شناسایی ترکیبات و بررسی فعالیت آنتی اکسیدانی اسانس دو جمعیت نوروزک ایرانی (تیره نعنائیان)

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چکیده. گیاه نوروزک (*Salvia leriifolia*) گونه ای از تیره نعنائیان است که بومی ایران و افغانستان بوده و کاربردهای متعددی در صنایع پزشکی، دارویی و غذایی دارد. هدف از این مطالعه بررسی ترکیبات تشکیل دهنده و خاصیت آنتی اکسیدانی اسانس نوروزک بود که به صورت خودرو در نیشابور و بجستان (استان خراسان رضوی، شمال شرق ایران) می روید. استخراج اسانس از بخش‌های هوایی این گیاه به روش تبخیر با بخار آب انجام و اسانس حاصل به روش GC/MS آنالیز شد. با توجه به نتایج، ۱و۸ سینئول (۲۰/۲۴٪ و ۲۶/۳۹٪) ، آلفاپینن (۱۵/۱۴٪ و ۱۴/۳۹٪) و بتاپینن (۲۴/۳۳٪ و ۲۶/۳۹٪) به ترتیب ترکیبات عمده اسانس این گیاه در منطقه بجستان و نیشابور بودند. فعالیت آنتی اکسیدانی اسانس به سه روش مختلف DPPH، TBARSو BCB مورد بررسی قرار گرفت. در همه سنجش‌ها اسانس کل و سه استاندارد (آلفاپینن، بتاپینن و ۱و۸ سینئول) اثر آنتی اکسیدانی معنی داری ($p<0.01$) را نشان دادند. فعالیت آنتی اکسیدانی به دست آمده در روش های TBARS و BCB بیش تر از میزان اندازه گیری شده با روش DPPH بود. علاوه بر این، سنجش BCB به عنوان مناسب ترین روش برای اندازه گیری فعالیت آنتی اکسیدانی اسانس معرفی شد. اسانس گیاهان رشد یافته در منطقه نیشابور، دارای فعالیت آنتی اکسیدانی بیش‌تری در مقایسه با اسانس گیاهان منطقه بجستان بود.

واژه‌های کلیدی. اسید تیوباربیتوریک، رنگ بری بتاکاروتن، روغن های فرار، سالویا، لاش خواری رادیکال

Essential oil (EO) composition and antioxidant activity of two *Salvia leriifolia* Benth. (Lamiaceae) populations from Iran

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Abstract. *Salvia leriifolia* Benth. from Lamiaceae family is a plant native to Iran and Afghanistan with significant applications in medical, pharmaceutical and food industries. The aim of current investigation was to evaluate the composition and antioxidant activity of essential oils (EOs) of *S. leriifolia* growing naturally in Neyshabur and Bajestan (Northeast of Iran). The aerial parts of the plant were subjected to hydro-distillation and the EOs were analyzed with GC/MS. According to the results, 1,8-cineole (20.24%, 26.39%), α-pinene (15.14%, 14.39%) and β-pinene (24.33%, 26.01%) were the main constituents of the EOs of the plant populations in Bajestan and Neyshabur, respectively. Antioxidant activity of the EOs was measured by three different methods, 2,2´-diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid reactive species (TBARS) and β-carotene bleaching (BCB). The total EO and three standards (α-pinene, β-pinene and 1,8-cineole) displayed a significant antioxidant effect in all assays. Antioxidant activities obtained by means of TBARS and BCB methods were higher than those measured by the DPPH assay. Moreover, BCB was proved to be the most appropriate method for measurement of antioxidant activity. The EOs of the plants grown in Neyshabur showed stronger antioxidant effects in comparison with those grown in Bajestan.

Keywords. BCB, DPPH, sage, TBARS, volatile oil

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INTRODUCTION

Oxidation of biomolecules with free radicals could lead to cell injury and death (Wang *et al*., 2007). Reactive oxygen species oxidize lipids containing polyunsaturated fatty acids readily. Coronary heart disease, aging, stroke, Parkinson, multiple sclerosis, carcinogenesis and Alzheimer are results of lipid peroxidation. An increasing investigations have been done to find anti-oxidative drugs which participate as radical scavengers in living organisms (Emami *et al*., 2007). Widely-used synthetic antioxidants in food products have been known to cause negative health effects. Studies on natural compounds as potential antioxidants have been of great interests for specialists (Fasseasa *et al*., 2007). Fruits, vegetables, nuts and whole grains have been studied in recent years as sources of natural plant antioxidants (Kulisic *et al*., 2004; Wang *et al*., 2007; Bohn *et al*., 2010). Essential oils (EOs) from various aromatic plants have been identified as strong natural antioxidants and a lot of studies on their antioxidant properties have been repeate- dly reported so far (Ruberto & Baratta, 2000; Kulisic *et al*., 2004; Kelen & Tepe, 2008; Okoh *et al*., 2011; Rowshan & Bejeli, 2013).

The EOs of some genera of the Lamiaceae family are potential candidates for exhibiting antioxidant and radical-scavenging activities (Emami *et al*., 2007). Numerous species of *Salvia* L. have been used in folk medicine for their wide variety of pharmacological properties. They were subjected to extensive investigations for identification of the biologically active compound (Bozan *et al*., 2002).

The *S. officinalis* L. EOs exhibited remarkable antioxidant activity (IC_{50} = 7.70 ± 0.90 µg/ml) (Bouaziz *et al*., 2009). The EO of *S. tomentosa* Miller was particularly found to possess strong antioxidant activity (Tepe *et al*., 2005). EOs of three different *Salvia* species (*S. aucheri* Benth. var. *aucheri*, *S. aramiensis* Rech.f and *S. pilifera* Montbret & Aucher) were screened for their possible antioxidant activity. Antioxidant activity of *S. aramiensis* was found to be higher than others (Kelen & Tepe, 2008).

Salvia leriifolia Benth. is a perennial herbaceous plant which is native to Razavi Khorassan and Semnan provinces of Iran. This plant has different vernacular names such as Norouzak and Jobleh. *Salvia leriifolia* has different pharmacological activities such as anticonvulsant (Hosseinzadeh & Arabsanavi, 2001), anti-ischemia (Hosseinzadeh *et al*., 2007), anti-inflammatory (Hosseinzadeh & Yavary, 1999; Hosseinzadeh *et al*., 2003) and antinociceptive (Hosseinzadeh *et al*., 2003), antioxidant (Farhoosh *et al*., 2004), antibacterial (Habibi *et al*., 2000) and antiulcer effects (Hosseinzadeh *et al*., 2000). Little information is available on the antioxidative nature of its EO for treatment of Alzheimer and acetyl-cholinesterase inhibitory. This plant has been introduced as an herbal medicine in toxicological and clinical trial evaluations (Hosseinzadeh *et al*., 2009; Savelev *et al*., 2004; Loizzo *et al*., 2009). An attempt was made in this study to identify the composition and to examine the antioxidant activity of *S. leriifolia* EOs in its flowering stage. In order to overcome possible methodology limitations, three different assay methods were employed, i.e. 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging me-thod, the thiobarbituric acid reactive species (TBARS) assay and the β-carotene bleaching (BCB) test.

MATERIAL AND METHODS

Mass spectrophotometric (GC-MS) analysis Plant material

Salvia leriifolia leaves were collected from the Bajestan and Neyshabur regions, Northeast of Iran, during the flowering stage. The specimen was identified by Mohammadreza Joharchi (FUMH) and deposited by the voucher number of A.A.Basiri 12835. The dried leaves were powdered. Three analytical standards (α-pinene, β-pinene and 1,8-cineole) were purchased from Sigma-Aldrich, USA.

Isolation of the essential oils

The powdered aerial parts of *S. leriifolia* L. (300 g) were subjected to hydro-distillation using a Clevenger-type apparatus for 3 hours. After dehydration by means of anhydrous sodium sulfate, the slightly yellow-colored oil was obtained and stored at -20°C prior to GC/MS and antioxidant analysis.

Gas chromatography-mass spectrometry

The GC-MS analyses were performed using an Agilent 5975 apparatus with HP-5ms column, interfaced with a quadruple mass detector and a computer equipped with Wiley 7 n.l library.

The constituents of the oil were identified by calculation of their retention indices under programmed temperature conditions for n-alkanes (C8-C20) and the oil on a CP-Sil 8CB column. The individual compounds were identified by comparing their mass spectra and retention indices (RI) with those of authentic samples and those being given in literatures (Adams, 2007).

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Determination of antioxidant activity by means of the DPPH radical scavenging method

Hydrogen atoms or electrons donation ability of the corresponding oils was measured by the bleaching of purple colored methanol solution of DPPH. Fifty microliter of various concentrations (0.1, 0.5, 1, 2, 4, 8, 16 µl/ml) of the EOs and three main components of the EO, as well as quercetin and ascorbic acid in methanol, was added to 2.5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I%) was calculated in following way:

I%: (Ablank _ Asample/Ablank) × 100

Determination of antioxidant activity with TBARS assay

A modified TBARS assay was used to measure the potential antioxidant capacity using homogenized egg yolk as lipid rich media. 0.5 ml of 10% (w/v) tissue homogenate and 0.1 ml of sample solutions to be tested in methanol. 0.05 ml 2,2′-azobis (2-amidinopropane) dihydrochloride solution (0.07 M) in water was added to induce lipid peroxidation. 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml 0.8% (w/v) thiobarbituric acid in 1.1% (w/v) sodium dodecyl sulfate solution was added and the resulting mixture vortexed, and then heated at 95°C for 60 min. After cooling, 5.0 ml of butanol was added to each tube, then extensively vortexed and centrifuged at 1200g for 10 min. The experiment was carried in triplicate. The absorbance of the organic upper layer was measured using a Shimadzu UV-3100 scanning spectrophotometer, set at 532 nm. All the values were based on the percentage antioxidant index (AI ٪):

AI% = (1- T/C) × 100

Where C is the absorbance value of the fully oxidized control and T is the absorbance of the test sample. Vitamin E and butylated hydroxyl toluene (BHT) were used as positive controls (Kulisic *et al*., 2004).

Determination of antioxidant activity with BCB test

Antioxidant activity of the *S. leriifolia* EO compounds was determined according to a slightly modified version of the β-carotene bleaching method. 0.5 mg of β-carotene in 1 ml of chloroform was added to 25 µl of linoleic acid and 200 mg of Tween 40 emulsifier mixture. After the evaporation of chloroform under vacuum, 50 ml of oxygenated distilled water was added and the mixture was sonicated with RPMI-1640 for 1 minute. Five milliliter of this mixture were transferred into deferent test tubes (200 µl) containing different concentrations of the sample (concentrations of stock solutions were 0.1, 0.5, 1.0, 2.0, 3.0 and 4.0 g/l). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. The emulsion system was incubated for 2 h at 50 °C and measured six time interval at 4 g/l. All determinations were performed in triplicate. The percentage inhibition was calculated from the data with the slightly modified formula:

$$\%\text{inhibition} = [\ (A_A(120) - A_C(120)) / (\ A_C(0) - A_C(120))]$$

Where AA (120) is the absorbance of the antioxidant at t=120 min, AC (120) is the absorbance of the control at t=120 min, and AC (0) is the absorbance of the control at t=0 min. BHT was used as positive control (Ozkan & Erdogan, 2011).

Statistical analysis

The data were analyzed statistically using INSTAT 3.0 software. The significant level was ascertained by one way analysis of variance (ANOVA), followed by Tukey multiple comparison test. Results were experienced as means ± SD and P values of <0.001 were considered as significant. Graphs were drawn by Graph Pad Prism3.0 and Microsoft Excel.

RESULTS AND DISCUSSION

Chemical composition of *S. leriifolia* essential oil

The dried *S. leriifolia* leaves yielded 0.6% (v/w) of EO. The constituents of the EO's were listed in Table 1. Thirthy nine compounds (96.95% of the EO), and thirthy five compounds (93.77% of the EO) were identified in the EO of *S. leriifolia* collected from Neyshabur and Bajestan. The fundamental compounds in the EO's were monoterpene hydrocarbons. The major constituent of Neyshabur and Bajestan *S. leriifolia* oils were α-pinene (14.39, 15.14%), β-pinene (26.01, 24.33%) and 1,8-cineol (eucalyptol) (26.39, 20.24%), respectively. These EO's contained (77.17, 70.66%) monoterpenes and (19.78, 23.11%) sesquiterpenes, respectively.

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It is certainly true that there are little differences in the chemical composition and main component of *S. leriifolia* EO among two localities. The different climatic conditions have a negligible impact on the chemical composition of the EO's. However, a number of compounds like nopinone, verbenone, β-copaene, α-calacorene and neryl isovalerate were not identified in Bajestan EO (Table 1). Hosseinzadeh *et al*., (2009) noted that β-pinene (31.5%), 1.8 cineole (24.7%) and α-pinene (17.5%) were the main components in the EO's of *S. leriifolia* plants grown in the southern regions of Khorassan and Semnan provinces.

Their finding is extremely similar to the results of the present study. In addition, Monfared and Ghorbanli (2009) recorded 1,8-cineole (20.04%), camphor (18.84%), α-pinene (16.49%) and camphene (10.94%) as the main constituents in the EO of plants grown in Bardaskan, Kashmar, Iran (Monfared & Ghorbanli, 2009). According to another study carried out by Loizzo *et al*. (2009), camphor (10.5%), 1, 8-cineol (8.6%), camphene (6.2%) and α-pinene (4.7%) were the main components of *S. leriifolia* EO from the southern regions of Khorassan (Loizzo *et al*., 2009).

These findings are not in agreement with the results presen-ted here. The changes in the EO compositions mi-ght be the consequence of several different aspe-cts including climatic, seasonal, geographical, and geological conditions (Perry *et al*., 1999).

Antioxidant activity

DPPH radical scavenging method

In the DPPH assay, the values of 17.8% and 37.2% were determined as free radical scavenging activity of *S. leriifolia* EO in Bajestan and Neyshabur, respectively (Fig.1), whereas the values of 3.1%, 11.6% and 2% were estimated for 1,8-cineole, α-pinene, β-pinene, respectively (in 16 µl/ml) (Fig. 2). The samples were less effective in comparison with ascorbic acid (95.2%) and quercetine (94.2%) as synthetic antioxidant agents. Higher antioxidant activity of *S. leriifolia* EO collected from Neyshabur (31.6%) than EO of plants grown in Bajestan (24.1%) might be partially due to the more amounts of oxygenated monoterpenes, which are strong antioxidant compounds (Ruberto & Baratta, 2000).

Thiobarbituric acid reactive species assay

As shown in Figures 3 and 4, at the concentration of 40 mg/ml in TBARS test, antioxidant activity of *S. leriifolia* oil from Bajestan was 59.2%. It was 57.8% for plants grown in Neyshabur. The values of 35.5%, 17.2% and 35.5% were measured as antioxidant indices for 1, 8-cineol, α-pinene and β-pinene, respectively. EOs exhibited almost the same antioxidant index. All the samples showed that less antioxidant activity as compared to vitamin E (91.5%) and BHT (71.24%) with the same concentration.

β-carotene bleaching method

Salvia leriifolia EO from Bajestan (48.7%) and Neyshabur (52.7%) and their major components including 1,8-cineole (32.2%), α-pinene (36.9%), β-pinene (38.8%) bleached β-carotene at the concentration of 4 mg/ml (Figs. 5 and 6) in BCB. Absorbance of β-carotene in the presence of total EO, as well as its constituents and positive control (sample with no antioxidant), showed a gradual decrease (Fig. 7).

A descending order in bleaching rate can be demonstrated as follows: 1,8cineole >β-pinene>α-pinene>EO of plants harvested from Bajestan>EO of plants grown in Neyshabur > BHT. *Salvia* species have been known as potent natural antioxidants (Rowshan & Bejeli, 2013). Antioxidant activities for the extracts of various *Salvia* species have been described so far. (Tepe *et al*., 2004, 2005).

Moreover, the anti-oxidant activities of EOs belonging to different *Salvia* species such as *S. officinalis* (IC_{50} values 22 mg/ml), *S. aramienesis* (IC_{50} values 12.26 mg/ml), *S. aucheri* (IC_{50} values 0.018 mg/ml) and *S. pilifera* (IC_{50} values 0.024 mg/ml) have been demonstrated by DPPH assay and for *S. microphylla* (IC_{50} values 0.77 mg/ml) have been measured by BCB test (Lima *et al*., 2012; Bouajaj *et al*., 2013; Kose *et al*., 2013). The EO of *S. eremophila* was almost inactive in DPPH and acting weakly in BCB test (Ebrahimabadi *et al*., 2010).

A number of studies on the antioxidant activity of *S. leriifolia* extract have been carried out (Farhoosh *et al*., 2004; Hosseinzadeh *et al*., 2009; Loizzo *et al*., 2010). However, there is only one study on the antioxidant activity of its EO.

According to a study executed by Loizzo *et al*. (2009), *S. leriifolia* oil exhibited a promising antioxidant activity by DPPH assay with an IC_{50} 2.26 µl/ml.

Table 1. Chemical composition (%) of the essential oil of *S. leriifolia* leaves, collected from Neyshabur & Bajestan

Compound	[a]RI	[b]RI	EO (N) %	EO (B) %
α-Thujene	932	930	0.65	0.75
α-Pinene	941	939	14.39	15.14
Camphene	957	954	1.46	3.3
Sabinene	979	975	0.38	0.79
β-Pinene	987	979	26.01	24.33
δ-3-Carene	1015	1011	1.1	1.25
1,8-Cineole	1039	1031	26.39	20.24
γ-Terpinene	1062	1059	1.08	0.83
Terpinolene	1091	1088	0.48	0.42
Linalool	1098	1090	0.1	0.12
Nopinone	1142	1140	0.1	—
Camphor	1150	1146	0.14	0.12
Pinocarvone	1167	1164	0.7	0.13
δ-Terpineol	1169	1166	0.7	0.73
Terpinen-4-ol	1181	1177	1.17	1.13
α-Terpineol	1193	1188	1.54	1.18
Myrtenol	1199	1195	0.58	0.31
α-Cubebene	1356	1348	0.23	0.26
α-Copaene	1385	1376	0.36	0.41
β-Cubebene	1396	1388	0.15	0.31
α-Gurjunene	1421	1409	1.36	1.8
β-Caryophyllene	1432	1419	1.18	1.51
β-Copaene	1440	1432	0.11	—
Aromadendrene	1451	1441	0.32	0.39
α-Humulene	1466	1454	0.33	0.41
Allo-Aromadendrene	1473	1460	0.22	0.28
γ-Muurolene	1483	1479	0.79	0.95
Germacrone-D	1491	1485	0.19	0.49
β-Selinene	1497	1490	0.59	0.49
α-Muurolene	1505	1500	1.3	1.71
γ-Cadinene	1522	1513	1.45	1.84
δ-Cadinene	1530	1523	4.83	5.49
α-Calacorene	1552	1545	0.05	—
Germacrone-D-4-ol	1590	1575	0.99	1.4
Caryophyllene oxide	1596	1583	0.28	0.46
Neryl isovalerate	1600	1583	0.17	—
Viridiflorol	1623	1592	0.99	1.21
α-Muurolol	1660	1646	1.16	1.21
α-Cadinol	1674	1654	2.93	2.65
Grouped compounds				
Monoterpene hydrocarbons			45.69	47.1
Oxygenated monoterpenes			31.48	23.56
Sesquiterpene hydrocarbons			13.26	16.18
Oxygenated sesquiterpenes			6.52	6.93
Total			96.95	93.77

[a]RI: The retention index calculated from retention times relative to C8-C20 n-alkanes on a CP-Sil 8 CB column.
[b]RI: The retention index from reference data (Adams, 2007).
N: Neyshabur; B: Bajestan

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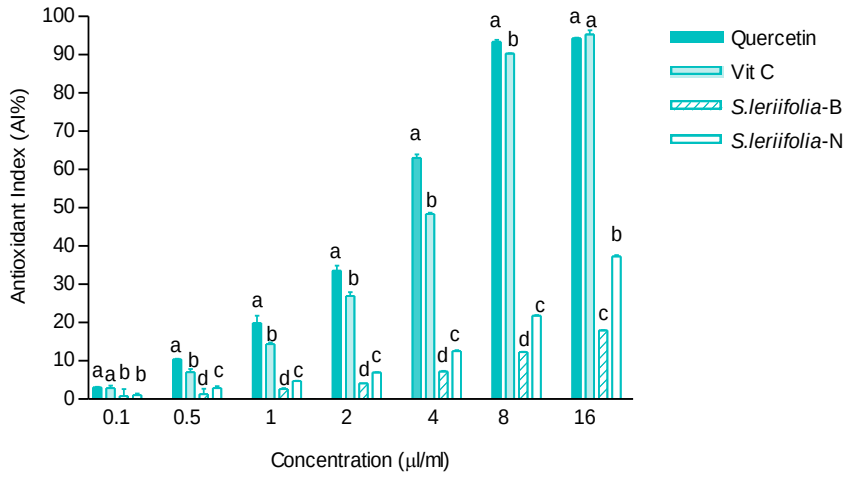


Fig. 1. Free radical-scavenging activity of *S. leriifolia* essential oil collected from Neyshabur (N) and Bajestan (B) in DPPH assay.

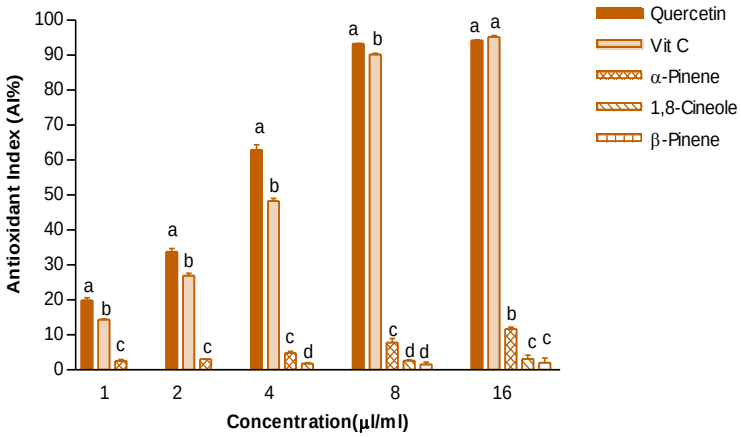


Fig. 2. Free radical-scavenging activity of α-pinene, β-pinene and 1,8-cineole in DPPH assay.

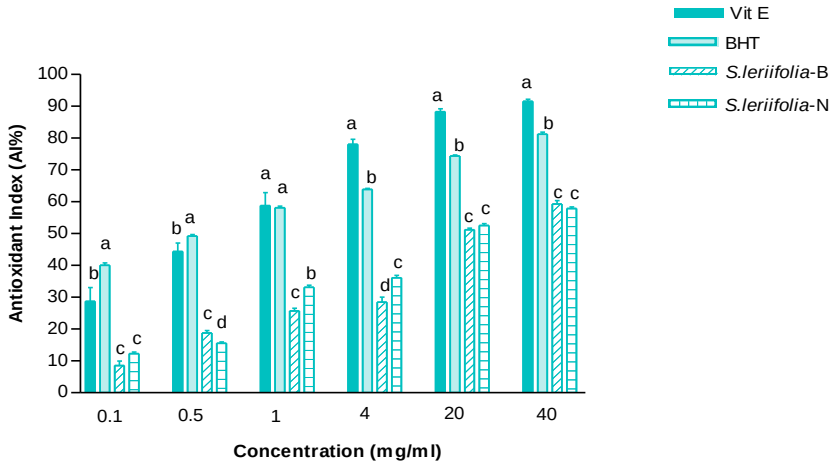


Fig. 3. Antioxidant activity of the *S. leriifolia* essential oil collected from Neyshabur (N) and Bajestan (B) in TBARS test.

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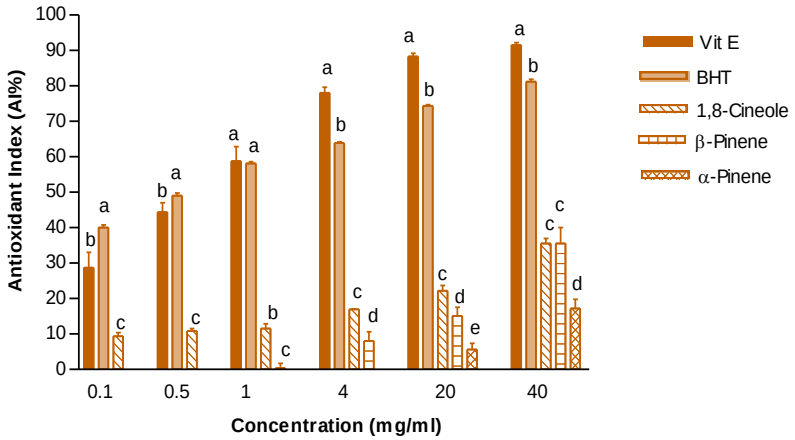


Fig. 4. Antioxidant activity of the of α-pinene, β-pinene and 1,8-cineole in TBARS test.

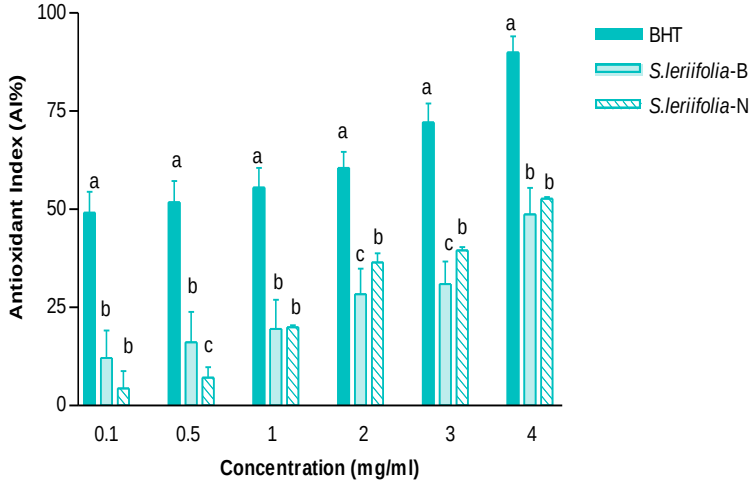


Fig. 5. Antioxidant activity of *S. leriifolia* essential oil collected from Neyshabur (N) and Bajestan (B) in BCB test.

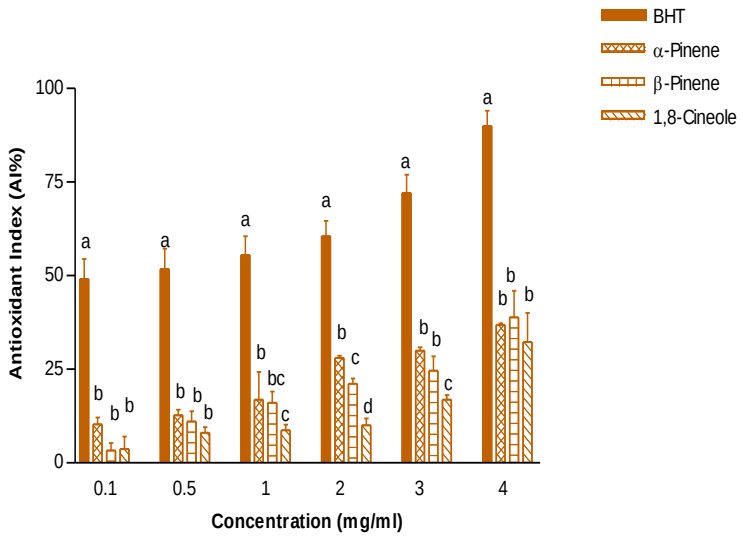


Fig. 6. Antioxidant activity of α-pinene, β-pinene and 1,8-cineole in BCB test.

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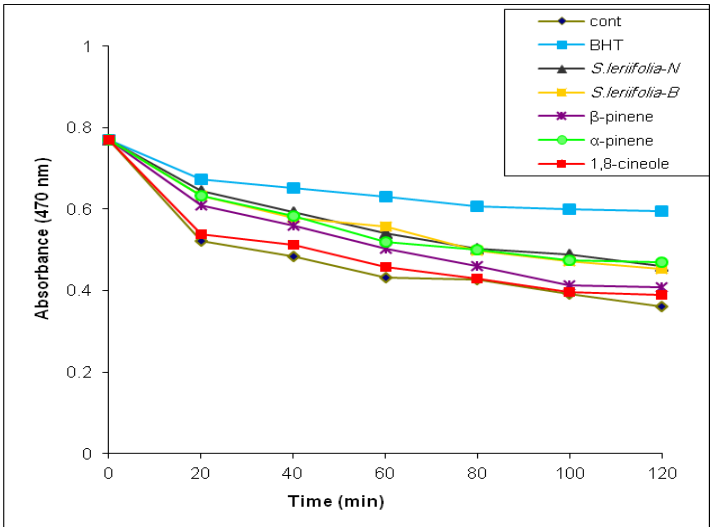


Fig. 7. Rate of β-carotene bleaching by essential oil extracted from *S.leriifolia* plants grown in Neyshabur and Bajestan compared with control, BHT, α-pinene, β-pinene and 1,8-cineole at 4 mg/ml concentration.

On the contrary, the EO of *S. leriifolia* was almost inactive in DPPH test of the current study and an inhibition percentage of less than 38% was recorded for the oil concentrations up to 16 µl/ml. EO's generally have low solubility in DPPH method; therefore, the DPPH test could not be reliable for the measurement of the antioxidant activities of such materials (Lima *et al*., 2012). In this survey, however, the EO's from *S. leriifolia* in Bajestan and Neyshabur had IC50 values of 5.7 and 2.7 mg/ml in BCB test and 18.9 and 15.2 mg/ml in TBARS assay. This study and the aforementioned ones confirm the presence of moderate to good antioxidant potentials for the EO's of the *Salvia*.

The EO of *S. leriifolia* contains some active components such as 1,8-cineole, α-pinene and β-pinene, which have been reported to exhibit an antioxidant activity. In general, *S. leriifolia* EO showed higher activity than its components in the three systems. It is very difficult to ascribe the antioxidant power of a total EO to one or some active fractions, which could be due to the fact that an EO always contains a mixture of different chemical components. Not only major but minor compounds also may make significant contributions to the oil activity (Wang *et al*., 2007).

α-Pinene and β-pinene are in monoterpene hydrocarbons classes. Presence of strongly activated methylene groups in these molecules probably accounts for their antioxidant behavior (Giweli *et al*., 2012). However, the relative high activity of the aforesaid monoterpenes is also confirmed by the TBARS assay. 1, 8- cineole is classified as an oxygenated monoterpene. Many examples of different functional compounds (alcohols, aldehydes, ketones, ethers, etc.) belong to this group. The antioxidant activity of these compounds depends on the presence of particular molecular moieties. Alcohols are the most active materials. Ethers (such as 1,8-cineole, the monoterpene cyclic ether) has less antioxidant activity (Ruberto & Baratta, 2000).

Since the specificity and sensitivity are different for each method used, application of an analytical eclectic method is ideal to evaluate the effectiveness of antioxidants accurately (Kelen & Tepe, 2008). This study also suggested that a single assay might not be sufficient to estimate the antioxidant activity of a plant extract or an EO sample. The co-application of three methods was turned out to be a good technique for evaluation of the antioxidant activity of *S. leriifolia* EO and could be recommended for similar investigations. In addition, the multi-concentration measurements provide a more comprehensive picture of a plant EO antioxidant activity in general.

In conclusion, the measured antioxidant power depends on the method employed and the concentration, intrinsic nature and physico- chemical properties of the materials studied (Kulisic *et al*., 2004; Ruberto & Baratta, 2000).

It was also confirmed that the exhibition of antioxidant activities of a single EO specimen may differ according to its concentration and the type of antioxidant assay (Kelen & Tepe, 2008).

DPPH and TBARS similarly allow testing of both hydrophilic and lipophilic substances (Magalhaes *et al*., 2008).

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Moreover, both model systems, BCB and TB-ARS, should be considered important since they allow us to follow the primary or secondary steps of the oxidative process.

It is necessary to assess antioxidant effectiveness in model systems dealing with the primary and secondary steps of lipid oxidation especially when food quality is under investigation (Ruberto & Baratta, 2000).

CONCLUSION

From the results above we could infer that the antioxidant activity of *S. leriifolia* EO is the cooperating result of its composition. In the extracted EO's, oxygenated monoterpenes and monoterpene hydrocarbons are mainly responsible for its antioxidant potential. The oxygenated monoterpens were found out to be the main components of *S. leriifolia* EO. The result could be of interest to those in charge of food industries in finding the possible alternatives to synthetic preservatives. In this context, *S. leriifolia* EO showed interesting results, being one of the best functioning antioxidants in terms of neutralizing the free radicals.

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