

Research Article

A study on some secondary metabolites and quantity and quality of essential oil of *Marrubium vulgare* L. grown in Guynik district (North Khorasan province)

Pooya Arvin* and Rana Firouzeh

Department of Agriculture, Payame Noor University, Tehran, Iran
(Received: 18/10/2022 - Accepted: 24/12/2022)**Abstract**

To evaluate the biological potential and medicinal properties, the biochemical compositions and quantity and quality of essential oil of *Marrubium vulgare* L., grown in the natural habitat located in Guynik region, North Khorasan province, were investigated. The content of secondary metabolites including phenol and flavonoids, and antioxidant capacity were measured in laboratory methods. Essential oil of plant was obtained by hydro distillation method and dehydrated with anhydrous sodium sulfate, then it was quantitatively and qualitatively analyzed by gas chromatography (GC) and gas chromatography / mass spectrometry (GC/MS). The results showed that the essential oil yield was 0.24% (w/w), and oxygenated monoterpenes made up 52.40% of essential oil. Also, 18 compounds were identified in the essential oil of *M. vulgare* plant that the main compounds were dodecanal (23.02%), β -bisabolene (15.65%) and thymol (9.60%). In this study, the phenol content of extract of *M. vulgare* was 72.01 (mg Gallic acid/g Dry weight), the flavonoids content was 6.25 (mg Quercetin/g Dry weight) and its antioxidant capacity was 81.12 ($\mu\text{g/ml}$). Overall, based on the results, it seems that *M. vulgare* grown in Guynik region can be a potential and rich source of natural antioxidant compounds.

Key words: Antioxidant capacity, Essential oil yield, *Marrubium vulgare* L., Phenol and flavonoid content

Introduction

The use of medicinal plants is common among people in most countries of the world. *Marrubium* is known as a medicinal plant from a genus of flowering plants in the Lamiaceae family (Mozaffarian, 2008). This plant includes about 97 species found along the Mediterranean, Asia, America, Australia, and temperate regions, also, nine of them are endemic in Iran. *Marrubium vulgare* L. is a perennial plant, herbaceous, C₃, with a height of 100 cm and stems that usually branched to form a rounded bushy (Golparvar *et al.*, 2015). Its leaves are oval and opposite to each other along the stem, and the flowers are also white and crowded in dense whorls (Mozaffarian, 2008).

M. vulgare plant contains various polyphenols and flavonoids and compounds such as apigenin, urosilic acid, betasisterol, luteolin, marobium, pectin and ascorbic acid, which have high antioxidant properties (Herrera-Arellano *et al.*, 2004). Many extensive pharmacological studies have shown that *M. vulgare* displays a series of activities including pain reliever, antioxidant, cardioprotective, reduction in tension of the blood vessel walls, gastro protective, anti-spasmodic, immune modulators, and anti-diabetic properties (Mnonopi *et al.*, 2012; Mnonopi *et al.*, 2011; Paula de Olivera *et al.*, 2011; Acimovic *et al.*, 2020; Salaj *et al.*, 2018). The research of Sahpaz *et al.* 2002 has shown that phenylpropanoid esters extracted from white

marrubium have antigenic properties through the ability of cyclooxygenase. The blood pressure lowering effect of this plant and the correction of anorexia, antitussive and its use in the treatment of acute bronchitis and abuse have also been confirmed (Acimovic *et al.*, 2020).

Although the human body has inherent antioxidant mechanisms to deal with the destructive effects of oxidative stress and reactive oxygen species produced during it, there is often a need to use diet or medicinal antioxidant supplements, especially during disease attacks as protective factors (Atmani *et al.*, 2009), therefore, the efforts related to the search for antioxidants from natural sources, especially medicinal plants, have increased today (Ayaz *et al.*, 2014). *M. vulgare* plant is expected to be a suitable option for the treatment or prevention of such diseases related to oxidative stress due to its high antioxidant capacity and strong potential in removing and scavenging free radicals (Abadi and Hassani, 2013; Yabrir, 2019). Many reports have shown that this plant has a high potential in removing reactive oxygen species due to having a special group of chemical compounds such phenol and flavonoid compounds, which are known to have antioxidant properties (Abadi and Hassani, 2013; Paula de Olivera *et al.*, 2011; Boulila *et al.*, 2015). Mainly, phenol and flavonoids can create different medicinal properties in plants, and it depend on the type of composition, the presence of the number of chemically

*Corresponding Author, Email: pooya.arvin@pnu.ac.ir

active groups, the presence of sugars or amino acids in their structure and also the spatial form of active groups in structure of phenol and flavonoids (Faramarzi Dozein *et al.*, 2022; Sweidan and Abu Zarga, 2016).

The essential oil of *M. vulgare* plant has special medicinal and antioxidant properties due to the presence of diverse and aromatic compounds. In the analysis of the essential oil obtained from the branches of this plant, a set of chemical compounds have been identified. In Algeria, Abadi and Hassani (2013) reported the main components of the oil of *M. vulgare* were tetramethyl heptadecan-4-ol (16.97 %), germacrene D-4-ol (9.61%), α -pinene (9.37 %), phytol (4.87%), dehydro-sabina ketone (4.12 %), piperitone (3.27%), δ -cadinene (3.13%), 1-octen-3-ol (2.35%) and benzaldehyde (2.31%). In Tunisian, Hamdaoui *et al.* (2013) reported the main components of the oil of *M. vulgare* were β -bisabolene (28.3%), *E*- β -farnesene (7.4%) and β -caryophyllene (7.8%). In Egypt, Salama *et al.* (2012) reported the main components of the oil of *M. vulgare* were thymol and γ -cadinene. In Iran, Asadipour *et al.* (2005) found that caryophyllene oxide (18.7%), β -caryophyllene (12.8%) and germacrene D (10.0%) were the major compounds of *M. vulgare* collected from Khaneshorkh of Sirjan. Also, Khanavi *et al.* (2006) showed that the major component of *M. vulgare* from Dashte Naz of Sari in Mazandaran province were β -bisabolene (25.4%), β -caryophyllene (11.6%), germacrene D (9.7%) and *E*- β -farnesene (8.3%). In order to investigate the effect of climate on the content of biochemical compounds and identification of active ingredients of *M. vulgare* essential oil collected from natural habitat in Guynik region, this study was carried out.

Materials and methods

Sampling of the flowering branches of *Marrubium vulgare* L. plant was done in the full flowering stage in a completely random way from one of its natural habitats located in Guynik region of North Khorasan province. Some soil characteristics of experimental location included pH= 7.5 and soil electrical conductivity (EC) = 1.12 dS.m⁻¹. Also, soil texture was sandy loam. Geographical coordinates of Guynik region was altitude =1221 meter above sea level, latitude = 37° 59' 51" N and longitude =57° 00' 56" E.

Plant sampling was done in such a way that three transects of 30 meters length were established. During each transect, 10 plots of one square meter were randomly placed and the samples of 10 plots were mixed together and considered as one sample, and finally 3 samples were prepared. Plants were identified according to the herbarium specimens of Ferdowsi University of Mashhad. The samples were dried out at shade and room temperature (22 to 25 °C) for one week. Then, the essential oil extraction was carried out.

Extraction: Extract was prepared by maceration method (Trusheva *et al.*, 2007). For this purpose, 10 g of dried powder (leaves, flowering branches) of

M. vulgare plant was added to 100 ml of methanol and stirred on a shaker for 24 hours. The obtained sample was filtered with Whatman paper and centrifuged at 10,000 rpm for 20 minutes, to remove suspended particles, completely. After removing the solvent by rotary evaporator, the obtained extract was stored in a refrigerator at 4°C until analyzed.

Total phenol assay: Total phenol content of the extract was determined using the Folin-Ciocalto reagent, according to the method described by Chun *et al.* (2003) and using gallic acid as a standard. First, 20 μ l of plant extract in the test tubes were mixed with 1.160 ml of distilled water and 100 μ l of Folin-Ciocalto reagent. After 8 min, 300 μ l of sodium carbonate solution (20% by w/v) was added to the contents of the test tube. After shaking, the test tubes were placed in a water bath with a temperature of 40 °C for 30 min, then, their absorbance were read at 760 nm and the results were expressed in terms of mg of Gallic acid per gr of extract.

Total flavonoid assay: The total flavonoids content were estimated according to the aluminum chloride colorimetric method (Chang *et al.*, 2002). In this method, 1 mg of extract (leaves, flowering branches) was dissolved in 1 ml of methanol. 0.5 ml of plant extract solution with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 molar potassium acetate and 2.8 ml of distilled water were mixed. After keeping the samples at room temperature for 30 min, the absorbance of the mixture was read at 415 nm and the results were expressed in terms of mg of Quercetin per gr of extract.

Evaluation of antioxidant activity: 50 μ l of the extract (leaves, flowering branches) were mixed with 5 ml of 0.004% DPPH solution dissolved in methanol. A mixture of methanol (without plant extract) with DPPH was used as a negative control, and a mixture of Glutathione with DPPH was used as a positive control in this experiment. After 30 min, the absorption of the samples was read at 517 nm. The inhibition percentage of DPPH free radicals was calculated using the following formula.

$$Sc (\%) = [(A_0 - A_s) / A_0] \times 100$$

A₀ = Control absorption (containing all reagents except the test sample), A_s = Absorption of the test sample, Sc (%) = DPPH free radical inhibition percentage (Burits and Bucar, 2000)

The results of this study were expressed as IC₅₀ (Half Maximal Inhibitory Concentration), which indicates the concentration of the extract that can inhibit 50% of free radicals (Khatamian *et al.*, 2019).

Extraction of the essential oil: To extract the essential oil, 30 g of the dried flowering branches were distilled with water in 3 replications using Clevenger apparatus for 3 hours and then dehydrated with anhydrous sodium sulfate. Essential oils were stored in capped dark jars and stored in refrigerator until analysis. The percentage of essential oil yield of the samples was calculated based on the weight of essential oil to the dry

weight of the plant material using the following formula.

Percentage yield of essential oil = weight of essential oil/dry weight of the plant \times 100

Identification of essential oil components:

Quantitative and qualitative identification of essential oil compounds was performed with gas chromatography (GC) and gas chromatography connected to mass spectrometer (GC/MS) model Shimadzu-QP2010SE equipped with Rtx-5MS column (column length 30 meters, inner diameter 0.25 mm and thickness stationary phase (0.25 μ m). The initial temperature of the oven was set at 60°C, the temperature was increased by 10 degrees every minute until the final temperature was 290°C, which remained at this temperature for 13 minutes. Helium gas was used as a carrier gas with a flow rate of 0.9 ml/min and a mass spectrometer with an ionization energy of 70 eV. Identification of the resulting spectra was done by drawing a chromatogram of a series of normal paraffins (C₅-C₃₀) under the same conditions as the sample injection. According to the inhibition time of these compounds, the inhibition index was calculated for each component in the sample chromatogram.

Results

The yield of essential oil in *M. vulgare* plant was 0.24% in terms of dry weight (w/w). Examining the obtained chromatogram and spectrum showed that there were 18 compounds in the essential oil content, which made up 92.22% of the total essential oil (Figure 1, Table 1). 52.40% of compounds were oxygenated monoterpenes, 26.21% hydrocarbon ses-qui-terpenes 8.5% oxygenated ses-qui-terpenes, and 5.11% hydrocarbon monoterpenes. Dodecanal with 23.02%, β -bisabolene with 15.65% and thymol with 9.60% were included the largest percentage of essential oil components. Carvacrol compound with 0.67% had the lowest amounts among essential oil constituents (Table 1).

Investigating biochemical contents of *Marrubium vulgare* L.: The measurement of antioxidant activity of *M. vulgare* plant extract in IC₅₀ was reported as 81.12 μ g/ml (Figure 2). The results also showed that the DPPH free radical inhibition rate was directly related to the increase in the extract concentration, in another words, the inhibition rate increased with the increase in the extract concentration. *M. vulgare* extract with IC₅₀ of about 81.12 μ g/ml was able to inhibit 50% of DPPH free radicals, while glutathione as a positive control at a concentration of 44 μ g/ml inhibited about 50% of free radicals (Figure 2). Comparison of DPPH free radical inhibition power of *M. vulgare* plant extracts with glutathione as a strong antioxidant confirms the high inhibitory effects of *M. vulgare* extract on DPPH free radical inhibition.

The results also showed that the phenol content of extract of *M. vulgare* was 72.01 (mg GA/g D.W), and the flavonoid content of the extract was 6.25 (mg QUE/g D.W).

Discussion

The medicinal properties of plant species depend on the biological compounds that are naturally present in their body. Therefore, in order to evaluate the biological potential and medicinal properties of any plant species, it is necessary to reveal their biochemical compounds first (Sabih Ozer *et al.*, 2016). On the other hand, the geographical conditions of the plant's growth environment, including soil structure, climatic conditions, and tensions in the region can affect the amount and type of biochemical compounds extracted from plants (Golparvar *et al.*, 2015; Yang *et al.*, 2018). In many studies, the effect of habitat and environmental conditions on content of secondary metabolites and herbal active ingredients has been confirmed (Abadi and Hassani, 2013; Bouterfas *et al.*, 2016; Arvin and Firouzeh, 2022). Other researchers argue that these changes may be related to various factors, such as moisture and altitude, solar radiation and temperature, which affect the physiological state of the plant, the phenolic biosynthesis and the production of antioxidants (Bouterfas *et al.*, 2016; Hayat *et al.*, 2020). Among the biochemical compounds found in *Marrubium*, can mention flavonoids, phenyl ethanoids, tannins and terpenoid compounds such as diterpenes, triterpenes, saponins and sterols (Hamedeyazdan *et al.*, 2013; Amri *et al.*, 2017; Yabrir, 2019). In the present study, 18 different chemical compounds were identified from 92.22% of the total essential oil, and dodecanal with 23.02%, β -bisabolene with 15.65% and thymol with 9.60% were the main components of essential oil in *M. vulgare* grown in the Guynik district. In the study conducted by Kadri *et al.* (2011), the main compounds obtained in the essential oil of *M. vulgare* were γ -eudesmol (11.93%), β -citronellol (9.90%), citronellyl formate (9.50%) and germacrene D (9.37%). In another research, the results indicated that major components of the oil of *Marrubium vulgare* L. collected from Kamu Mountain of Isfahan province were β -caryophyllene (32.19%), *E*- β -farnesene (11.39%), 1,8-cineole (8.17%) and α -pinene (6.64%) (Golparvar *et al.*, 2015). During studies Said-Al Ahl *et al.* (2015) reported that the major constituents of the *M. vulgare* essential oil cultivated in Egypt were carvacrol (36.28%), β - phellandrene (15.49%), as well as carvyl acetate (11.52%). Zawislak also (2012) reported that the main components of the oil of *M. vulgare* were *E*-caryophyllene (25.91–32.06%), germacrene D (20.23–31.14%) and δ -amorphene (8.38–10.22%). Although secondary metabolites in medicinal plants are basically synthesized by genetic control and guidance, their production is significantly influenced by environmental factors (Isah, 2019) and it has been repeatedly reported that the climatic conditions of habitat in different phenological stages, can change the content of secondary metabolites such as phenol, flavonoids and essential oil properties (concentration and type of compounds) of plants (Farrokhi *et al.*, 2021; Pourhosseini *et al.*, 2018). For example, during the investigation of the content of secondary metabolites of

Table 1. Percentage and type of essential oil compounds in *Marrubium vulgare* L.

NO.	Compounds	Percentage of compounds	Inhibition index RI ^{exp}	Type of compounds
1	<i>p</i> -cymene	2.78	1033	Hydrocarbon monoterpene
2	limonene	2.33	1035	Hydrocarbon monoterpene
3	decanal	6.03	1209	Oxygenated monoterpene
4	4 <i>E</i> -decen-1-ol	1.98	1240	Oxygenated monoterpene
5	<i>n</i> -decanol	3.75	1269	Oxygenated monoterpene
6	thymol	9.60	1292	Oxygenated monoterpene
7	carvacrol	0.67	1304	Oxygenated monoterpene
8	geranyl acetate	6.03	1385	Oxygenated monoterpene
9	dodecanal	23.02	1409	Oxygenated monoterpene
10	<i>E</i> -caryophyllene	1.64	1422	Hydrocarbon sesquiterpene
11	<i>E</i> - β -ionone	1.14	1482	Oxygenated monoterpene
12	β -bisabolene	15.65	1504	Hydrocarbon sesquiterpene
13	δ -cadinene	1.70	1515	Hydrocarbon sesquiterpene
14	<i>E</i> - γ -bisabolene	2.34	1532	Hydrocarbon sesquiterpene
15	<i>E</i> -nerolidol	0.82	1548	Oxygenated sesquiterpene
16	caryophyllene oxide	5.14	1580	Oxygenated sesquiterpene
17	hexahydrofarnesyl acetone	2.54	1955	Oxygenated sesquiterpene
18	<i>n</i> -tricosane	4.88	2300	Hydrocarbon sesquiterpene
Total		92.22		
Hydrocarbon monoterpene		5.11		
Oxygenated monoterpene		52.40		
Hydrocarbon sesquiterpene		26.21		
Oxygenated sesquiterpene		8.5		

RI^{exp}: experimental retention index given for RTX-5MS column in reference to *n*-alkane

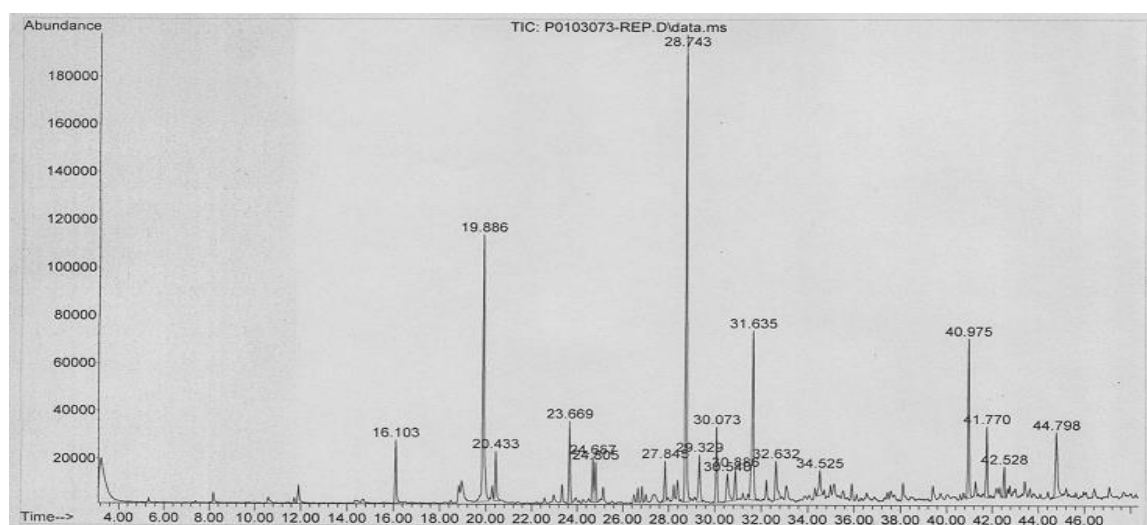


figure 1. Chromatogram related to the essential oil of *M. vulgare* plant in Guynik region

the root of licorice plant (*Glycyrrhiza glabra* L.), it was seen that the content of the secondary metabolites of the plant varied depending on different climatic and weather conditions (Oloumi and Hasibi, 2012). Also, it was found that there is a direct relationship between the environmental conditions of the habitat and the amount of phenolic and flavonoid active substances in the extracts of angelica (*Heracleum persicum*), chicory (*Cichorium intybus* L.) and artichoke (*Cynara scolymus* L.) plants (Mazandarani *et al.*, 2011). Therefore, although the mechanism of the effects of the environment on the accumulation of secondary metabolites is not clear, but it has been confirmed that the environment is effective on the production process of metabolites and related enzymes, the type and

intensity of chemical reactions and the synthesized final product (Srivastava and Shym, 2002; Arvin and Firouzeh, 2022).

Investigation of the capacity and antioxidant potential of *Marrubium vulgare* L.: Today, the effect and importance of natural antioxidants in medicinal plants have been proven in preventing or reducing oxidative damage caused by the action of free radicals and side effects of chemical drugs in the human body. Antioxidants are substances capable of delaying, slowing down and even stopping oxidation processes (Sardarodiyani and Arian Far, 2019). The antioxidant property of *M. vulgare* has been reported in the study of the antioxidant activity of sixty plants from Iran done by Souri *et al.* in 2004. In the present research, the

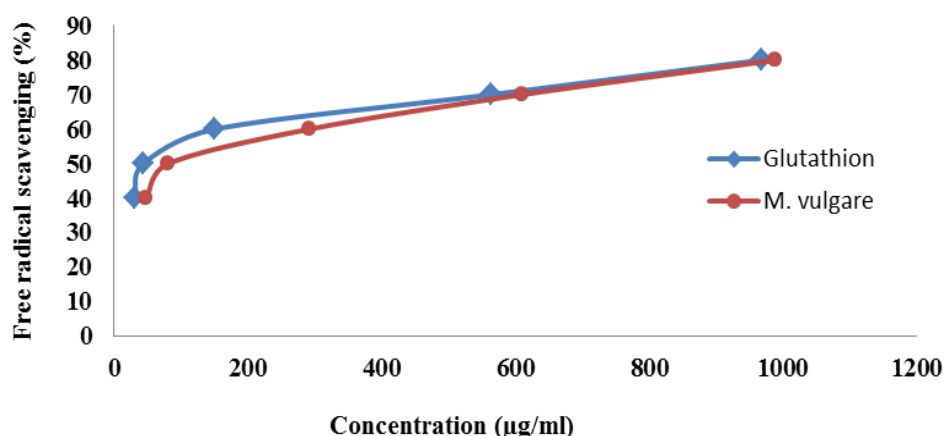


Figure 2. DPPH free radical scavenging in reaction with *Marrubium vulgare* extracts compared to glutathione

antioxidant properties of *M. vulgare* were investigated by DPPH free radical inhibition test method and the IC_{50} of its extract was reported as 81.12 µg/ml. This amount means that *M. vulgare* extract at a concentration of 81.12 µg/ml was able to inhibit half of the free radicals. IC_{50} has an inverse relationship with the antiradical activity of antioxidant compounds, so the lower the IC_{50} , the higher the antioxidant activity and potential. In a study, Jamshidi *et al.* (2010) compared the antioxidant activity of six species from Lamiaceae family, native of Mazandaran province, and concluded the *M. vulgare* species with IC_{50} of 52.55 had the highest antioxidant properties compared to other species. Chouaieb *et al.* (2013) observed in the ethanol extracts of *M. vulgare* an IC_{50} ranging between 0.25 and 0.68 µg/ml, which indicated the high antioxidant power of this plant. Also, Pukalskas *et al.* (2012) stated that the methanol extract of *M. vulgare* aerial parts has high antioxidant activity. This can be due to the presence of higher amounts of phenol and flavonoid compounds in the extract of *M. vulgare*, and according to this issue, it can be suggested to use it as a natural antioxidant to improve health in order that these compounds prevent the oxidation process of biological molecules by scavenging free radicals or mechanisms such as shutting down single oxygen (Amri *et al.*, 2017).

Investigation of the content of phenol and flavonoid compounds of *Marrubium vulgare* L.: Studies on different species of the genus *Marrubium* have shown that the presence of phenolic compounds can be considered as a special characteristics of this plant species, which is also valuable from the point of view of chemosystematics (Hennebelle *et al.*, 2007). The results of many of these studies also indicated that the potential of antioxidant of this plant was directly related to its phenolic content (Boulila *et al.*, 2015). In a research on antioxidant effect of *M. vulgare* extract was carried out, significant antioxidant activity against DPPH and ABTS+ free radicals were recorded. This activity was related to phenolic glycosides such as luteolin, 7-glucuronyl luteolin, ladanin, verbascoside

and forcytoside B, that were introduced as plant active ingredients (Pukalskas *et al.*, 2012). Vermerris and Nicholson (2006) also reported that the remarkable antioxidant effect of the plant extract is due to its complexity on polyphenols and flavonoids. In the present research, the phytochemical analysis of the methanolic extract confirmed the presence of phenol and flavonoid compounds in *M. vulgare*. The comparison of phenol and flavonoid compounds of six species from Lamiaceae family, was studied by Jamshidi *et al.* (2010) and they reported that the *M. vulgare* species with 182.23 (mg QUE/g) flavonoid content and 58.45 (mg GAE/g) phenol content had the highest amount of these compounds compared to other species. In research of Salaj *et al.* (2018) on biomedical potential of *Marrubium vulgare* extract, the phenolic content was 59.87 (mg GAE/g) and the flavonoid content was 14.47 (mg QUE/g). In the review of phenolic content and allelopathic potential of leaves extracts of White Horehound (*Marrubium vulgare* L.) were collected from national park of Djebel Zaghouan, phenol and flavonoid contents were obtained as 44.89 (mg GAE/g) and 24.60 (mg QUE/g), respectively (Dallali *et al.*, 2017). A study of Chouaieb *et al.* (2013), on the effect of geographic location on the antioxidant activities of Tunisian Horehound (*M. vulgare*), showed that the ethanolic extract had phenol content (267-325 mg GAE/g) and flavonoids (212-245 mg QUE/g). Also, the results obtained in the investigation of the content of polyphenols, flavonoids and antioxidant activities of *Marrubium vulgare* L. from two different locations in north eastern Morocco showed that the concentrations of total phenols and flavonoids varied respectively between 0.27 and 86.91 (µg GA/mg), and 6.08 and 33.82 (µg QUE/mg) (Hayat *et al.*, 2020). Overall, these results show that the environmental and climatic conditions of region affect the content of secondary metabolites such as phenol and flavonoid compounds.

Conclusions

The results of this research show that *Marrubium*

vulgare L., which grows wild in Guynik regions of North Khorasan province, has high amounts of natural antioxidant compounds, including phenol and flavonoids, thus, it seems this plant has valuable medicinal properties that can be used in food and pharmaceutical industries. Considering the medicinal importance of this plant and the effect of climatic and

environmental conditions on their value and medicinal properties, it is suggested to evaluate the medicinal and agronomic yield of this plant in other regions of the country.

References

- Abadi, A. and Hassani, A. (2013) Essential oil composition and antioxidant activity of *Marrubium vulgare* L. growing wild in Eastern Algeria. *International Letters of Chemistry, Physics and Astronomy* 9: 17-24.
- Acimovic, M., Jeremic, K., Salaj, N., Gavaric, N., Kiproviski, B., Sikora, V. and Zeremski, T. (2020) *Marrubium vulgare* L.: A phytochemical and pharmacological overview. *Molecules* 25: 1-24.
- Amri, B., Martino, E., Vitulo, F., Corana, F., Kaab, L. B. B., Rui, M., Rossi, D., Mori, M., Rossi, S. and Collina, S. (2017) *Marrubium vulgare* L. leave extract: Phytochemical composition, antioxidant and wound healing properties. *Molecules* 22: 1851.
- Arvin, P. and Firouzeh, R. (2022) Study of phenolic and flavonoid compounds and essential oil content of medicinal plant of *Proveskia abrotanoides* Karel in three natural habitats of Raz and Jargalan County. *Plant Process and Function* 47: 69-82.
- Asadipour, A., Mehrabani, M., Nazeri, V. and Tabarraii, M. (2005) Composition of the essential oil of *Marrubium vulgare* L. *Ulum-i-Daroei* 2: 77-82.
- Atmani, D., Chaher, N., Berboucha, M., Ayouni, K., Lounis, H., Boudaoud, H., Debbache, N. and Atmani, D. (2009) Antioxidant capacity and phenol content of selected Algerian medicinal plants. *Food Chemistry* 112: 303-309.
- Ayaz, M., Junaid, M., Ahmed, J., Ullah, F., Sadiq, A., Ahmad, S. and Imran, M. (2014) Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from *Polygonum hydropiper* L. *BMC complementary Medicine and Therapies* 14: 145.
- Boulila, A., Sanaa, A., Salem, I. B., Rokbeni, N., Mrabet, Y., Hosni, K. and Fernandez, X. (2015) Antioxidant properties and phenolic variation in wild populations of *Marrubium vulgare* L. (Lamiaceae). *Industrial Crops and Products* 76: 616-622.
- Bouterfas, K., Mehdadi, Z., Elaoufi, M. M., Latreche, A. and Benchiha, W. (2016) Antioxidant activity and total phenolic and flavonoids content variations of leaves extracts of white Horehound (*Marrubium vulgare* Linne) from three geographical origins. *Annales Pharmaceutiques Françaises* 453-462.
- Burits, M. and Bucar, F. (2000) Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research* 14: 323-328.
- Chang, C., Yang, M., Wen, H. and Chern, J. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Food and Drug Analysis* 10: 178-82.
- Chouaieb, H., Ayadi, I., Zouari, S., Fakhfakh, N., Zaidi, S. and Zouari, N. (2013) Effect of phenological stage and geographical location on antioxidant activities of Tunisian Horehound: *Marrubium vulgare* L. (Lamiaceae). *Journal of Biologically Active Products from Nature* 232-238.
- Chun, O. K., Kim, D. O. and Lee, C. Y. (2003) Superoxide radical scavenging activity of the major polyphenols in fresh plums. *Journal of Agriculture and Food Chemistry* 51: 867-872.
- Dallali, S., Rouz, S., Aichi, H. and Ben Hassine, H. (2017) Phenolic content and allelopathic potential of leaves and rizosphere soil aqueous extracts of white horehound (*Maribum vulgare* L.). *Journal of new Sciences, Agriculture and Biotechnology* 39: 2106-2120.
- Faramarzi Dozein, R., Karimi, A., Karimi, E., Oskoueian, E. and Ghasemi, M. (2022) Comparative study of phytochemical, antioxidant and anti-inflammatory properties of *Anethum graveolens* L., *Bunium persicum* L., *Achillea millefolium* L. and *Syzygium aromaticum* extracts. *Ecophytochemical Journal of Medicinal Plants* 4: 43-58.
- Farrokhi, E., Samadi, A. and Rahimi, A. (2021) Evaluation of antioxidant activity, total phenol and flavonoid content of lemon balm (*Melissa officinalis* L.) in different cultures under hydroponic conditions. *Ecophytochemical Journal of Medicinal Plants* 19-33.
- Golparvar, A. R., Hadipanah, A., Mehrabi, A. and Armin, A. (2015) Essential oil composition of *Marrubium vulgare* L. from Iran. *Journal of Herbal Drugs* 6: 1-5.
- Hamdaoui, B., Aidi Wannas, W., Marrakchi, M., Ben Brahim, N. and Marzouk, B. (2013) Essential oil composition of two Tunisian Horehound species: *Marrubium vulgare* L. and *Marrubium aschersonii* Magnus. *Journal of Essential Oil Bearing Plants* 16: 608-612.
- Hamedeyazdan, S., Fathiazad, F. and Asnaashari, S. (2013) Chemical composition of the essential oil from *Marrubium persicum* CA Mey. (Lamiaceae). *Pharmaceutical Sciences* 19: 35-38.
- Hayat, J., Akodad, A., Moumen, A., Baghour, M., Skalli, A., Ezrari, S. and Belmalha, S. (2020) Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. *Heliyon* 1-9.

- Hennebelle, T., Sahpaz, S., Skaltsounis, A. and Bailleul, F. (2007) Phenolic compounds and diterpenoids from *Marrubium peregrinum*. *Biochemical Systematics and Ecology* 35: 624-626.
- Herrera -Arellano, A., Aguilar Santamar, B., Garc Ia -Hern andez, B. P., Nicasio -Torres, P. and Tortoriello, J. (2004) Clinical trial of *Cecropia obtusifolia* and *Marrubium vulgare* leaf extracts on blood glucose and serum lipids in type 2 diabetics. *Science Direct* 11: 561-566.
- Isah, T. (2019) Stress and defense responses in plant secondary metabolites production. *Biological Research* 52: 39.
- Jamshidi, M., Ahmadi Ashtiani, H. R., Reza Zadeh, S. A., Fathi Azad, F., Mazandarani, M. and khaki, A. (2010) Investigation and comparison of phenolic compounds and antioxidant activity of several plant species native to Mazandaran. *Journal of Medicinal Plants* 177-183.
- Kadri, A., Zarai, Z., Bekir, A., Gharsallah, N., Damak, M. and Gdoura, R. (2011) Chemical composition and antioxidant activity of *Marrubium vulgare* L. essential oil from Tunisia. *African Journal of Biotechnology* 10: 3908-3914.
- Khanavi, M., Ghasemian, L., Motlagh, E. H., Hadjiakhoondi, A. and Shafiee, A. (2006) Chemical composition of the essential oils of *Marrubium parvi florum* Fisch. and C. A. Mey. and *Marrubium vulgare* L. from Iran. *Flavour and Fragrance Journal* 20: 324-326.
- Khatamian, N., Homayouni Tabrizi, M. and Ardalan, P. (2019) Effect of *Carum carvi* essential oil nanoemulsion on tubo cancer cells and L929 normal cells and evaluation of antioxidant activity. *Study of Medical Science* 30: 315-321.
- Mazandarani, M., Makari, S. and Bajian, G. R. (2011) Evaluation of phytochemical and antioxidant activity in different parts of *Heracleum gorganicum* Rech. in Golestan province, North of Iran. *Journal of Plant Physiology* 2: 381-388.
- Mnonopi, N., Levendal, R. A., Davies-Coleman, R. T. and Frost, C. L. (2011) The cardioprotective effects of marrubiin, a diterpenoid found in *Leonotis leonurus* extracts. *Journal of Ethnopharmacology* 138: 67-75.
- Mnonopi, N., Levendal, R. A., Mzilikezi, N. and Frost, C. L. (2012) Marrubiin, a constituent of *Leonotis leonurus*, alleviates diabetic symptoms. *Phytomedicine* 19: 488-493.
- Mozaffarian, V. (2008) A pictorial dictionary of botany botanical taxonomy Latin-English-French-Germany Persian. Germany: Koeltz Scientific Books.
- Oloumi, H. and Hasibi, N. (2012) Evaluation of secondary metabolites in roots of *Glycerizia glabra* L. in some of natural habitats in Kerman province. *Journal of Medicinal Plants* 11: 55-68.
- Paula de Olivera, A., Santin, J. R., Lemos, M., Klein, L. C. J., Couto, A. G., Bittencourt, C. M. S., Cechinel, F. and Valdir, F. A. (2011) Gastroprotective activity of methanol extract and marrubiin obtained from leaves of *Marrubium vulgare* L. (Lamiaceae). *Journal of Pharmacy and Pharmacology* 63: 1230-1237.
- Pourhosseini, S. H., Mirjalili, M. H., Nejad Ebrahimi, S. and Sonboli, A. (2018) Essential oil quantity and quality of different plant organs from *Perovskia abrotanoides* Karel in natural habitat of north khorasan province. *Journal of Plant Productions* 40: 53-62.
- Pukalskas, A., Venskutonis, P. R., Salido, S., Waard, P. and Van Beek, T. A. (2012) Isolation, identification and activity of natural antioxidants from horehound (*Marrubium vulgare* L.) cultivated in Lithuania. *Food Chemistry* 130: 695-701.
- Sabih Ozer, M., Sarikurcu, C. and Tepe, B. (2016) Phenolic composition, antioxidant and enzyme inhibitory activities of the ethanol and water extracts of *Chenopodium botrys*. *Royal Society of Chemistry* 1-21.
- Sahpaz, S., Garbacki, N., Tits, M. and Bailleul, F. (2002) Isolation and pharmacological activity of phenylpropanoid esters from *Marrubium vulgare*. *Journal of Ethnopharmacology* 79: 389-92.
- Said-Al Ahl, H. A. H., Gendy, A. S. H., Mahmoud, A. A. and Mohamed, H. F. Y. (2015) Essential oil composition of *Marrubium vulgare* L. cultivated in Egypt. *International Journal of Plant Research* 1: 138-141.
- Salaj, N., Barjaktarovic, J., Kladar, N., Gavaric, N. and Bozin, B. (2018) Biomedical potential of horehound extract (*Marrubium vulgare*, Lamiaceae). *Medicinski Pregled* 71: 21-26.
- Salama, M. M., Taher, E. E. and El-Bahy, M. M. (2012) Molluscicidal and mosquitocidal activities of the essential oils of *Thymus capitatus* HOFF. ET LINK. and *Marrubium vulgare* L. *Revista do Instituto de Medicina Tropical de Sao Paulo* 54: 281-286.
- Sardarodiyani, M. and Arian Far, A. (2019) Investigation of chemical components, antibacterial and antioxidant properties of essential oil *Artemisia khorasanica* in north khorasan area. *Journal of Innovation in Food Science and Technology* 11: 37-55.
- Souri, E., Amin, G., Dehmobed-Sharifabadi, A., Nazifi, A. and Farsam, H. (2004) Antioxidative activity of sixty plants from Iran. *Iranian Journal of Pharmaceutical Research* 3: 55-59.
- Srivastava, A. W. and Shym, S. (2002) *Citrus: Climate and soil*. International Book Distributing Company.
- Sweidan, N. I. and Abu Zarga, M. H. (2016) Acylated flavonoid glucoside from *Marrubium vulgare*. *Letters in Organic Chemistry* 13: 277-282.
- Trusheva, B., Trunkova, D. and Bankova, V. (2007) Different extraction methods of biologically active components from propolis: A preliminary study. *Chemistry Central Journal* 1: 1-4.
- Vermerris, W. and Nicholson, R. (2006) *Phenolic Compound Chemistry*. USA: Springer Editions.

- Yabrir, B. (2019) Essential oil of *Marrubium vulgare*: Chemical composition and biological activities. A review. *Natural Product Sciences* 25: 81-91.
- Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F. and Wang, Q. (2018) Response of plant secondary metabolites to environmental factors. *Molecules* 23: 762.
- Zawislak, G. (2012) Chemical composition of essential oils of *Marrubium vulgare* L. and *Marrubium incanum* Desr. grown in Poland. *Chemija* 23: 136-140.