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Antioxidant Activity of Flavonoids Isolated from *Rosmarinus officinalis L.*

Research article

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Abstract

Rosmarinus officinalis L. (Lamiaceae), Rosemary is recognized several years ago in the traditional medicine by its therapeutic virtues and its ability to treat some diseases, in this context the present study aim to determine the content of phenolic and flavonoid compounds contained in Rosmary leaves and to evaluate their antioxidant activity. The total phenolic and flavonoid contents were estimated by the methods of Folin-Ciocalteu and $AlCl_3$ methods respectively. Diagnosis and isolation of flavonoids have been carried out by various chromatographic techniques (Thin layer chromatography and Column chromatography) and their structural identification was carried out by spectral analysis by the spectrophotometer (UV-Vis). The antioxidant capacity was evaluated by *in vitro* trapping of free radical DPPH. The total polyphenols and flavonoids contents in rosemary were 10.42 mg GAE/g of dry material for polyphenols and 9,075±0,002 mg QE/g of dry material for flavonoids. The chromatographic and spectral identification of extracts of thyme revealed the presence of three flavonoids characterized by their antioxidant activity in the order of 90%, 88%, 87% respectively for quercetin 3OR, quercetin, and luteolin compared to quercetin standard characterized by its percentage of scavenging DPPH equivalent to 93.05%.

In conclusion, we report that the flavonoids of *R. officinalis* exhibit good antioxidant activity against free radicals. The molecules may be used as antioxidants and for therapeutic applications.

Keywords: *Rosmarinus officinalis*; Flavonoids; Thin layer chromatography; Column chromatography, spectrophotometer (UV-Vis); antioxidant activity

Introduction

Species and herbs have been added to foods since ancient times, mainly to modify or to improve their flavor. Since 1952, the antioxidant properties of some of these species have been recognized. Antioxidants are compounds that when present in foods at low concentrations, compared to the concentration of an oxidizable substrate markedly delay or prevent oxidation of the substrate. Among the species with antioxidant properties, usage of rosemary has increases tremendously in many food applications [1].

Rosmarinus officinalis L. belongs to the Lamiaceae family of herbs, which is not only useful as a food flavoring agent and preservative but also for its powerful antibacterial, antimutagenic properties,and as a chemopreventive agent [2].

Owing to its antioxidant properties, *R.officinalis* has been widely accepted as one of the species with the highest antioxidant activity [3].

In this paper, we report the results of a study aimed to evaluate and compare the *in vitro* antioxidant properties of some *R. officinalis* using quercetin standard.

Material and Methods

Plant material, extraction and isolation

R. officinalis leaves were collected from Constantine, Algeria in March 2008. Taxonomic identification was performed by Prof. Merghem R, department of Biology and Ecology, at University Mentouri of Constantine, Algeria.

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The plant material was dried at room temperature and ground in a mortar. Sample of 100 g of dried leaves of *Rosmarinus officinalis* was extracted by ethanol/water (30:70) for 24 h at room temperature, the remaining vegetal marerial was extracted twice under identical condition [4]. The combined extract was concentrated under reduced pressure and dissolved in distilled H_2O. The solution successively was partitioned with petroleum ether, ethyl acetate (EtOAc), and butanone (BuOH). The ethyl acetate (EtOAc) and butanone (BuOH) dried extracts were dissolved in methanol, combined together and subjected to column chromatography on polyamide. The column was eluted with a gradient of toluene-MeOH, provided 172 fractions of 25 ml each. All fractions were chromatographed on thin layer chromatography polyamide DC_6. As result, six flavonoids were obtained and their purity was monitored by thin layer chromatography on polyamide or silica gel.

Total phenol determination

The total phenolic compounds were determined by Folin-Ciocalteu reagent [5]. Calibration curve was prepared by mixing ethanolic solution of tannic acid (1ml; 0.01-0.1 mg/ml) with 5ml Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4ml, 0. 7M). We measured absorbance at 765 nm and drew the calibration curve. One ml of ethanolic extract (0.05 mg/ml) was also mixed with the reagents above and after two hours the absorbance was measured to determine total plant phenolic contents. All determinations were carried out in triplicate. The total content of phenolic compounds in the extract in Gallic acid equivalent (GAE) was calculated by the following formula:

T=C.V/M

Where T: Total content of phenolic compounds, milligram per gram extract, in TAE.

C: Concentration of tannic acid established from the calibration curve, mg/ml.

V: Volume of extract (ml).

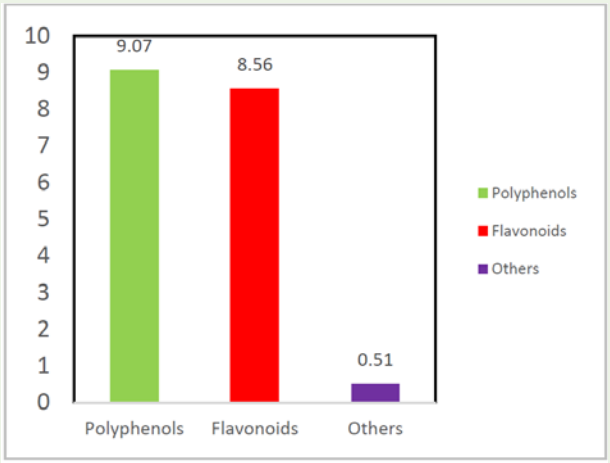

Figure 1: Phenolic and flavonoids contents in rosemary (*R. officinalis*) extract.

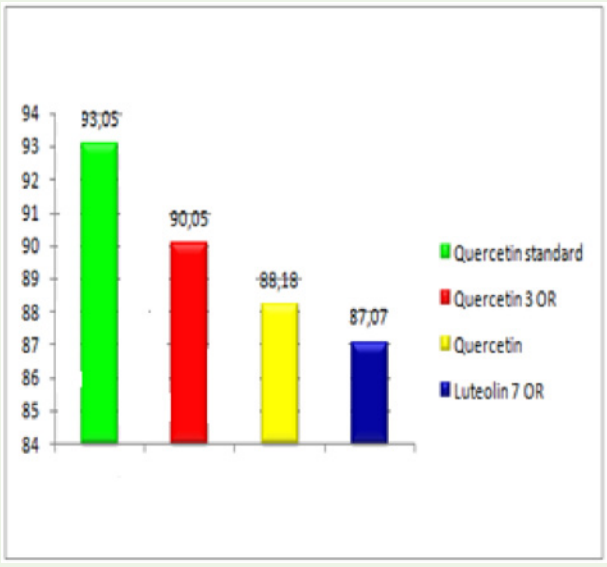

Figure 2: Percentage of the scavenging activity of DPPH by the isolated flavonoids from *R. officinalis*.

M: Weight of ethanolic plant extract (g).

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination [6]. Two ml of 2% $AlCl_3$ in ethanol was added to 2 ml of the test sample and the UV absorption was measured at 420 nm after one hour at room temperature. Sample solution of concentration of 0.05 mg/mL was used while quercetin concentrations of (0,01- 0,1) mg/ml were used to obtain a calibration curve. Determinations were performed in triplicates. Total flavonoid contents were obtained from the regression equation of the calibration curve of quercetin (Y=0,1085x, r^2=0,96).

vvThe stable DPPH 1,1-diphenyl-2-picryl hydrazyl radical was used for determination of free radical scavenging activity of the isolated flavonoids from the extract of *Rosmarinus officinalis* [7]. Briefly, 50 µl of methanolic solution containing the compound to be tested were added to 5 ml of a 0,004% methanolic solution of DPPH. The studied compounds were tested with MeOH as control and quercetin as antioxidant reference and absorbance at 517 nm was determined after 30 min. The absorbance (A) of the control and samples was measured and the DPPH scavenging activity in percentage was determined as follow:

$$\textbf{DPPH scavenging activity (\%)} = [(A_{control}-A_{sample})/ A_{control}] \times 100$$

The data are presented as mean of triplicate.

Results and Discussion

Total phenolic and flavonoids contents determination

The amount of total phenolic contents was 10.42mg GAE/g of dry material. This result is comparable to one obtained by Muchuweti et al. [8], who studied the content of phenolic compounds in

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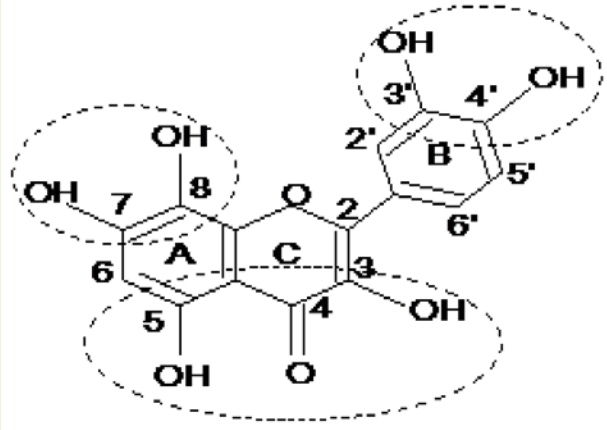

Figure 3: Phenolic and flavonoids contents in rosemary (*R. officinalis*) extract.

Zimbabwe's rosemary and obtained 10.83 mg GAE/g of dry material. The flavonoid contents in the extract of *R. officinalis* in terms of quercetin equivalent were 9.075±0.002 mg QE/g of dry material. Our experiment revealed flavonoids were present in the highest amount in the rosemary plant and this compound which contain hydroxyls is responsible for the radical scavenging effect in plants (Figure 1), [9,10].

Antioxidant activity of the isolated flavonoids

The stable free radical DPPH method was used to determine the antioxidant activity of the isolated flavonoids. This method is an easy, rapid, and sensitive way to survey the antioxidant activity of a specific compounds or plant extracts [11].

The isolated flavonoids obtained were tested for their antioxidant scavenging effects on DPPH radical and their activity was compared to quercetin standard used as antioxidant reference. The results obtained at a concentration of 5 mg/ml are given in figure 2 and expressed as the percentage of the scavenging activity of DPPH.

The result demonstrated that all the compounds tested presents a good radical scavenging activity.

The major flavonoids isolated from *R.officinalis* are quercetin and luteolin, to which are attributed many of the antioxidant properties, due to their hydrogen donation ability, and their structural requirement considered to be essential for effective radical scavenging, it has been reported that this activity may result from:

- The presence of a 3', 4'-dihydroxy, *i.e.,* an *o*–dihydroxy group (catechol structure) in the B ring, possessing electron donating properties and being a radical target.
- The 3-OH moiety of the C ring is also beneficial for the antioxidant activity of flavonoids.
- The C2-C3 double bond conjugated with a 4-keto group, which is responsible for electron delocalization from the B ring, enhances further the radical-scavenging capacity.
- The presence of both 3-OH and 5-OH groups in combination with a 4-carbonyl function and C2-C3 double bond.
- The presence of hydroxyl substituents in a catechol structure on the A-ring were able to compensate the absence of the *o*-dihydroxy structure in the B-ring, and became a larger determinate of flavonoid antiradical activity (Figure 3).

Conclusion

R.officinalis is an important source of phenolic compounds and the present study confirms the extract of this plant contains a high amount of flavonoids consisting of quercetin 3 OR, quercetin, and luteolin 7 OR. In this context, rosemary can be used as an easily accessible source of natural antioxidants in commercial food products and drugs.

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