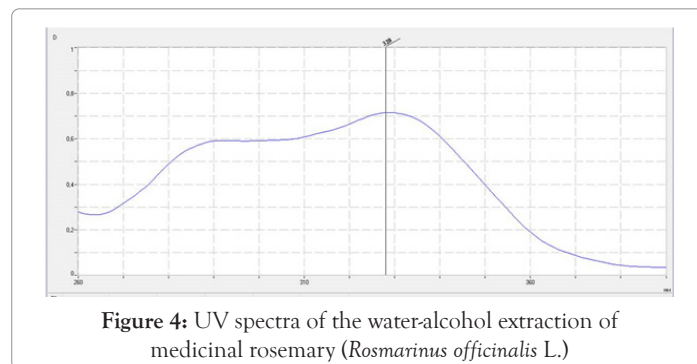


Figure 4: UV spectra of the water-alcohol extraction of medicinal rosemary (*Rosmarinus officinalis* L.)

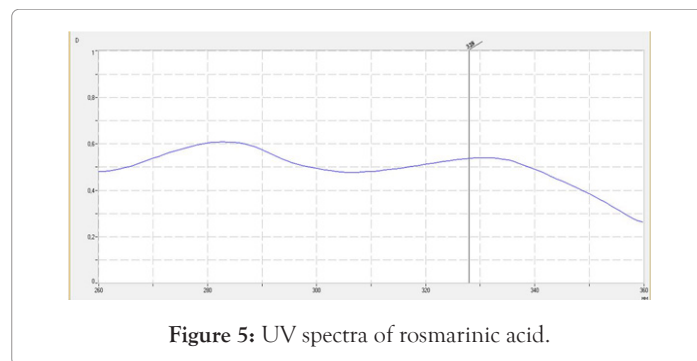


Figure 5: UV spectra of rosmarinic acid.

The content of the sum of hydroxycinnamic acids (X,%) in terms of rosmarinic acid was calculated by the formula:

$$X = \frac{A \cdot 500}{m \cdot w} \cdot V_3$$

A is the optical density of the test solution;

m - mass of raw materials;

500 - specific absorption index of rosmarinic acid (at  $\lambda = 328$ );

w - loss of mass during drying of raw materials: moisture

V1 is the volume of solution A

V2 is the volume of solution B

V3 is the volume of the extractant (40 ml).

The results of the determination of the amount of hydroxycinnamic acids in terms of rosmarinic acid in the aerial parts of rosemary officinalis growing under natural conditions in the northern and northwestern parts of Tunisia.

As a result of the qualitative and quantitative analysis of the aerial part of rosemary, the presence of caffeic acid and rosmarinic acids was detected. The quantitative content of the sum of hydroxycinnamic acids in terms of rosmarinic acid in the aerial organs of rosemary officinalis (*Rosmarinus officinalis* L.) is  $5.88 \pm 0.25\%$ .

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