Antioxidant activity and total phenolic and flavonoid content of the extract and chemical composition of the essential oil of *Eremostachys laciniata* collected from Zagros

Iman Bajalan¹*, Masoumeh Zand², Masoud Goodarzi³, Mahin Darabi⁴

¹Young Researchers and Elite Club, Borujerd Branch, Islamic Azad University, Borujerd, Iran
²Department of Agriculture, College of Agriculture, Borujerd Branch, Islamic Azad University, Borujerd, Iran
³Department of Biology, Borujerd Branch, Islamic Azad University, Borujerd, Iran
⁴Department of Chemistry, Faculty of Basic Science, Borujerd Branch, Islamic Azad University, Borujerd, Iran

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**Objective:** To examine the chemical component of the essential oil of *Eremostachys laciniata* and evaluate antioxidant activity of the extract.

**Methods:** The hydrodistilled essential oil was analyzed by gas chromatography-flame ionization detector and gas chromatography–mass spectrometry. Also, antioxidant activity, total phenolic and flavonoid content were determined by 2,2-diphenyl-1-picrylhydrazyl, Folin-Ciocalteu and colorimetric method, respectively.

**Results:** The major components of the essential oil were *p*-cymene (21.64%), linalool (17.93%), and *α*-pinene (11.42%). Also, the extract obtained by methanol showed a good antioxidant activity. The same extract also exhibited high phenolic and flavonoid contents.

**Conclusions:** These results indicate that *Eremostachys laciniata* can be used in dietary applications with a potential to reduce oxidative stress.

**Keywords:**

*Eremostachys laciniata*

GC–MS/FID

DPPH

*p*-Cymene

1. Introduction

Medicinal plants are the most important source of folk medicine for the majority of the world's population [1]. The use of plants in traditional medicine for treating various ailments remains an integral part of the culture and traditions of a majority of the world's population. In addition, factors such as the availability, affordability and accessibility of medicinal plants have led to their high demand and usage. Secondary metabolites such as essential oils, alkaloids, flavonoids, tannins, saponins generally produced by plants for their defense mechanisms have been implicated in the therapeutic properties of most medicinal plants [2]. *Eremostachys laciniata* (L) Bunge (*E. laciniata*) is a perennial medicinal herb with a thick root and pale purple or white flowers. It is one of the fifteen endemic Iranian species of the genus *Eremostachys*. Traditionally, aerial and underground parts of this plant have been used to treat allergies, headache and liver diseases. *E. laciniata* is also used to alleviate inflammatory conditions. It is usually given as a remedy in the form of herbal teas, or tisanes of the roots and flowers [3]. Literature survey revealed that, there is no study about antioxidant activity of the extract and essential oil component of *E. laciniata*. Therefore, this present study was designed to evaluate the essential oil component and antioxidant activity of *E. laciniata* in the Zagros area, for the first time.

2. Materials and methods

2.1. Plant material and site description

Aerial parts of plants were collected at the early flowering stage from plants growing wild in the Zagros region from Iran (GPS coordinates: 33°56'15.54" N, 48°48'51.69" E) in May 2014. The plant was identified by Prof. Mohammad Mehdi Dehshiri, Department of Biology, Islamic Azad University, Borujerd, Iran. A voucher specimen (L417) had been deposited at the herbarium of Islamic Azad University from Borujerd.
2.2. Essential oil extraction

The extraction of the essential oils was carried out by hydrodistillation in a Clevenger-type apparatus (100 g for 3 h). The essential oils were stored at 4 °C in the dark and in the presence of anhydrous sodium sulfate [4].

2.3. Preparation of plant extracts

The plant sample was extracted with methanol solvent described by Mohammadi et al. [5] with some modifications. Twenty grams were extracted with 200 mL methanol solvent by using Soxhlet extractor for 4 h. After complete extraction, the methanol solvent was evaporated by using rotary evaporator. Extracts were concentrated, dried and kept in the dark at 4 °C until tested.

2.4. Gas chromatography (GC) and GC–mass spectrometry (GC–MS) analyses

GC and GC–MS analyses were carried out by using an Agilent Technologies 7890 GC apparatus equipped with a flame ionization detector. The analysis was carried out on fused silica capillary HP-5MS column (30.00 m × 0.25 mm inner diameter; film thickness 0.25 μm). The injector and detector temperatures were kept at 250 °C and 300 °C, respectively. GC–MS analysis was done on an Agilent Technologies 5975 Mass system. Mass spectra were recorded at 70 eV. Mass range was from m/z 50 to 550. The identification of constituents was performed on the basis of comparison of their retention indices and mass spectra with those given in the literature [6].

2.5. Total phenolic and flavonoid content

The concentration of the phenolics in the plant extracts was determined using the Folin-Ciocalteu assay. In brief, 0.1 mL of each phenolic extract was mixed with 2 mL of 2% sodium carbonate and then the mixture was allowed to stand at room temperature for 5 min. After addition of 100 mL of Folin-Ciocalteu reagent, the mixture was left in the dark room for 30 min at room temperature for 5 min. After addition of 100 mL of Folin-Ciocalteu reagent, the mixture was left in the dark for 30 min and thereafter read at 515 nm using a spectrophotometer. The results were expressed as mg catechin equivalent per gram of sample. All samples were analyzed in triplicate [7].

The total flavonoid content of the extracts was determined using a colorimetric method [8]. A volume of 200 mL of hydrodistilled water was mixed with 500 mL of plant sample. Then, it was mixed with 150 mL of a NaNO2 solution (15%). After 6 min, 150 mL of aluminum chloride solution (10%) was added and allowed to stand for 60 min. Then, 2 mL of NaOH solution (4%) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 mL and the mixture was thoroughly mixed and allowed to stand for another 15 min. The absorbance against blank was determined at 510 nm (spectrophotometer). The sample concentration providing 50% inhibition (IC50) was calculated by plotting inhibition percentages against concentrations of the sample. Samples were analyzed in triplicate.

3. Results

3.1. Essential oil composition

The compositions of essential oil were studied and twenty-two compounds, which accounted for 92.42% of the total compositions, are reported in Table 1.

Results indicated that essential oil of E. laciniata was a complex mixture of monoterpene hydrocarbons (62.08%), oxygenated monoterpenes (26.19%), sesquiterpene hydrocarbons and alkane hydrocarbon. p-Cymene with 21.64%, α-pinene with 11.42%, limonene with 9.72% and E-β-ocimene with 8.71% were the most important constituents presented among the monoterpene hydrocarbons compounds of the essential oil. Besides, linalool, 1,8-cineole and terpinen-4-ol with 17.93%, 3.96% and 2.14%, respectively, were the main oxygenated monoterpenes found in the E. laciniata essential oil. Additionally, germacrene D with 1.88% and β-caryophyllene with 0.16% were the major sesquiterpene hydrocarbons.

<table>
<thead>
<tr>
<th>Components</th>
<th>RI</th>
<th>E. laciniata (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Thujene</td>
<td>925</td>
<td>0.11</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>933</td>
<td>11.42</td>
</tr>
<tr>
<td>Camphene</td>
<td>950</td>
<td>0.62</td>
</tr>
<tr>
<td>Sabinene</td>
<td>974</td>
<td>3.14</td>
</tr>
<tr>
<td>Myrcene</td>
<td>991</td>
<td>1.74</td>
</tr>
<tr>
<td>Decane</td>
<td>996</td>
<td>2.11</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>1025</td>
<td>21.64</td>
</tr>
<tr>
<td>Limonene</td>
<td>1028</td>
<td>9.72</td>
</tr>
<tr>
<td>β-Phellandrene</td>
<td>1029</td>
<td>0.84</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>1032</td>
<td>3.96</td>
</tr>
<tr>
<td>E-β-ocimene</td>
<td>1035</td>
<td>8.71</td>
</tr>
<tr>
<td>(Z)-β-ocimene</td>
<td>1045</td>
<td>0.25</td>
</tr>
<tr>
<td>γ-Terpine</td>
<td>1061</td>
<td>3.78</td>
</tr>
<tr>
<td>α-Terpinolone</td>
<td>1089</td>
<td>0.11</td>
</tr>
<tr>
<td>Linalool</td>
<td>1100</td>
<td>17.93</td>
</tr>
<tr>
<td>Camphor</td>
<td>1145</td>
<td>0.15</td>
</tr>
<tr>
<td>Bornol</td>
<td>1166</td>
<td>0.21</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1179</td>
<td>2.14</td>
</tr>
<tr>
<td>α-Terpinol</td>
<td>1191</td>
<td>0.63</td>
</tr>
<tr>
<td>Geranyl acetate</td>
<td>1384</td>
<td>1.17</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>1422</td>
<td>0.16</td>
</tr>
<tr>
<td>Germacrene D</td>
<td>1483</td>
<td>1.88</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>92.42</td>
</tr>
</tbody>
</table>

RI: Retention indices. a - Relative to C5–C24 n-alkanes on HP-5MS capillary column.
3.2. Total phenolics and flavonoids contents

The total phenolics content from samples by the Folin-Ciocalteu reagent was determined. The analysis detected a high phenolic content for sample (45.39 mg GAE/g). Also, the total content of flavonoids was evaluated using a colorimetric assay method (23.75 mg GAE/g).

3.3. Antioxidant activity

Plants with radical scavenging property and antioxidant capacity are useful for medicinal applications and as food additive [10]. So, in this research, the antioxidant activity of E. laciniata was evaluated using DPPH radical scavenging method by comparing with the activity of the butylated hydroxytoluene as a known antioxidant. In general, the antioxidant capacity (IC50) of the extract was 54.64 μg/mL (IC50 for butylated hydroxytoluene was 19.03 μg/mL). Therefore, the antioxidant properties of E. laciniata extract could play a beneficial role in the food preservation.

4. Discussion

Plants contain numerous phytochemical constituents, many of which are known to be biologically active compounds and are responsible for exhibiting diverse pharmacological activities. Some of these secondary metabolites of plants are important source of natural antioxidants that are preferred over synthetic ones because of safety concerns. The bioactive secondary metabolites have been shown to reduce the risk and progression of diseases such as cancer, cardiovascular, neurodegenerative diseases, etc. by scavenging free radicals through various biological mechanisms [11]. Results of this study showed that monoterpene hydrocarbons, oxygenated monoterpene and sesquiterpene hydrocarbons were the main constituent groups. Results were in agreement with Al-Jaber et al. on variation in the essential oil composition of E. laciniata from Jordan [12]. In another study, Navaei and Mirza investigated the essential oil of air-dried E. laciniata growing wild in Iran and detected 19 components representing 91.2% of the total oil composition. The major constituents in the essential oil of E. laciniata from Iranian origin including dodecanol (72.5%), widdrol (2.4%), germacrene B (1.9%) and thujopsene (1.9%) were not detected in the essential oil of E. laciniata from this study [13]. Nevertheless, similar to Al-Jaber et al. study, different types of hydrocarbons and their oxygenated derivatives were the main constituents of the essential oils obtained from E. laciniata [12].

Phenols are compounds that have the ability to destroy radicals because they contain hydroxyl groups. These important plant components give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals; hence, they play an important role in antioxidant activity. Therefore, determination of the quantity of phenolic compounds is very important in order to determine the antioxidant capacity of plant extracts [14].

Excess free radical formation caused cellular damage and induced many dysfunctions like atherosclerosis, myocardial infarction, cancer and neurodegenerative disorders in human beings. But, natural antioxidant compounds are useful in repairing free radical formation in cells and manage various chronic diseases. Antioxidant tests are highly specific and sensitive to temperature and incubation period. Also, physicochemical properties of the sample are very important for analyzing antioxidant properties [15].

Therefore, this paper can be a guideline for researchers in the field of pharmacology to make more investigations about these plants from other points of view.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References