



GC-MS analysis of the essential oil composition and antioxidant activity of *Perovskia abrotanoides* Kar. from different growth stages

Ebrahim Gholamalipour Alamdari*

Department of Plant Production, Faculty of Agriculture and Natural Resources, Gonbad Kavous University, Gonbad Kavous, Golestan, Iran, IR 4971799151

Received 04 May 2020; Revised 21 March 2021

The objective of this study was to evaluate the changes in the chemical composition of essential oils (EO) and the correlation between some secondary metabolites and antioxidant activity of different plant parts of *Perovskia abrotanoides* Kar. at the vegetative and flowering stages in Vamnan (Iran) in 2018. The EO of this plant was analyzed using the GC-Mass Spectrometry method. As the findings showed, *P. abrotanoides* leaf, during the flowering stage, had a greater amount of phenolic compounds, flavonoids, and anthocyanins than other parts and even the vegetative stage. At the flowering stage, the maximum antioxidant activity was obtained in the leaf (69.10%), based on the DPPH method. During the vegetative stage, the root was also in the next rank (62.10%). In this research, aerial parts of *P. abrotanoides* were different in terms of EO composition percentage and retention time. Likewise, 21 and 26 constituents were identified during the vegetative and flowering stages, respectively. The major constituent was Carotol (31.105%) at the flowering stage, where IR-alpha-pinene (0.268%) had the lowest value. Overall, the results showed that *P. abrotanoides* can be introduced as an important source of natural antioxidant for pharmaceutical and natural food supplements due to its acceptable amount of bioactive compounds such as phenolic, flavonoids, anthocyanins, and EO.

Keywords: Antioxidant activity, Carotol, Flowering stage, GC-Mass Spectrometry method, *Perovskia abrotanoides* Kar.

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 36/53, A61K 125/00, A61K 127/00, A61K 133/00, A61K 135/00, A61P 39/00

Introduction

Phytoconstituents are chemical compounds produced by plants through primary or secondary metabolites, generally to help them thrive or thwart competitors, predators, or pathogens. The secondary molecules are produced occasionally in living plant cells. They do not play any significant role in the primary life of plants and producing them at a low concentration is commensurate with the growth physiology of a plant species¹. However, plants constitute a source of different chemical compounds which may be used in medicine and other applications. The plant parts such as leaves, flowers, barks, seeds, fruits, stem and root contain bioactive compounds like alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids². Medicinal plants contain various ranges of chemical molecules with pharmacological applications. In recent years, botanists, ethnopharmacologist and natural-product chemists have analyzed the available medicinal plants for

extracting various phytochemicals against different emerging drug-resistance fungi and bacteria³. Most medicinal plants are known to derive their characteristic benefits from their phytochemical constituents. The phytochemical research approach is considered effective in discovering the bioactive profile of plants of therapeutic importance⁴. However, more than 350,000 species are yet to be investigated for the presence of biopharmaceuticals⁵. Owing to the significance of the aforementioned materials, such preliminary phytochemical screening of plants is needed to discover and develop novel therapeutic agents with improved efficacy. Numerous research groups have also reported such studies throughout the world⁶. Many weeds and wild plants are also economically important as they could be used for fodder, medicine, weedicide, insecticide, biofertilizer and other purposes. Borazambol medicinal plant, with the scientific name of *Perovskia abrotanoides* Karel, belongs to the Lamiaceae family⁷ and consists of three wild species which grow in Iran, Afghanistan, Pakistan, and Turkmenistan⁸. A review of previous research shows that there are some important chemical compounds in the Lamiaceae family. In one

*Correspondent author
Email: eg.alamdari@gonbad.ac.ir

of the related reports, ethanol extracts of aerial parts of *P. abrotanoides*, which were collected from Semnan province, Iran, had sufficient antioxidant activity with an IC₅₀ of 15.03±1.2 mg/mL when using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assay. In addition, the evaluation of reducing power assay resulted in an IC₅₀ of 32.3±0.31 mg/mL⁹. In a study on the essential oil (EO) composition of *P. abrotanoides*, 19 compounds were identified by GC-MS analysis, which (E)-9-dodecenal and hexadecanoic acid were the major components in stem and leaves, respectively. In contrast, GC-MS analysis of fixed oil showed 40 constituents, with α -amyrin being the major component in stem and α -copaene in leaves. The antioxidant activity also showed the highest value of 76.7% in EO from leaves in comparison with fixed oil from the stem (45.9%) through inhibition of peroxidation in the linoleic acid system¹⁰. Many scientists reported that plant EO composition and bioactive components are affected by such varied factors as a collection site, growth stage, and genetic makeup¹¹. Due to the large geographical distribution of *P. abrotanoides* in Iran, special emphasis was not given to on study of the correlation between secondary metabolites and antioxidant activity. Moreover, no research has so far conducted to find out the presence of secondary metabolites from different parts of this species from Golestan province, especially in the Vamenan region. Therefore, the main objective of this study was to assess the changes in the chemical composition of EO, secondary metabolites and antioxidant activity of different plants parts of *P. abrotanoides* from different growth stages.

Materials and Methods

Plant collection and sample processing

First, plant samples of *P. abrotanoides* were collected from the Vamnan region of Azadshahr County, located in Golestan province in Iran, during the vegetative and flowering stages in April and June 2018. The collected plant materials of *P. abrotanoides* were botanically authenticated by coloured flora of Iran^{12,13} with the assistance of a systematic expert of Gonbad Kavous University. Herbarium specimen of *P. abrotanoides*, with the herbarium code No. GKU/803894, was registered in the herbarium of Gonbad Kavous University. Then plant samples of *P. abrotanoides* were divided into various parts such as root, stem and leaf at the vegetative stage, and root,

stem, leaf and flower at the flowering stage, and were washed with distilled water to remove soil and dust particles and were half-dried up in the shade for 3 days at room temperature. Finally, plant samples were dried at 40 °C in the oven until they reached a fixed weight. They were then crushed into a uniform powder using a grinder with mesh 8.

Preliminary phytochemical screening

It includes a number of qualitative analysis that allows identifying secondary metabolites present in a certain sample. The detection of these chemical groups is performed through colour and precipitation reactions occurring with adding specific reagents^{14,15}.

Qualitative tests for tannins

Water extracts of various plant parts were treated with a few drop of ferric chloride (acidic) and observed for brownish green or a blue-black coloration¹⁶ which indicates the presence of tannins.

Qualitative tests for saponins

Water extracts of various plant parts were vigorously shaken with few drops of neutral water. A permanent lather (foam) indicates the presence of saponins¹⁶.

A portion of the residue obtained after evaporating ethanol extract was dissolved in water and shaken vigorously. A honeycomb, froth persisting for 15 minutes, indicated the presence of saponins. A portion was further dissolved in chloroform and then filtered. A few drops of concentrated sulfuric acid with 1 mL of acetic anhydride were then added to the resulting frozen solution (1 mL). The appearance of the blue or bluish-green or reddish-brown colour showed the presence of saponins¹⁷.

Qualitative test for terpenoids (Salkowski test)

Aqueous extracts of plants obtained from different plant parts (5 mL) were mixed with 2 mL chloroform and concentrated H₂SO₄ (3 mL) was carefully added to form a layer. A radish brown colouration of the interface was formed to show positive results for the presence of terpenoids¹⁸⁻²⁰.

Qualitative test for flavonoids

Ethanol extract (1 mL) from various plant parts was mixed with few drops of concentrated HCL and Mg. The development of pink or magenta colour indicated the presence of flavonoids¹⁷.

Qualitative test for glycosides

Take 10 mL of the filtrated ethanol extract in a test tube. Add warm benzene slowly in the filtrate through

the side of the test tube. The formation of white precipitate at the junction of both the filtrate and benzene indicates the presence of glycosides²¹.

Qualitative test for steroids

Acetic anhydride (2 mL) was added to 0.5 mL ethanol extract of each sample with 2 mL H₂SO₄. Changing the colour from violet to blue or green in some samples indicated the presence of steroids¹⁸⁻²⁰.

Quantitative estimation of the chemical constituency

In this experiment, all determinations were carried out in triplicate.

Determining total phenolic content

Measuring total phenolics is based on the *Folin-Ciocalteu* method²². For this purpose, 2.5 mL of ethanol 80% was added to 0.25 g of sample and centrifuged at 2°C for 10 minutes. The obtained supernatant was then preserved. In the following, the sample was re-extracted with 2.5 mL of 80% ethanol and centrifuged. The pooled supernatant was first evaporated, and 3 mL of distilled water was added to the dried supernatant. 0.5 mL of Folin's phenol reagent and 2 mL of sodium carbonate (20%) were added to it. The reaction mixture was kept in a boiling water bath for 1 minutes. The absorbance was measured at 650 nm in a spectrophotometer (Biochromlibera-S22). The phenolic content was calculated by gallic acid equivalents mg GAE per g of dry plant material based on the standard curve of gallic acid [$y = 0.0027x - 0.0055$, $R^2 = 0.999$].

Determining total flavonoids content

The aluminium chloride colourimetric method was used to determine the total flavonoid content of the sample^{23,24}. For total flavonoids estimation, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 5 mg quercetin in 1 mL methanol, then using methanol (5–200 µg/mL), the standard quercetin solutions were prepared by serial dilutions. An amount of 0.6 mL diluted standard quercetin solutions or extracts was separately mixed with 0.6 mL of 2% aluminium chloride. After mixing, the solution was incubated for 60 minutes at room temperature. The absorbance of the reaction mixtures was measured at 420 nm with a spectrophotometer (Biochromlibera-S22). The concentration of total flavonoids content in the test samples was calculated from the calibration plot ($y = 0.0162x + 0.0044$, $R^2 = 0.999$) and expressed as mg quercetin equivalent (QE)/g of dried plant material.

Determining anthocyanin content

To determine anthocyanin content, 0.5g of a fresh sample of each plant part was ground with 4 mL of 1% hydrochloric acid solution in methanol in a ratio of 99:1 (pure methanol (99) and pure hydrochloric acid (1)). The extract was poured into refluxing tubes and was kept for 24 hours in the dark condition at 25 °C. It was then centrifuged at 4000 rpm for 10 minutes. Light absorption was then read at 550 nm with a spectrophotometer (Biochromlibera-S22). The anthocyanin content was calculated according to $A = \epsilon BC$ formula, where ϵ is the extinction coefficient, equal to 3300 cm/mole, B, the width of the cuvette, equal to 1 cm and C, the complex concentration in terms of µg/g fresh weight²⁵.

Determining antioxidant activity

The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method as a free radical. To conduct this experiment, 3.9 mL of DPPH made of stock (0.0004 g DPPH in 100 mL methanol), was poured inside the tube and then 0.1 mL of methanol extract of each part was added. It was put in dark condition for 30 minutes and then the absorbance point was measured at 517 nm with a spectrophotometer (Biochromlibera-S22). Radical inhibition of DPPH was calculated using the following equation²⁶.

$$I(\%) = 100 \times (A_0 - A_s) / A_0 \quad \text{equation 1}$$

Where A_0 is Control absorbance (containing all reactive components without sample) and A_s is sample absorbance.

Extracting EO and EO components of aerial parts of *P. abrotanoides* at the vegetative and flowering stages

To extract EOs, 50 g of plant parts from aerial parts of *P. abrotanoides* were extracted by the distillation method at the vegetative and flowering stages using the Clevenger for 3 hours. The EO was dehydrated by dry sodium sulfate and kept in the refrigerator for further use. Using the GC-MS system, EO components from aerial plant parts were measured. All analyses were run in triplicate and mean and standard error (SE) was later calculated.

Results

Results of preliminary phytochemical screening

The preliminary phytochemical screenings of different parts of the *P. abrotanoides* at the vegetative

and flowering stages are summarized in Table 1. At the vegetative stage, tannins and saponins were detected in the water extract of root, stem and leaf of *P. abrotanoides*. Whereas flavonoids, steroids, and anthocyanins were found in the ethanol extract of all the parts under the study, terpenoids were not detected in the water extract from plant parts in the vegetative phase. It was also found out that all constituents, except glycosides, were confirmed in *P. abrotanoides* plant parts at the flowering stage.

Results of quantitative estimation of some chemical constituents

Results of the quantitative estimation of total phenolic in various parts of *P. abrotanoides* during the vegetative and flowering stages are presented in Table 2, where leaf, at the flowering stage, possesses most of the total phenolic content (8.17 mg GAE per

100 g dry weight of the sample) compared to the other parts. Meanwhile, the stem contained the lowest total phenolic (0.37 mg GAE acid per 100 g dry weight of sample) at the vegetative stage.

The average amount of flavonoids content indicated that the leaf sample (2.26 mg QE/g dry sample) at the flowering stage had a maximum quantity of this compound. Indeed, it did not show a significant difference with plant part of flower and root at the flowering and vegetative stages, respectively, so they were in a group statistically. However, the root sample (0.11 mg QE/g dry sample) of *P. abrotanoides* at the flowering stage contained the lowest mentioned constituents (Table 2).

Based on the results, the highest content of anthocyanins was found in the flower part at the flowering stage, which contained 0.66 µg/g fresh weight sample. However, there was no significant difference in the level of this compound compared to leaf at the same stage. The least significant accumulation of anthocyanins was observed in both stem (0.11 µg/g fresh weight sample) and root samples (0.13 µg/g fresh weight sample) during the vegetative and flowering stages, respectively (Table 2).

Antioxidant activity

At the flowering stage, the maximum antioxidant activity was obtained in the leaf part (69.10%), followed by the root at the vegetative stage (62.10%), while the stem part at the vegetative stage contained the lowest antioxidant activity, with a value of 3.59% (Table 2).

Results of EO constituents of *P. Abrotanoides* aerial parts at the vegetative stage

GC analysis was performed to identify various constituents in the EO from aerial parts of *P. abrotanoides*. According to the results, 21 chemical compounds were identified in the EO from

Table 1 — Phytochemical tests of different parts of the *Perovskia abrotanoides* at flowering stages

Growth stages	Phytochemicals	Parts		
		Root	Stem	Leaf
Vegetative stage	Water extract			
	Tannins	+	+	+
	Saponins	+	+	+
	Terpenoids	-	-	-
	Alcoholic extract			
	Flavonoids	+	+	+
	Glycosides	+	+	+
	Steroids	+	+	+
	Anthocyanins	+	+	+
Flowering stage	Water extract			
	Tannins	+	+	+
	Saponins	+	+	+
	Terpenoids	+	+	+
	Alcoholic extract			
	Flavonoids	+	+	+
	Glycosides	-	-	-
	Steroids	+	+	+
	Anthocyanins	+	+	+

+ indicates presence, - indicates absence

Table 2 — Qualitative estimation (Mean±SE) of total phenolic, flavonoids, anthocyanins and antioxidant activity of various parts of *Provskia abrotanoides* during the vegetative and flowering stages

Various stages	Organic constitute/ various organs	Total phenolic (mg GAE /100 g dry sample)	Flavonoids (mg QE/g dry sample)	Anthocyanins (µg/g fresh weight sample)	Antioxidant activity (%)
Vegetative	Root	6.15±0.11 ^b	2.12±0.10 ^a	0.50±0.03 ^b	62.10±1.09 ^b
	Stem	0.37±0.06 ^d	0.22±0.02 ^c	0.11±0.003 ^d	3.59±0.62 ^f
	Leaf	3.02±0.04 ^c	0.87±0.05 ^b	0.36±0.02 ^c	32.10±1.91 ^d
Flowering	Root	0.59±0.05 ^d	0.11±0.01 ^d	0.13±0.09 ^d	5.98±0.56 ^f
	Stem	2.69±0.07 ^c	0.87±0.05 ^b	0.41±0.60 ^c	27.14±0.71 ^e
	Leaf	8.17±0.74 ^a	2.26±0.60 ^a	0.58±0.017 ^{ab}	69.10±0.83 ^a
	Flower	6.03±0.08 ^b	2.11±0.08 ^a	0.66±0.03 ^a	53.53±2.90 ^c

Data with different letters in the same column indicate mean (±) standard error and groups based on the least significant difference compared with 95% confidence interval (n=3)

P. abrotanoides aerial parts. The major compounds were Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7 dimethyl-1-(1-methylethyl)-, (1S-cis) (15.807%) followed by Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (1R) (14.754%) and (-)- Isolongifolol, acetate (7.972%). In general, these constituents included 38.40% of EO at the vegetative stage. On the contrary, composition of Tricyclo [2.2.1.0 (2, 6)] heptane, 1, 7, 7- trimethyl (0.863%) and Bornyl acetate (0.998%) had the lowest values (Table 3).

Results of EO constituents of *P. abrotanoides* aerial parts at the flowering stage

A total of 26 constituents were identified in *P. abrotanoides* aerial parts at the flowering stage. According to the results, the major constituent found in the EO was Carotol (31.105%), followed by Naphthalene, 1, 2, 3, 5, 6, 8 a-hexahydro-4, 7 dimethyl-1-(1-methylethyl) (1S-cis) (14.251) and Agarospirol (7.233%), which included 52.59% of all EO constitutes at this stage. It was also observed that the composition of IR- alpha-pinene (0.268%) had the lowest value (Table 4).

Discussion

The findings of our study indicated that at the flowering stage, the leaf part of *P. abrotanoides* had

higher amounts of secondary metabolites such as phenolic compounds, flavonoids, anthocyanins as compared to other parts and the vegetative stage. A significant relation between phenolic content, flavonoids and anthocyanins with antioxidant activity of plant constituents have been reported by many researchers²⁷⁻³². Phenolic compounds are secondary metabolites that can be categorized as flavonoids and non-flavonoids³³. Their free radical-scavenging properties help to prevent oxidative stresses³⁴. Antioxidants reduce the negative effects of free radicals in living organisms. There are two major antioxidant groups, i.e., enzymatic and non-enzymatic antioxidants, in the cells of living organisms³⁵. Several findings have been reported regarding the antioxidant and antimicrobial activities of *P. abrotanoides*^{36,37}. Anthocyanins are a class of naturally occurring antioxidants that belongs to the group of polyphenolic compounds. They are known as pigments abundant in highly coloured plants and impart red, blue, purple, or black colour. The GC-MS analysis confirmed the presence of various chemical components in aerial parts of *P. abrotanoides* with different retention times. As findings showed, the highest constituent of EO belonged to the flowering stage. The major constituent was Carotol (31.105%) at the flowering stage, where IR-alpha-pinene was

Table 3 — GC-MS analysis of aerial parts of *Provsikia abrotanoides* at the vegetative stage

S. No	Name of the compound	Composition percentage	Retention time
1	Tricyclo [2.2.1.0(2,6)] heptane, 1, 7, 7-trimethyl	0.863	3.440
2	Camphene	2.441	4.054
3	Eucalyptol	7.843	4.879
4	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)	14.754	7.281
5	2-Furoic acid, tridec-2-ynyl ester	2.111	7.835
6	Bornyl acetate	0.998	10.220
7	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	3.107	11.708
8	Copaene	1.370	12.408
9	Delta.-Selinene	2.207	13.185
10	Longifolene-(V4)	5.234	13.545
11	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethen yl)-, [1S (1.alpha.,4.alpha.,7.alpha.)]	4.737	14.414
12	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl), (1.alpha.,4a.beta.,8a.alpha.)	4.336	15.751
13	Benzamide, 4-tert-butyl-N-[2-(2-isopropyl-5-methylphenoxy)ethyl]	6.552	15.837
14	Cubanol	1.779	18.181
15	Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl),[1R-(1.alpha.,7.beta.,8a.alpha.)]	2.156	18.733
16	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7 dimethyl-1-(1-methylethyl)-, (1S-cis)	15.807	18.872
17	(-)-Isolongifolol, acetate	7.972	18.967
18	1-Naphthalenol,decahydro-1,4a-dimethyl-7-(1-methylethylid[1R-(1.alpha.,4a.beta.,8a.alpha.)]-	3.577	19.157
19	Patchoulene	2.733	19.551
20	alpha.-Bisabolol	7.228	19.730
21	Carotol	2.095	20.062

Table 4 — GC-MS analysis of aerial parts of *Provsikia abrotanoides* at the flowering stage

S. No.	Name of the compound	Composition percentage	Retention time
1	IR- alpha-pinene	0.268	3.302
2	Eucalyptol	1.396	4.705
3	Bicyclo (2,2,1) heptan - 2-one 1, 7, 7- trimethyl (1S)	1.024	7.040
4	Bornyl acetate	1.654	10.026
5	cyclohexene -1- methanol , alpha 4- trimethyl- propanoate	3.395	11.537
6	Copaene	0.779	12.208
7	1 H- cycloprop(e) azulene, 1a .2,3,4 ,4a ,5, 6, 7 b-octahydro 1, 1, 4, 7 tetramethyl-[1 a R1a alpha .4.alpha, 4 a. beta, 7 balpha]	0.952	12.981
8	Caryophyllene	2.370	13.343
9	Azulene,1, 2, 3, 4, 5, 6, 7, 8-octahydro1,4-dimethyl 7 (1- methyle) - (1S)- (1 alpha , 4.alpha, 7 alpha)	2.220	14.213
10	1 H cycloprop (e) azulene, decahdro-1,1, 7- trimethyl 4- methylene (1 a, alpha, 4 a.beta, 7 alpha, 7 beta 7 b. alpha)	0.788	14.306
11	Naphthalene,1,2, 3, 4, 4 a, 5, 6, 8 a- octahydro -7- methyl 4- methylene-1-1 methylethyl) - (1 alpha , 4 a beta , 8 a alpha)	2.858	15.570
12	Naphthalene, 1, 2, 3, 5, 6, 8 a- hexahydro 4, 7- dimethyl-1, (1- methylethy) (1S-cis)	3.736	15.655
13	2, 3, 4- Trifluorobenzoic acid ,2, 4, 6 trichlorophenyl ester	1.908	15.773
14	Isoarom aden drene epoxide	1.473	17.279
15	Aristolene epoxide	1.720	17.936
16	Cubenol	1.597	18.013
17	beta – Guaiene	1.204	18.276
18	1- Naphthalenol, decahydro 1, 4 a – dimethyl - 7, (1-methylethylid) 1 R- (1. alpha, 4a . beta , 8 a alpha)	3.644	18.575
19	Naphthalene,1, 2, 3, 5, 6, 8 a – hexahydro -4, 7 dimethyl - 1-(1-methylethyl) (1S-cis)	14.251	18.773
20	Patchoulene	5.516	18.847
21	Ir, 4 s,7 s.11 R -2, 2, 4, 8 - Tetramethyltricyclo [5,3,1,0(4,11)] undec - 8ene	1.805	19.042
22	Agarospinol	7.233	19.419
23	1 H cyclopropeazulene, 1 a,2, 3, 4, 4 a, 5, 6, 7 b-octahydro 1,1,4, 7- tetramethyl-(1 a. alpha. 4 alpha., 4 a beta), 7 b alpha	2.865	19.765
24	Carotol	31.105	20.009
25	7-1 sopropyl- 1,1, 4 a,trimethyl, 1, 2, 3, 4, 4 a,9, 10, 10 a - octa hydro phenan threne	0.904	26.918
26	1 R, 4 s,7 s,11 R-2, 2, 4, 8- Tetramethyl tricyclo [5, 3, 1,0 (4,1)] undec-8-ene	1.017	27.511

available in a very small amount (0.268%). In a study on chemical profiling of *P. abrotanoides* EO by GC/MS analysis, camphor (4.05-35.94%), 1, 8-cineole (7.15-24.34%), borneol (0- 21.75%), and alpha-pinene (2.05-10.33%) had been reported as the main constituents in this species³⁸. The reason for the changes in EO components could be due to fluctuations in plant metabolic activities under the influence of environmental factors during the growth period or the expression of different genes at different stages of plant growth. Literature review in studies related to the EO obtained from the plants belonging to the Lamiaceae family showed that high variability in their chemical composition depended on stages of plant development, location and genetic³⁹. Given these findings, it can be realized that our findings are in accordance with those of previous researchers³⁸⁻⁴¹.

Conclusion

Based on the findings, various parts of the *P. abrotanoides*, especially the leaf at the flowering

stage, contained higher amounts of phenolic, flavonoids and anthocyanins content. It was also observed that the leaf of the *P. abrotanoides* contained the highest composition of EO at the flowering stage. In general, based on the various criteria including antioxidant activity and EO composition of different parts of *P. abrotanoides* in Vamnan habitat may be recommended to exploit the huge biomass generated by this luxuriantly growing plant as drug and food supplement with biological origin, antimicrobial and bioherbicides compounds in sustainable agriculture.

Acknowledgement

The author is grateful to the experts of the Herbarium, University of Gonbad Kavous for identifying the sample. In addition, the author expresses special gratitude to the chemistry experts of Islamic Azad University, Gorgan Branch (Iran) for their technical assistance.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this article.

References

- Ncube B and Van Staden J, Tilting plant metabolism for improved metabolite biosynthesis and enhanced human benefit, *Molecules*, 2015, **20**(7), 12698-12731.
- Joseph B, Ph K and Mc K, Preliminary phytochemical screening of selected medicinal plants, *Int Res J Sci Eng*, 2013, **1**(2), 55-62.
- Selvakumar T A and Rajasekar P, *Aerva lanata* mediated phytofabrication of silver nanoparticles and evaluation of their antibacterial activity against wound associated bacteria, *J Taiwan Inst Chem Eng*, 2017, **78**, 539-551.
- Seriki S A, Odetola A O and Adebayo O F, Analysis of phytoconstituents of *Desmodium adscendens* in relation to its therapeutic properties, *Am J Biomed Sci*, 2019, **2**(4), 158-162.
- Isah T, Stress and defense responses in plant secondary metabolites production, *Biol Res*, 2019, **52**(1), 39.
- Watal G, Yadav M, Chatterji S and Gupta S K, Preliminary phytochemical screening of six medicinal plants used in traditional medicine, *Int J Pharm Pharm Sci*, 2014, **6**(5), 2-14.
- Mahboubi M, Iranian medicinal plants as antimicrobial agents, *J Microbial Biotechnol Food Sci*, 2013, **2**(4), 2388-2405.
- Arabi F, Moharramipour S and Sefidkon F, Chemical composition and insecticidal activity of essential oil from *Perovskia abrotanoides* (Lamiaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae), *Int J Trop Insect Sci*, 2008, **28**(3), 144-150.
- Ghafourian M and Mazandarani, M, Ethnopharmacology, ecological requirements, antioxidant and antimicrobial activities of *Perovskia abrotanoides* Karel, extract for vaginal infections from semnan province, *Int J Women's Health Reprod Sci*, 2016, **5**(4), 295-300.
- Ashraf S N, Zubair M, Rizwan K, Tareen R B, Rasool N, *et al.*, Compositional studies and biological activities of *Perovskia abrotanoides* Karel, oils, *Biol Res*, 2014, **47**(1), 1-9.
- Akbarian A, Rahimmalek M and Sabzalian M R, Variation in essential oil yield and composition of *Dorema aucheri* Boiss., an endemic medicinal plant collected from wild populations in natural habitats, *Chem Biodivers*, 2016, **13**(12), 1756 – 1766.
- Qahreman A, *Iranian cormophytes (plant systematic)*, vol I, (University Publishing Center, Tehran), 1994, 752.
- Qahreman A, *General code of families and genera of flora in Iran*, (Publication of Iran Forests and Rangelands Research Institute), 1996, 222.
- Harborne J B, *Textbook of phytochemical methods, a guide to modern techniques of plant analysis*, 5th edn, (Chapman and Hall Ltd, London), 1998, 21-72.
- Perry Marie Claude M C, Evaluation of the curcumin as cancer agent in the treatment of brain tumors, Memoire of Master's Degree in chemistry, (University of Quebec in Montréal), 2008, 97.
- Trease G E and Evans W C, Flavone and related flavonoid glycoside, *Pharmacognosy*, 10th edn, (Brailliar Tiridel London), 1972.
- Fransworth *et al*, *Handbook der Dragenkunde*, (Wilhem Mudrich Verlag, Wein Band V), 1960, 279-298.
- Trease G E and Evans W C, *Pharmacognosy*, 11th edn, (Brailliar Tiridel Can, Macmillian Publishers), 1989, 45-50.
- Harborne J B, *Phytochemical methods*, (Chapman and Hall Ltd, London), 1973, 49-188.
- Edeoga H O, Okwu D E and Mbaebie B O, Phytochemical constituents of some Nigerian medicinal plants, *Afr J Biotechnol*, 2005, **4**(7), 685-688.
- Trease G E and Evans W C, *Pharmacognosy*, (Spottiswoode Ballantyne Ltd., London), 1980, 366.
- Malick C P and Singh M B, *Plant enzymology and histo-enzymology*, (Kalyani Publishers, New Dehli), 1980, 286.
- Marinova D, Ribarova F and Atanassova M, Total phenolic and total flavonoids in Bulgarian fruits and vegetables, *J Univ Chem Technol Metallurgy*, 2005, **40**(3), 255-260.
- Miliauskas G, Venskutonis P R and Van Beek T A, Screening of radical scavenging activity of some medicinal and aromatic plant extracts, *Food Chem*, 2004, **85**(2), 231-237.
- Wagner G J, Content and vacuole/extra vacuole distribution of neutral sugars, free amino acids, and anthocyanins in protoplast, *Plant Physiol*, 1979, **64**(1), 88-93.
- Brand-Williams W, Cuvelier M E and Berset C, Use of a free radical method to evaluate antioxidant activity, *LWT Food Sci Technol*, 1995, **28**(1), 25-30.
- Agregan R, Munekata P E, Franco D, Dominguez R, Carballo J, *et al.*, Phenolic content and antioxidant activity of extracts from *Bifurcata alga*, obtained by diverse extraction conditions using three different techniques (hydrothermal, ultrasounds and supercritical CO₂), *Environ Eng Manag J*, 2019, **18**(7), 1535–1542.
- Roselló-Soto E, Barba F J, Lorenzo J M, Munekata P E S, Gómez B, *et al.*, Phenolic profile of oils obtained from horchata by products assisted by supercritical-CO₂ and its relationship with antioxidant and lipid oxidation parameters: Triple TOF-LC-MS-MS characterization, *Food Chem*, 2019, **274**, 865–871.
- Rebaya A, Belghith S I, Baghdikian B, Leddet V M, Mabrouki F, *et al.*, Total phenolic, total flavonoid, tannin content, and antioxidant capacity of *Halimium halimifolium* (Cistaceae), *J Appl Pharm Sci*, 2016, **5**(1), 52–57.
- Borrás-Linares I, Fernández-Arroyo S, Arráez-Roman D, Palmeros-Suárez P A, Del Val-Díaz R, *et al.*, Characterization of phenolic compounds anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*), *Ind Crops Prod*, 2015, **69**, 385–394.
- Ashwathanarayana R and Naika R, Antioxidant and cytotoxic properties of *Pavatta crassicaulis* Bremek, leaf crud extract and its isolated pure compound, *Indian J Nat Prod Resour*, 2017, **8**(4), 335-350.
- Chhetri A, Pokhrel Y, Shahi N, Lama D and Chhetri D R, Antioxidant and cytotoxic activities of *Isaria amoenerosea* Henn.: An entomopathogenic fungus from Darjeeling Hills, Eastern Himalaya, *Indian J Nat Prod Resour*, 2019, **10**(2), 111-118.
- Caleja C, Ribeiro A, Barreiro M F and Ferreira I C F R, Phenolic compounds as nutraceuticals or functional food ingredients, *Curr Pharm Des*, 2017, **23**, 2787-2806.
- Bhuyan D J and Basu A, Utilisation of bioactive compounds derived from waste in the food industry, In *Utilisation of bioactive compounds from agricultural and food production waste*; edited by Vuong Q V, (CRC Press: Boca Ratón, FL, USA), 2017, 342-357.

- 35 Selamoglu Z, Akgul H and Dogan H, Environmental effects on biologic activities of pollen samples obtained from different phytogeographical regions in Turkey, *Fresenius Environ Bull*, 2016, **25**(7), 2484-2489.
- 36 Anggraini T, Wilma S, Syukri D and Azima F, Total phenolic, anthocyanin, catechins, DPPH radical scavenging activity, and toxicity of *Lepisanthes alata* (Blume) Leenh, *Int J Food Sci*, 2019, **3**, 1-7.
- 37 Tan C, Celli G B, Selig M J and Abbaspourrad A, Catechin modulates the copigmentation and encapsulation of anthocyanins in polyelectrolyte complexes (PECs) for natural colorant stabilization, *Food Chem*, 2018, **264**, 342–349.
- 38 Ghaffari Z, Rahimmalek M and Sabzalian M R, Variations in essential oil composition and antioxidant activity in *Perovskia abrotanoides* Kar, Collected from different regions in Iran, *J Chem Biodivers*, 2018, **15**(6), e1700565.
- 39 Hadian V, Bigdeloo M, Nazeri V and Khadivi-Khub A, Assessment of genetic and chemical variability in *Thymus caramanicus*, *Mol Biol Rep*, 2014, **41**, 3201–3210.
- 40 Ale Omrani Nejad S M H, Badi H N, Mehrafarin A, Abdossi V and Khalighi-Sigaroodi F, The impact of macro environmental factors on essential oils of *Oliveria decumbens* Vent. from different regions of Iran, *Jundishapur J Nat Pharm Prod*, 2019, **14**(2), e59456.
- 41 Dehghani M S, Naeemi M, Gholamalipour Alamdari E and Jabbari H, Effects of chitosan foliar application on quantitative and qualitative characteristics of German chamomile (*Matricaria chamomilla* L.) under water deficit stress conditions, *Iran J Med Aromat Plants*, 2019, **35**(1), 121-133