HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.11.022

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 Bajalan[1*], Masoumeh Zand[2], Masoud Goodarzi[3], Mahin Darabi[4]

[1]*Young Researchers and Elite Club, Borujerd Branch, Islamic Azad University, Borujerd, Iran*

[2]*Department of Agriculture, College of Agriculture, Borujerd Branch, Islamic Azad University, Borujerd, Iran*

[3]*Department of Biology, Borujerd Branch, Islamic Azad University, Borujerd, Iran*

[4]*Department of Chemistry, Faculty of Basic Science, Borujerd Branch, Islamic Azad University, Borujerd, Iran*

ARTICLE INFO

Article history:
Received 25 Sep 2015
Received in revised form 16 Oct 2015, 2nd revised form 26 Jan, 3rd revised form 1 Feb 2016
Accepted 9 Nov 2016
Available online 23 Nov 2016

Keywords:
Eremostachys laciniata
GC–MS/FID
DPPH
p-Cymene

ABSTRACT

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

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 mech-anisms 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.

*Corresponding author: Iman Bajalan, Young Researchers and Elite Club, Borujerd Branch, Islamic Azad University, Borujerd, Iran.
Tel: +98 9396184417
E-mails: bajalan_iman@yahoo.com, ibajalan@yahoo.com

Foundation Project: Supported by Islamic Azad University from Borujerd, Iran (Grant No. P–151–930319).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

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, and the absorbance was determined against a blank at 750 nm using a spectrophotometer. The results were expressed as mg gallic acid equivalent per gram (mg GAE/g) of dry weight of extract. 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 hydrodistillated water was mixed with 500 mL of plant sample. Then, it was mixed with 150 mL of a $NaNO_2$ 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 results were expressed as mg catechin equivalent per gram of dry weight of extract. All samples were analyzed in triplicate.

2.6. Antioxidant activity

The ability of the plant extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was assessed using the standard method. About 10 μL of the extracts were dissolved in 90 μL of distilled water and DPPH (3.9 mL of 25 mmol/L DPPH•). The solution was placed in the dark at room temperature for 30 min and thereafter read at 515 nm using a spectrophotometer (4802 Doubly Beam, Unico), against a blank of methanol without DPPH•.

$$\% \text{ DPPH free radical} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of test compound [9].

The sample concentration providing 50% inhibition (IC_{50}) 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 1
Chemical composition of the essential oil of *E. laciniata*.

Components	RI[a]	*E. laciniata* (%)
α-Thujene	925	0.11
α-Pinene	933	11.42
Camphene	950	0.62
Sabinene	974	3.14
Myrcene	991	1.74
Decane	996	2.11
p-Cymene	1025	21.64
Limonene	1028	9.72
β-Phellandrene	1029	0.84
1,8-Cineole	1032	3.96
E-β-ocimene	1035	8.71
(Z)-β-ocimene	1045	0.25
ɣ-Terpinene	1061	3.78
α-Terpinolene	1089	0.11
Linalool	1100	17.93
Camphor	1145	0.15
Borneol	1166	0.21
Terpinen-4-ol	1179	2.14
α-Terpinol	1191	0.63
Geranyl acetate	1384	1.17
β-Caryophyllene	1422	0.16
Germacrene D	1483	1.88
Total		92.42

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 (IC_{50}) of the extract was 54.64 μg/mL (IC_{50} 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 monoterpenes 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, physiochemical 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

This work was a university project and supported by Islamic Azad University from Borujerd, Iran (Grant No. P–151–930319).

References

[1] Samejo MQ, Sumbul A, Shah S, Memon SB, Chundrigar S. Phytochemical screening of *Tamarix dioica* Roxb. Ex Roch. *J Pharm Res* 2013; **7**(2): 181-3.
[2] Ghribia L, Ghouilaa H, Omrib A, Besbesb M, Janneta HB. Antioxidant and anti-acetylcholinesterase activities of extracts and secondary metabolites from *Acacia cyanophylla*. *Asian Pac J Trop Biomed* 2014; **4**(Suppl 1): S417-23.
[3] Said O, Khalil K, Fulder S, Azaizeh H. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. *J Ethnopharmacol* 2002; **83**(3): 251-65.
[4] Moussaoui F, Alaoui T. Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants. *Asian Pac J Trop Biomed* 2016; **6**(1): 32-7.
[5] Mohammadi M, Alaei M, Bajalan I. Phytochemical screening, total phenolic and flavonoid contents and antioxidant activity of *Anabasis setifera* and *Salsola tomentosa* extracted with different extraction methods and solvents. *Orient Pharm Exp Med* 2016; **16**(1): 31-5.
[6] Bajalan I, Ghasemi Pirbalouti A. Variation in chemical composition of essential oil of populations of *Lavandula* × *intermedia* collected from Western Iran. *Ind Crops Prod* 2015; **69**: 344-7.
[7] Stankovic MS. Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujev J Sci* 2011; **33**(1): 63-72.
[8] El-Haci IA, Bekkara FA, Mazari W, Gherib M. Phenolics content and antioxidant activity of some organic extracts of endemic medicinal plant *Anabasis aretioides* Coss. & Moq. from Algerian Sahara. *Pharmacogn J* 2013; **5**(3): 108-12.
[9] da Costa LS, Andreazza NL, Correa WR, da Silva Cunha IB, Gois Ruiz ALT, Ernesto de Carvalho J, et al. Antiproliferative activity, antioxidant capacity and chemical composition of extracts from the leaves and stem of *Chresta sphaerocephala*. *Rev Bras Farmacogn* 2015; **25**(4): 369-74.
[10] Sepahvand R, Delfan B, Ghanbarzadeh S, Rashidipour M, Veiskarami GH, Ghasemian-Yadegari J. Chemical composition, antioxidant activity and antibacterial effect of essential oil of the aerial parts of *Salvia sclareoides*. *Asian Pac J Trop Med* 2014; **7**: S491-6.
[11] Al-Owaisi M, Al-Hadiwi N, Khan SA. GC/MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. *Asian Pac J Trop Med* 2014; **4**(12): 964-70.
[12] Al-Jaber HI, Al-Qudah MA, Barhoumi LM, Abaza IF, Afifi FU. Variation in the essential oil composition of *Eremostachys laciniata* from Jordan at different flowering stages. *J Essent Oil Res* 2012; **24**(3): 289-97.
[13] Navaei MN, Mirza M. Chemical composition of the oil of *Eremostachys laciniata* (L.) Bunge from Iran. *Flavour Fragr J* 2006; **21**(4): 645-6.
[14] Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak J Pharm Sci* 2009; **22**(1): 102-6.
[15] Kuppusamy P, Yusoff MM, Parine NR, Govindan N. Evaluation of *in-vitro* antioxidant and antibacteria properties of *Commelina nudiflora* L. extracts prepared by different polar solvents. *Saudi J Biol Sci* 2015; **22**(3): 293-301.