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ANALYSIS OF THE ESSENTIAL OIL OF *SALVIA BRACHYCALYX* BOISS BY NANO SCALE INJECTION AND ANTIOXIDATIVE ACTIVITY OF METHANOL EXTRACT OF THIS PLANT

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The chemical composition of the essential oil obtained from aerial parts of *Salvia brachycalyx* Boiss has been analyzed by a combination of GC and GC/MS during the flowering period. Twenty one constituents accounting to 94.9% of the total oil were identified. The major components of the oil of *Salvia brachycalyx* B. were 1,8-cineole (76.58%) and geraniol (15.08%). The methanolic extract of *Salvia brachycalyx* B. also was examined for free radical scavenging activity. Antioxidant activity was examined using 2, 2-diphenyl-1-picrylhydrazyl assay. The result indicated the free radical scavenging activity of methanol extract (M) (IC_{50}=27.2 µ g/ml) and phenol content of sample (Gallic acid equivalent=266 mg/l).

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1. Introduction

There is at present increasing interest in the dusty and in scientific research for the essential oils and various extracts of plants as sources of natural products [1]. Herbs have been used for a large range of purpose including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, charms, smoking and industrial uses. the importance of antioxidants in the maintenance of health and in protection from the damage induced by oxidative stress (implicated in the risk of chronic diseases) ,is coming to the forefront of dietary recommendations, the development of functional foods and the extraction of novel potentially therapeutic compounds from medicinal plants. Moreover antioxidants offer an effective way to prevent a variety of lifestyle-related diseases and aging that result from lipid per oxidation and active oxygen [2]. Usually synthetic antioxidants such as butyl hydroxyl anisole (BHA) and butyl hydroxyl toluene (BHT) are used to decelerate these processes because of the possible toxicities of the synthetic antioxidants, increasing attention has been directed toward natural antioxidants [3]. Salvia, the largest genus of the Lamiaceae family, includes about 900 species wide spread over the world. Fifty-eight species of the genus Salvia are found in Iran [4, 5]. Marked morphological and genetic variation was observed in the plants of this genus according to their geographical origin. Several species of Salvia are cultivated because of their aromatic nature[6] and are used as a flavor and food condiments, in cosmetics, perfumes [7]. and in folk medicine [8, 9, 10 and 11]. In this paper, we report the chemical composition and antioxidant activity of the methanolic extract of Salvia *brachycalyx boiss.* from Iran . The chemical composition of the essential oil was evaluated by using GS–MS analysis. The antioxidant property of methanolic extract of the plant

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was determined by using the 1, 1- diphenyl-2-picrylhydrazyl (DPPH) assay. Furthermore, the phenol content is evaluated.

2. Experimental

Plant material

Aerial parts of the plant were collected from the Zagruse Mountain in Lorestan state in south west of Iran in June 2008 at flowering stage. The voucher specimen was deposited at the Herbarium of the Department of Biology, Lorestan University.

Isolation of the essential oil

Air-dried plant material (100g) was hydro-distilled for 4 hr using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulfate, and then, was kept in a sealed vial at 4 ºC until analysis.

Preparation of the methanolic extract

The air-dried and finely ground samples were extracted by using the method described elsewhere [12]. Briefly, the sample, weighing about 150 g, was extracted in a Soxhlet apparatus with methanol at about 60 °C for 12 h. The extract was then filtered and concentrated in vacuo at 45 °C, yielding a waxy material .3.87%, w/w. Finally, the extracts were then lyophilized and kept in the dark at 4 °C until tested.

GC–MS analysis conditions

The oil was analyzed by GC/MS using a gas chromatography analysis. GC analysis of the oil was conducted using a Varian CP-3800 instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm id., film thickness 0.25 μm). Nitrogen was used as the carrier gas at the constant flow of 1 mL/min. The oven temperature was kept at 60°C for 1 min, then programmed to 250°C at a rate of 4°C/min, and kept constant at 250°C for 10 min. The injector and detector (FID) temperatures were kept at 250°C and 280°C, respectively. The amount of the sample injected was 1.0 nL (diluted 1.0 μL of sample in 1000 ml of n-pentane, v/v) in the splitless mode [13]. GC-MS instrument equipped with a DB-1 fused silica column (60 m× 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from 40^{o}C to 250^{o}C at a rate of 4^{o}C/min, and then held at 250^{o}C for 10 min. with the transfer line temperature adjusted at 250^{o}C. The flow rate of helium as carrier gas was 1.1 mL/min. split ratio was, 1/50. The quadrupole mass spectrometer was scanned over the 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA. The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions for Identification of individual n-alkanes (C_6–C_{24}) and the oil on DB-1 compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds or with those of reported in the literature [1]. Quantitative data was obtained from FID area percentages without the use of correction factors. . The list of compounds identified in the oil of *S. brachycalyx* can be seen in Table 1.

Table 1. Percentage composition of the oil of the aerial parts of Salvia brachycalyx from south west of Iran

Compound[a]	RI[b]	%[c]
α-pinene	933	0.78
myrcene	974	0.08
p-cymene	1014	0.16
1,8-cineole	1024	76.58
linalool	1082	0.18
α-pinene epoxy	1175	0.05
nerol	1208	0.06
citronellol	1216	0.13
geraniol	1235	15.08
citral	1244	0.18
Formate geranyl	1280	0.11
Geranyl acetate	1357	0.06
β-cubebene	1389	0.06
γ-muurolene	1480	0.09
Buthyl hydroxyl toluene	1492	0.33
pentadecane	1496	0.08
Spathulenol	1570	0.2
caryophylleneoxide	1579	0.11
Geranyl pentanate	1584	0.09
Benzyl benzoate	1733	0.45
Palmitic acid	1943	0.06
Total		94.9

[a] RI(retention index) measured relative to n-alkanes (C_6–C_{24}) on the DB-1 capillary column
[b]. Compounds listed in order of their RI
[c] %, Relative percentage obtained from peak area

Antioxidant activity: Free Radical Scavenging Capacity (RSC)

RSC was evaluated by measuring the scavenging activity of examined extract on the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical . The DPPH assay was carried out as described elsewhere [14]. Various concentrations of the sample were mixed with 1 ml of 90 μM DPPH. Solution and filled up with 95% methanol to a final volume of 4 ml. After a 60-min incubation period at room temperature. The absorbance of the resulting solutions and blank (with same chemicals, except for the sample) were recorded against tert-butylated hydroxy toluene (BHT) as positive control. Three replicates of sample were recorded. The disappearance of DPPH was measured spectrophotometrically at 517 nm on a Shimadzu 2501UV spectrophotometer.The percentage of RSC was calculated in following way:

$$RSC(\%) = 100\times (A_{blank} - A_{sample}) / A_{blank}$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

The IC_{50} value, which represented the concentrations of the essential oil and extracts that caused 50% inhibition, was determined by linear regression analysis from the obtained RSC values.

Assay for phenolic content

The amount of phenolic content in the herb extract was determined with the Folin-Ciocalteu reagent according to the method of slinkard and slingleton using gallic acid as standard [15-18]. Twenty microliters of extract solution was taken in cuvette, then 1.58 ml of distilled water and 100 µl of Folin-Ciocalteu reagent were added, and cuvette was shaken thoroughly. After 3 min, 300 µl of the sodium carbonate solution (7% w/v) was added, and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm.

Table 2. Effects of S. brachycalyx Boiss methanol extract and positive control on the in vitro free radical (DPPH) assay

Extract	Gallic acid Equivalent(mg/l)[a]	IC_{50} (µg/ml)
Methanol extract	266.2 ± 3.1	27.2±0.3
(BHT) control	19.8± 0.5	26.5±1.0

[a]Result is given as mean ± S.D. of three different experiments.

3. Results and discussion

Chemical composition of the essential oil

The results of the GC/MS analysis of the oil of aerial parts of *S. brachycalyx* (at flowering) are listed in Table I. Twenty-one compounds were identified representing 94.9 % of total oil. The major components of the oil were 1,8-cineole (76.58%), geraniol (15.08%) and α-pinene (0.78%). Thus the oil consisted mainly of oxygenated monoterpenes (92.52%), hydro- carbonated monoterpenes (1.02%) and sesquiterpenes (0.83%) .Comparing these results with previous studies on Salvia species revealed that in contrast to the oil of *S. nethiopis*, S. hypoleuca and *S. hydrangea* [19, 20]. In S. brachycalyx oil, monoterpenes predominated over sesquiterpenes, the same as *S. multicaulis* and *S. sahendica* oil [21].

Antioxidant activity

The antioxidative capacity of *S. brachycalyx* methanolic extract was determined by comparing with the activity of BHT as antioxidant. Free radical scavenging capacity of the extract, measured by DPPH assay. The result indicated the free radical scavenging activity of methanol extract (IC_{50}=27.2 µ g/ml**)**. Effect of *S. brachycalyx* methanol extract and positive control (BHT) on the in vitro free radical (DPPH) are given in (Table 2).

Amount of Phenolic content

Typical Phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids [22]. Phenolic acids have been repeatedly implicated as natural antioxidants in fruits, vegetables, and other plants. Amount of Phenolic content based on the absorbance value the extract solution ,reacting with Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents, as described above,the phenolic content of methanol extract was high (266.2 ± 3.1 mg/l) as measured by gallic acid test (Table 2) .

In conclusion, we have determined the chemicalcomposition of the essential oil of *S. brachycalyx* Boiss, and have evaluated its antioxidative activity of methanol extract. The results reported here can be considered as the first information on antioxidant property of *S. brachycalyx*. The molecular mechanism of radical scavenging activity of methanol extract of *S. brachycalyx*

could be attributed to the presence of polyphenolic compounds. It has already been shown that polyphenolic compounds were responsible for radical scavenging activity in lamiacea family due to ease of their hydrogen atom donation to active free radical [23].

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