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Research Article

Chemical composition and Antioxidant activities of the ethanolic extract *of Salvia libanotica* growing in Lebanon

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Abstract

The total phenolic and flavonoidal composition of the ethanolic extract of *salvia libanotica* was determined using the (FC) and aluminium chloride spectrophotometric methods. Quantitative HPLC was used for the identification and quantification of each phenolic compound present in the extract. Chlorgenic acid, Naringenin, quercetin and hesperetin were found to be the major phenolic compounds in the extract.

The antioxidant activity of the extract was tested using the DPPH scavenging assay. The extract has shown a strong scavenging activity with an IC_{50} = 8.73 µg/ml compared to the synthetic antioxidant BHT (IC_{50} = 91.40 µg/ml).
Key Words: *Salvia libanotica*, antioxidant activity, ethanolic extract, HPLC

1.Introduction

Salvia is the largest genus of plants that belong to the mint family, Lamiaceae, or previously named, Labiatae, which encompasses a variety of about 1000 species. It is one of several genera commonly referred as sage. The Salvia genus is distributed throughout the world, mainly in three different regions: Central and South America (housing nearly half the species), Central Asia and the Mediterranean which inhabits about 250 species, and the rest grows in Eastern Asia[1]. Many species have been used for different purposes including food, drugs, medical treatments, and decorations. However, the main use of Salvia has involved medical treatments due to some medicinal properties of a variety of its species. Such medical uses included treatments of coughs, cold, bronchial infections, stomach ache, and sore throats[2]. Increase in use of Salvia species as folk medicine in various parts of the world has sparked research interest in extracting their essential oils and studying their biological activities, which exhibited antibacterial, antioxidant, antitumor, cardioactive, antidiabetic, and anti-inflammatory activities[3,4].

Salvia libanotica is a member of the salvia family that grows in Lebanon. In addition to the biological activities caused by compounds constituting the essential oils of Salvia species and other herbs, the presence of flavonoids and phenolic compounds in such herbs greatly contributes to the biological activities of the plants. Flavonoids are polyphenolic compounds that exhibit some important properties such as inhibition of hydrolytic and oxidative enzyme and anti-inflammatory actions[5]. Studies have shown that, in addition to the biological activities mentioned above, phenolic compounds have shown anti-oxidant activities that may have therapeutic benefits.

Attributed to the presence of a plethora of S*alvia lebanotica* in Lebanon[6] (commonly known as *mariamia*), and knowing its wide use in food and in folk medicine by the people in the region, we became interested in extracting the essential oil, as well as the phenolic compounds and flavanoids of the species and studying its antimicrobial as well as antioxidant activities.

2. Materials and Methods

2.1 Plant material: *Salvia libanotica* was collected from north of Lebanon (Danyeh) authenticated by Dr. Ali Chakas, a botanist, Lebanese University, faculty of Science III, in July 2010. The sample was dried at room temperature. The plant was air dried and reduced to moderately fine powder.

2.2 Solvents and reagents: The solvent used for extraction of *Salvia* was analytical grade ethanol; while those used for the HPLC analysis were HPLC grade acetonitrile and Milli-Q (Millipore Australia Pty. Ltd) distilled water.

Aluminium chloride, potassium acetate, FC reagent, Na_2CO_3, DPPH and the standards Gallic acid, chlorogenic acid, syringic acid, vanillic acid, cafeic acid, hydroxybenzoic acid, sinapic acid, ferulic acid, p-coumaric acid, cinammic acid, myrcetin, hesperetin, quercetin, naringenin, chrysin and ellagic acid were all purchased from Sigma Aldrich (Steinheim,Germany) .

2.3 Standards: All standards were prepared as a stock in acetonitrile. Working standards were made by diluting stock solutions in acetonitrile to yield concentrations ranging between 25-125 mg/L

2.4 Sample preparation: The collected sample of *Salvia* libanotica was percolated with ethanol (70%) at room temperature for two days, filtered, then solvent dried by vacuum evaporation. The extract was kept at 20°C until usage. For HPLC analysis, 10.5 mg of the extract was dissolved in 1 mL of acetonitrile and directly injected into the HPLC.

2.5 Determination of total flavonoids: The aluminum chloride spectrophotometric method was used for the quantification of the total phenolic content of the extract as described by Hossian & Mizanur Rahman[7] with slight modifications. 0.5 ml of ethanolic extract (6 mg/ml), 0.1 ml of aluminium chloride (10%), 0.1 ml of potassium acetate (1M) and 4.3 ml of distilled water were mixed together then incubated for 30 minutes. The absorbance was measured at 415 nm and Quercetin was used to make the calibration curve .Results were expressed in mg of quercetin/ g dry sample

2.6 Determination of total phenolic compositions: Folin-Ciocalteu (FC) spectrophotometric method was used for the quantification of the total flavonoidal content of the extract based on a procedure described by Barros *et al.*[8] with slight modifications. 1.0 ml of the ethanolic extract (6 mg/ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent (1N) and diluted by 5ml of distilled water. After 5 minutes, 350 µl of 15 %Na_2CO_3 was added. The tubes were allowed to stand for 1.5 h in dark for color development. Absorbance was measured at 725nm and Gallic acid was used to make the calibration curve. Results were expressed in mg of gallic acid/ g dry sample.

2.7 Antioxidant activity of the ethanolic extract: The radical scavenging activity of the BHT and a prepared solution of the ethanolic extract (S_0 = 6 mg/ml) were tested. DPPH radical (1.75 mM) was mixed with a range of (20 µl – 60 µl) of S_0 and the total volume was adjusted to 4 ml by ethanol leading to a range of 0.048 - 0.145 mg/ml solutions of ethanolic extract. The reaction mixture was shaken and then incubated at room temperature in the dark for 45 minutes. The DPPH radical inhibition was measured at 517 nm by using a Shimadzu UV spectrophotometer. Using the same conditions, BHT was used as a reference to compare its results to those of the ethanolic extract.

2.8 HPLC Analysis: A simple and quick reversed phase method for determination of phenolic acids and flavonoids was developed. Chromatography analysis was performed with the use of liquid chromatography system, which consisted of Prominence Liquid Chromatography Shimadzu instrument with UV- Detector-SPD-20 A. The separation was carried out on Ascentis RP-Amide (15 cm x 4.6 mm ID, 5 µm particles) reversed phase column. Column temperature was maintained at 25°C. The mobile phase was a gradient elution of water containing 0.085 % orthophosphoric acid (solvent A) and acetonitrile (solvent B) at a flow rate 1 ml/min. The gradient program of solvent A in B (v/v) was as follows: 0 – 30 min 85 % A; 30 – 35 min 65 % A; 35 – 60 min 15 % A. the injection volume of all samples was 20 µl. For detection, chromatograms were monitored at 280 nm. Identification of phenolic acids and flavonoids was based on retention times in comparison with standards. The quantification was carried out using the external standard method. A Stock solution of standard compounds at concentration 1 mg/ml each was prepared in acetonitrile, and several dilutions with acetonitrile were made. The solution of standards at various concentrations (25-125 mg/L) was injected into the HPLC system and the calibration curves were established for each standard compound. The concentration of the compound was calculated from the peak area according to calibration curves. The amount of each phenolic acid and flavonoid was expressed as milligram per gram dry sample.

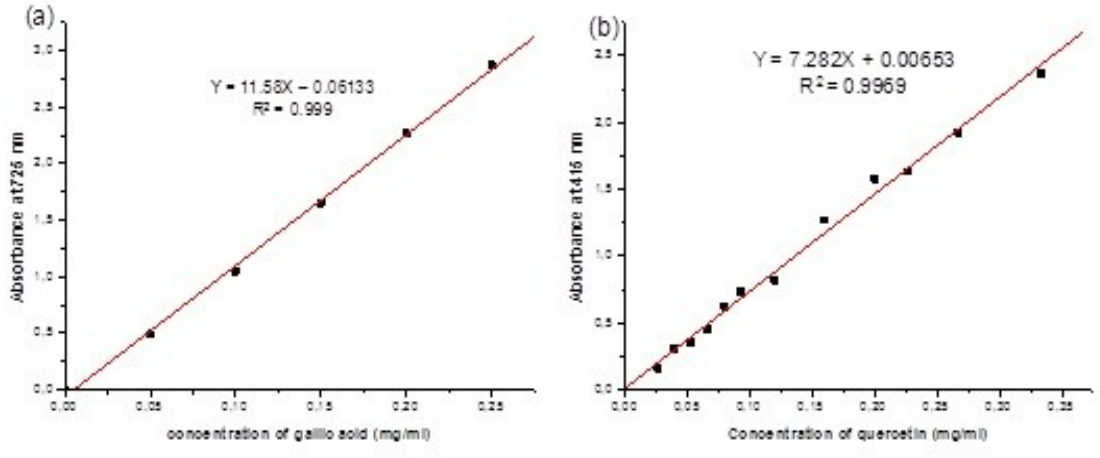
3. Results

3.1 Total phenolic and total flavonoid content: The calibration curves of gallic acid and quercetin showed linearity of the method over the concentration range analyzed with values of correlation coefficient 'R' of 0.999 and 0.9969 respectively (Figure 1). Using the calibration curves, the total phenolic content of the *Salvia libanotica* is 50.56 mg gallic acid/g extract where as its total flavonoid content was 17.4 mg quercetin/g extract (Table 1). The results show that the berries contain significant amounts of phenolic compounds including flavonoids.

Table 1: Total Phenolic and Total Flavonoidal Content of the Ethanolic Extract

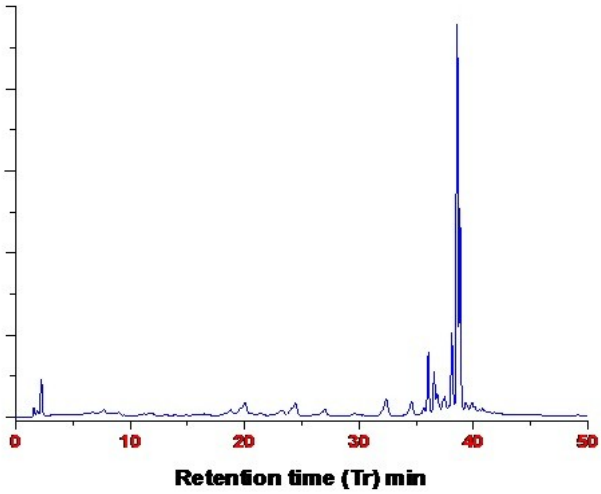
	Phenolic content	**Flavonoidal content**
Standard curve	Gallic acid	Quercetin
Absorbance	725nm	415 nm
Content/g extract	50.56 mg /g	17.4 mg/g
R^2 value	0.999	0.9969

Figure 1: Calibration curves of Gallic acid (a) and Quercetin (b)



3.2 HPLC Analysis: After extraction, the content of phenolic substances was determined by HPLC quantitative analysis. The HPLC chromatogram of *Salvia libanotica* is presented in Figure 2.

Figure 2: HPLC Chromatogram of S*alvia libanotica* ethanolic extract.



The amounts of identified phenolic compounds detected in the extract are represented in Table 2

Table 2: Chemical Composition of the Ethanolic Extract Using HPLC

Compound	mg/g of extract	Retention time
Gallic acid	0.186	1.993
Chlorogenic acid	12.98	2.29
Syringic acid	0.243	6.759
Vanilic acid	1.43	8.48
Caffeic acid	0.11	9.35
Hydroxybenzoic acid	2.95	13.222
Sinapic acid	2.95	15.096
Ferulic acid	0.35	16.507
p-coumaric acid	0.19	17.489
Cinnamic acid	0.09	30.902
Myrcetin	0.27	34.127
Hesperetin	3.11	36.321
Narnigenin	3.53	37.072
Quercetin	6.35	37.387
Ellagic acid	1.62	40.353

As indicated by Table 2, the major phenolic acids are chlorogenic acid whereas the major flavonoids are hesperetin, naringen and quercetin.

3.3 Antioxidant capacity: The degree of inhibition was calculated using the following equation:

$$\% \text{ DPPH inhibition} = (A_{\text{Blank}} - A_{\text{Sample}}) / A_{\text{Blank}} \times 100$$

The results are indicated in Figure 3 and Table 3.

Figure 3: % of DPPH Inhibition of the Ethanolic Extract

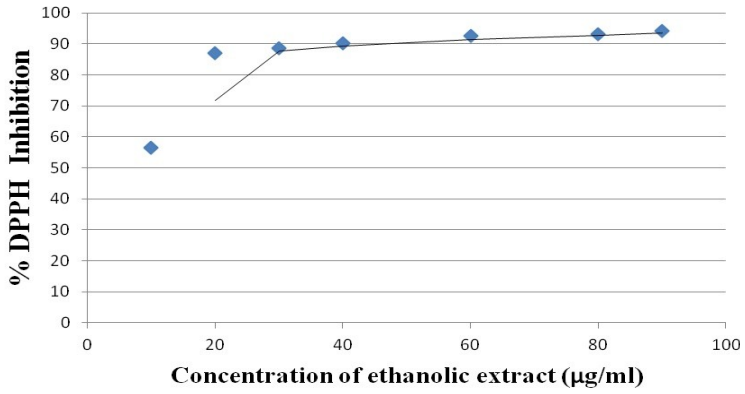


Table 3: Radical scavenging activity of ethanolic extract and BHT

Extract	IC_{50} µg/ml
Ethanolic extract	8.73
BHT	91.40

The ethanolic extract of the *Salvia libanotica* inhibited the activity of DPPH radical in a dose response relationship. In this case, the IC_{50} of the extract was 8.73 µg/mL which was significantly less than that of BHT (91.40 µg/ml).

4. Discussion

The chemical composition of the ethanolic extract of *salvia libanotica* was determined using quantitative HPLC and other spectrophotometric methods revealing the presence of significant amounts of phenolic compounds, including flavonoids, of important medicinal impact in the extract. Naringenin has an anti-inflammatory effect and is considered an immune system modulator as it reduces hepatitis C virus production by infected hepatocytes (liver cells) in cell culture[9]. Hesperetin suppresses tumor cell proliferation and bioactive hormone production making this compound a potential candidate for treatment of carcinoid cancer[10].

The DPPH radical scavenging activity of the extract has shown a remarkably better antioxidant activity of the extract than the commonly used antioxidant BHT that can be correlated to the high phenolic content of the extract. So the ethanolic extract is a better antioxidant than the commonly used synthetic BHT. This can be justified by the presence of phenolic acids and flavonoids in the extract as it was shown by the HPLC analysis results (Table 2). The antioxidant activity of some plants is mainly attributed to their Phenolic compounds that act as reducing agents[11].

This study supports the use of this herb in herbal medicines, and suggests that more work should be done on this herb as it contains several phenolic compounds of important medicinal impact and has remarkable antioxidant activity.

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