J. BIOL. ENVIRON. SCI.,
2019, 13(39), 151-159

Original Research Article

Evaluation of Some Phytochemical Properties of Three Medicinal Plants from Northwest of Iran

Amir Rahimi[1], Gülen Özyazıcı[2*], Fatemeh Ahmadi[3] and Latifeh Pourakbar[4]

[1] Urmia University, Faculty of Agriculture, Department of Agronomy, Urmia, IRAN
[2] Siirt University, Faculty of Agriculture, Department of Field Crops, Siirt, TURKEY
[3] Urmia University, Faculty of Agriculture, Department of Soil Science, Urmia, IRAN
[4] Urmia University, Faculty of Agriculture, Department of Biology, Urmia, IRAN

Received: 13.12.2019; Accepted: 26.12.2019; Published Online: 31.12.2019

ABSTRACT

Traditional medicine has a long history of serving peoples all over the world. In Iran, because of the geographical location and climatic conditions, the large area of cultural and biological properties, there is a suitable environment for various medicine plants growth. The aim of this study was evaluate some phytochemical properties of three common medicinal plants from northwest of Iran. Standard methods were used to determine the protein percentage, oil content, essential oil percentage, total phenol and flavonoid contents. In *Trigonella foenum graecum* L. seeds showed statistically significant differences in protein and total phenol contents according to regions. In *Nigella sativa* L. seeds, protein, oil, essential oil and total phenol contents were obtained significant differences between studied regions. In total phenol and total flavonoid contents of *Lallemantia iberica* Fisch. et C.A. Mey. seeds were found significant differences according to regions. Different climate changes significantly affect the phytochemical composition of the plants that should be attended in medical studies. Identification and introducing of medicinal plants flora and traditional uses of these plants, provide useful information concerning the distribution and medicinal plants usage in the region and causes to various pharmacological activities in connection with this matter.

Keywords: Medicinal plants, Phytochemical properties, *Trigonella foenum graecum* L., *Nigella sativa* L., *Lallemantia iberica* Fisch. et C.A. Mey.

INTRODUCTION

Traditional medicine has a long history of serving peoples all over the world. In many countries and cultures of different nations, the use of medicinal plants to treat diseases and maintain public health is highly prevalent (Amirmohammadi *et al.*, 2014). Natural products play an important role in the field of new drugs research and development since years ago. In Iran, because of the geographical location and climatic conditions, the large area of cultural and biological properties, there is a suitable environment for various medicine plants growth (Yuan *et al.*, 2000). Different regions of Iran have different cultures and customs in the use of medicinal plants and thus for gathering valuable information in the field of medicinal plants among the tribes, further investigation is needed (Nazemi *et al.*, 2012). Especially, northwest of Iran is among those regions with a long and rich history in traditional medicine, as shown by 'The Canon of Medicine' of Avicenna or 'The Continents' by Rhazes (Forouzan *et al.*, 2012). Some early Iranian physicians were already engaged in the study and treatment of cognitive disorders and successfully used different plants to treat these diseases in northwest of Iran (Bahmani *et al.*, 2012). The *Trigonella foenum graecum* L., *Nigella sativa* L., and *Lallemantia iberica* Fisch. et C.A. Mey. are three main medicinal plants that widely grown in northwest of Iran (Zargari, 1998). Several studies has reported the medicinal effects of the species (Zolfeghari *et al.*, 2012). Studies show that *T. foenum graecum* seeds have antioxidant properties. Supplementation of *T. foenum graecum* seed powder in the diet leads to a reduction in biomarkers of oxidative damage in alloxandiabetic rats (Aljabre *et al.*, 2015). Further *T. foenum graecum* seed polyphenols prevented oxidative hemolysis and lipid peroxidation induced by H_2O_2 in vitro in human erythorcytes (Cherkaoui-Tangi *et al.*, 2016). The promotion of antioxidants as food additives or as therapeutic agents requires proof on their efficacy in various in vitro systems besides their pharmacological, toxicological properties and in vivo benefits. The antioxidant activity of the seeds in vivo and the traditional medicinal uses has prompted us to investigate the in vitro antioxidant activity of the seeds in detail (Beheshti *et al.*, 2016). Much work has been done on the beneficial effects of *T. foenum graecum* seeds in diabetic and hypercholesterolemia states (Al-Gayyar *et*

* Corresponding author: gulenozyazici@siirt.edu.tr

al., 2016). The *N. sativa* commonly known as black seed or black cumin, is used in folk medicine as a natural remedy for a number of disease and condition such as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and gastrointestinal disturbances (Farooqui *et al.*, 2017). Furthermore, modern pharmacological and toxicological studies have demonstrated that crude extracts of the seeds and some of its active constituents (essential oil, thymoquinone) might have protective effect against nephrotoxicity and hepatotoxicity induced by either disease or chemicals (Gholamnezhad *et al.*, 2016). Very interesting is the isolated oil of the oilseed crop of *Lallemantia*, better known as lberian dragonhead, showing a very high content of linoleic acid exceeding that of linseed oil, and showed high theoretical iodine values. Unsaturation in the oils were used to introduce epoxides by epoxidation with in situ generated proxy acetic acid (Ghannadi *et al.*, 2015). Nowadays herbal science has advanced and medicinal plants along with chemical drugs are used to treat some diseases (Sivanesan *et al.*, 2016). During the past decade the use of complementary medicines, such as herbal medicinal substances in dementia therapy, has been studied (Bose *et al.*, 2019) based on traditional medicine, which has been practiced in many parts of the world. The knowledge of these important sources could profitably apply to allopathic science (Khan *et al.*, 2016). Knowledge of the phytochemical properties of medicinal plants is essential to improve their medicinal effect and facilitate the design of harvesting, processing, and storing of the seed. Various types of cleaning, grading and separation equipment may be designed on the basis of the physical properties of the seed. So, the purpose of this study was to determine and compare of some phytochemical properties of three types of local medicinal plants seeds (*T. foenum graecum*, *N. sativa* and *L. iberica***)** collected from different regions northwest of Iran.

MATERIALS AND METHODS

The seeds (*T. foenum graecum, N. sativa, and L. iberica***)** used in this study were obtained from the local markets in different parts of northwest of Iran. The samples were cleaned manually to get rid of all foreign matter, broken and immature seeds. The initial moisture content of seeds was determined by oven drying at 105±1 ºC for 24 h (Selvi *et al.*, 2006). The seeds at the different moisture levels were prepared by adding calculated quantity of water mixing thoroughly and then sealing in separate polyethylene bags. The seeds were kept at 5 ºC in a refrigerator for a week to allow the moisture to distribute uniformly throughout the sample. Before each test, the required quantities of the samples were taken out of the refrigerator and allowed to warm up to room temperature. Laboratory experiments were performed in agriculture department of Urmia University.

The extraction with ether method was used for measure of total oil content (Fokina *et al.*, 2018). One gram of each samples were transferred in test tubes and 10 mL ether were added them, again. Tubes were placed in 40 ºC for 12 h and above solutions were transferred in balanced tubes. Tubes were placed in 40 ºC oven for 4 h to evaporation. Weight difference of tubes before and after experience was used for oil content. Measuring of the total phenolic compounds in flowers was performed by Folin- Ciocalteau method adapted from Singleton *et al.* (1999). in details, 10 µL of methanolic extracts and 1600 µL of distilled water was mixed together then 200 µL of Folin-Ciocalteau reagent (10% V/V prepared in distilled water) were added and left at 25°C for 5 min, then 200 µL of sodium carbonate (7.5%) was added and kept for 30 min (at 25°C in dark place). The absorbance of the solution was determined at 760 nm using a spectrophotometer (DB-20/DB-20S UV/Visible Spectrophotometer, USA) for quantitative analysis of TPC, the gallic acid was used as an external standard, and TPC was expressed as mg gallic acid g^{-1} DW.

The analysis of total flavonoid content in the extracts was carried out by aluminum chloride colorimetric method. Briefly, 30 µL of the extract was mixed with 150 µL of sodium nitrate (5% W/V) and was allowed to stand for 5 min, followed by the addition 3 mL of Aluminum chloride hexahydrate (10% W/V) and incubated for 5 min, Then, 1 mL of NaOH (1.0 M) was added and the mixture was diluted to the mark with distilled water. After incubation at 25°C in dark place for 30 min, the absorbance of the solution was measured at 510 nm by spectrophotometer. For the quantification of TFC, the Quercetin (QE) was used as an external standard, and TFC was expressed as mg QE g^{-1} DW (Chantiratikul *et al.*, 2009). The radical scavenging activity of extracts was evaluated using the colorimetric method described by Brand-Williams et al (1995). Briefly, 15 µL of methanolic extract was mixed with 2.0 mL of the DPPH solution and the mixture was incubated in dark place at 20°C for 30

min. Then the absorbance of the solution was measured at 517 nm. The following equation was used to calculation of DPPH inhibition (Khalighi-Sigaroodi *et al.*, 2012):

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad \text{Eq. (1)}$$

Where $A_{control}$ and A_{sample} are the absorbance of the control and the sample respectively.

Super oxide radical scavenging activity of samples was determined according to Jing and Zhao (1995). Briefly, 1 ml of extract was added to 9 ml of 5 mM Tris-HCl buffer (pH 8.2). Then, 40 µL of 4.5 mM pyrogallol was added to the mixture. The mixture was shaken for 3 min and the absorbance of the solution was measured at 420 nm by spectrophotometer (Similar concentration extract was used as the blank to eliminate interference). Super oxide radical scavenging activity was expressed by the oxidation degree of a test group in comparison to that of the control. The percentage of scavenging effect was calculated using the following equation (Ebrahimzadeh *et al.*, 2008):

$$\text{Super oxide radical scavenging (\%)} = [(A_0 - A_1/A_0)] \times 100 \quad \text{Eq. (2)}$$

Where A_0 is theabsorbance of the Tris-HCl buffer with pyrogallol, $A_1$is the absorbance of the extract addition.

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction (Garrat, 1964). In this assay, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). 3 mL of the solution containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL) and Artemisia vulgaris extract (25 to 125 mg/mL) or standard solution (rutin, 0.5 mL) was incubated at 25°C for 150 min. After incubation, 0.5 mL of the solution mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and left for 5 min for completing diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochloride was added, mixed and left for 30 min at 25°C. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions were measured at 540 nm against the corresponding blank solutions using spectrophotometer. The following equation was used to calculation of nitric oxide radical inhibition (Fadzai Boora *et al.*, 2013):

$$\text{Nitric oxide radical inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad \text{Eq. (3)}$$

Where $A_{control}$ is absorbance of control sample and A_{sample} absorbance in the presence of the samples of extracts or standards.

Dried seeds were subjected to hydrodistillation for 3 h in 500 mL water, using a Clevenger-type apparatus for determination of essential oil in all samples. Protein percentage and oil content percentage were determined according to standard method (Ram *et al.*, 2005).

All experimental sections were performed in triplicate, results were expressed as mean ± SE. Analysis of variance was performed by ANOVA procedure, and significant differences were calculated according to Duncan's multiple range tests ($p < 0.05$) using SAS (version 9.1.3) software.

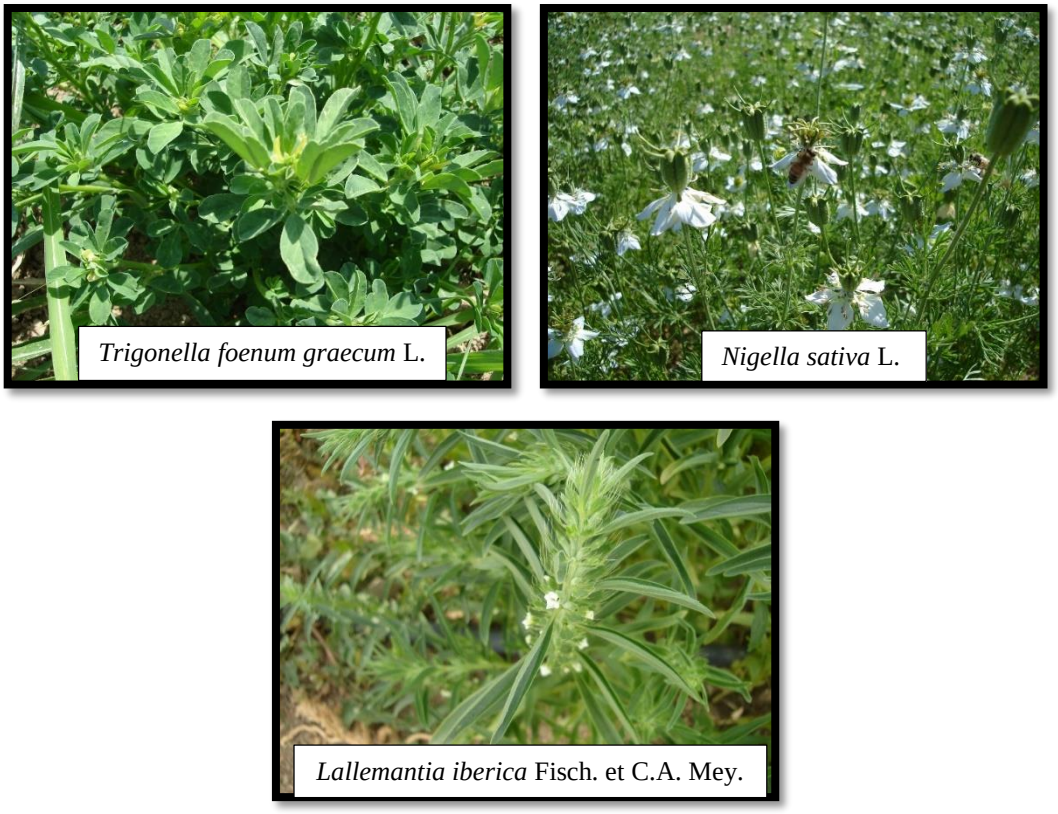

Figure 1. Studied medicinal plants.

RESULTS AND DISCUSSION

Phytochemical properties of studied *T. foenum graecum*, *N. sativa, and L. iberica* from different regions of northwest of Iran were shown in Tables 1, 2, and 3 respectively. The results obtained from our study indicates that there was not very significant differences between each phytochemical properties in each studied area (Tables 1, 2, and 3). According to Table 1, the fenugreek grown in Tekab (22.57 %), Marand (22.52 %) and Bokan (20.94 %) showed the highest protein. There were not statistically significant differences in essential oil and oil contents in *T. foenum graecum* between different regions. Oil content in *T. foenum graecum* ranged from 13.19 to 14.87 %. Essential oil ranged from 0.11 to 0.15 %. The highest total phenol content was determined in *T. foenum graecum* seeds collected from Tekab (19.90 mg GAE g^{-1} DW), Marand (19.30 mg GAE g^{-1} DW) and Bokan (18.25 mg GAE g^{-1} DW) regions. According to different regions, total flavonoid content ranged from between 4.78 and 5.52 mg QE g^{-1} DW (Table 1, Figure 2). Comparing of our results with other studies on Turkey showed that the 2 times higher amounts of total phenolic and flavonoid contents in Iranian grow studied plants than other species (Alves *et al.*, 2016).

In *N. sativa*, statistically significant differences were found in phytochemical properties except for total flavonoid. The highest protein content was found in *N. sativa* seeds collected from Marand (12.29 %), Urmia (11.81 %) and Tekab (10.36 %) regions respectively. Oil content and total phenol contents are similar to protein content. The highest essential oil content was found in *N. sativa* plants grown in Marand (0.55 %) and Urmia (0.42 %) regions. Total flavonoid content of *N. sativa* seeds varied between 1.61-1.94 mg QE g^{-1} DW (Table 2, Figure 3). The average values of some phytochemical properties of *L. iberica* seeds are given in Table 3. The *L. iberica* seeds collected from different regions of Iran were not statistically significant difference in terms of protein, oil and essential oil contents. However, the highest protein content was determined in seeds collected from Urmia (12.47%) regions numerically. Oil and essential oil contents of *L. iberica* seeds varied between 23.87-26.91% and

0.13-0.15%, respectively. A statistically significant difference was found between regions in terms of total phenol and flavonoid contents. The highest total phenol content was determined in plants grown in Tekab (17.57 mg GAE g^{-1} DW) and Urmia (17.25 mg GAE g^{-1} DW) regions. The highest total flavonoid content in Tekab (5.03 mg QE g^{-1} DW), Urmia (4.99 mg QE g^{-1} DW) and Marand (4.58 mg QE g^{-1} DW) was found in the seeds of plants grown in the region. The results were shown in Table 3 and Figure 4. Genetic background and growth conditions may be responsible for phenolic compounds changes in different species (Veberic, 2016). Environmental factors (such as soil composition, temperature, rainfall, and ultraviolet radiation) are the most effective factors on the phenolic content (Ghasemzadeh and Ghasemzadeh, 2011).

Increasing the area under cultivation of common oilseeds, identifying and cultivating new sources is a necessary step to provide oil. Oil and fat are the main components of food, with a gram of about 9.2 kcal of energy producing a good taste in the body (Ram *et al.*, 2005). Recently, with the growth of public knowledge, people's demand for oils that are useful in addition to providing energy and creating a healthier taste has increased (Carrier *et al.*, 2003). The oil seeds contain betaine, trimethylglycine, and a large amount of oil that has been implicated in the anti-inflammatory and anti-hepatitis effects of the extract (Kim *et al.*, 2009). Previous researches have been reported the anti-hepatitis effect of oil seeds (Sivanesan *et al.*, 2016). Also, oil from marigold seed contains high levels of certain nutrients such as phospholipid and E supply (Capasso *et al.*, 2009; Doehmer *et al.*, 2011).

Various studies have shown that silymarin is widely used to combat oxidative stress due to its antioxidant and protective properties against a variety of free radical species (Khan *et al.*, 2009; Doehmer *et al.*, 2011). Phenolic compounds with antioxidant and antiradical properties can play an important role in preserving food products and maintaining human health (Capasso *et al.*, 2009). Due to the native nature of marigold in Iran and its easy and inexpensive access to food and medicinal use of this plant from time immemorial in Iran, the study of phenolic and flavonoid compounds could be a prelude to the practical use of this plant extract as an antioxidant in industry. Be food and medicinal so as to enable both a convenient and affordable source of food and to promote health and food security (Ram *et al.*, 2005). It is well known today that oxidative degradation caused by the activity of these molecules causes and promotes a number of chronic diseases such as cardiovascular disease, cancer disease (Carrier *et al.*, 2003). Antioxidant compounds are needed to counteract the toxic effect of oxygen free radicals. Plant cells usually use enzymatic antioxidant systems such as super oxidase dismutase, catalase, antioxidant metabolites, phenol, etc. to solve this problem (Carrier *et al.*, 2003; Kim *et al.*, 2009).

The results of radical scavenging activity of *T. foenum graecum*, *N. sativa* and *L. iberica* seeds are given in Tables 4, 5 and 6. According to Table 4, the highest DPPH was determined in the seeds of Tekab region and the seeds of the other regions were statistically in the same group. There were not differences between the regions in terms of nitric oxide and super oxide radical scavenging activities (Table 4). There was a statistically significant difference between the regions in terms of nitric oxide radical inhibition and super oxide radical scavenging activity in *N. sativa* seeds. Seeds of Tekab (29.65 %) and Marand (25.00 %) were the first group statistically in terms of nitric oxide radical inhibition. In terms of super oxide radical scavenging activity, there was no statistically significant difference between the regions except the seeds of the Marivan region (Table 5).

In terms of DPPH and nitric oxide radical inhibition, *L. iberica* seeds collected from Tekab and Bokan regions gave better results than other regions. The lowest superoxide radical scavenging activity was determined in the seeds of *L. iberica* collected from Urmia region.

Active oxygen radicals can attack the best cellular constituents such as fatty acids, proteins, nucleic acids and pigments (Capasso *et al.*, 2009). Oxygen radicals are capable of destroying cell membrane lipids, proteins, and hereditary substances (Khan *et al.*, 2009). It is well known today that oxidative degradation caused by the activity of these molecules causes and promotes a number of chronic diseases such as cardiovascular disease, cancer disease (Carrier *et al.*, 2003). Antioxidant compounds are needed to counteract the toxic effect of oxygen free radicals. Plant cells usually use enzymatic antioxidant systems such as super oxidase dismutase, catalase, antioxidant metabolites, phenol, etc. to solve this problem (Carrier *et al.*, 2003; Kim *et al.*, 2009). Oxidative stress is caused by the overproduction of free radicals and reactive oxygen species and the weakening of the antioxidant system due to the low production of endogenous antioxidants (Ram *et al.*, 2005).

Table 1. Average of some phytochemical properties of *Trigonella foenum graecum* L.

Study area	Protein (%)	Oil content (%)	Essential Oil (%)	Total phenol (mg GAE g^{-1} DW)	Total flavonoid (mg QE g^{-1} DW)
Marivan	19.03 [b]	14.04	0.11	16.73 [b]	4.93
Tekab	22.57 [a]	14.87	0.15	19.90 [a]	5.52
Marand	22.52 [a]	13.19	0.12	19.30 [a]	5.27
Bokan	20.94 [a]	13.36	0.14	18.25 [a]	4.92
Urmia	19.24 [b]	13.68	0.12	17.40 [b]	4.78

Different letters indicate significant differences at P < 5%.

Table 2. Average of some phytochemical properties of *Nigella sativa* L.

Study area	Protein (%)	Oil content (%)	Essential oil (%)	Total phenol (mg GAE g^{-1} DW)	Total flavonoid (mg QE g^{-1} DW)
Marivan	8.94 [b]	34.73 [b]	0.36 [b]	4.38 [b]	1.65
Tekab	10.36 [a]	42.07 [a]	0.39 [b]	6.52 [a]	1.94
Marand	12.29 [a]	42.34 [a]	0.55 [a]	6.24 [a]	1.76
Bokan	9.61 [b]	36.60 [b]	0.36 [b]	4.27 [b]	1.61
Urmia	11.81 [a]	39.73 [a]	0.42 [a]	5.35 [a]	1.66

Different letters indicate significant differences at P < 5%.

Table 3. Average of some phytochemical properties of *Lallemantia iberica*.

Study area	Protein (%)	Oil content (%)	Essential oil (%)	Total phenol (mg GAE g^{-1} DW)	Total flavonoid (mg QE g^{-1} DW)
Marivan	10.25	24.87	0.14	11.47 [b]	3.29 [b]
Tekab	11.26	25.71	0.15	17.57 [a]	5.03 [a]
Marand	12.32	23.87	0.13	13.65 [b]	4.58 [a]
Bokan	10.56	26.91	0.15	13.05 [b]	3.99 [b]
Urmia	12.47	25.15	0.15	17.25 [a]	4.99 [a]

Different letters indicate significant differences at P < 5%.

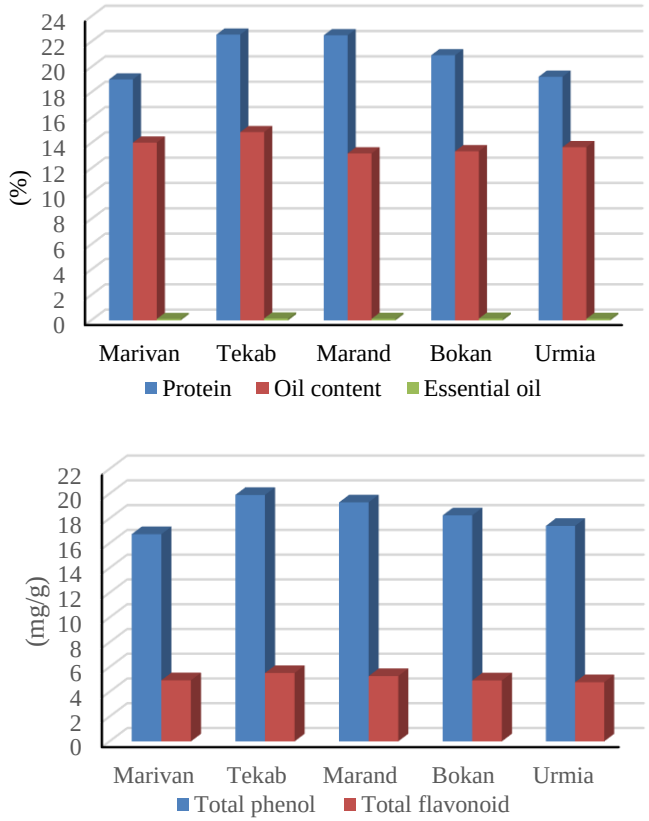

Figure 2. Comparison of different phytochemical properties of *Trigonella foenum graecum* among studied sites.

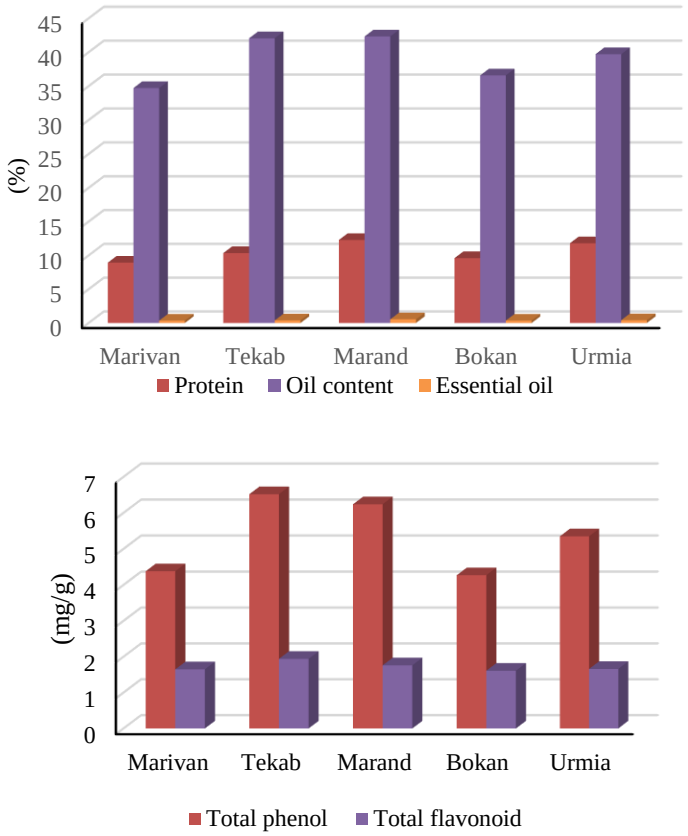

Figure 3. Comparison of different phytochemical properties of *Nigella sativa* among studied sites.

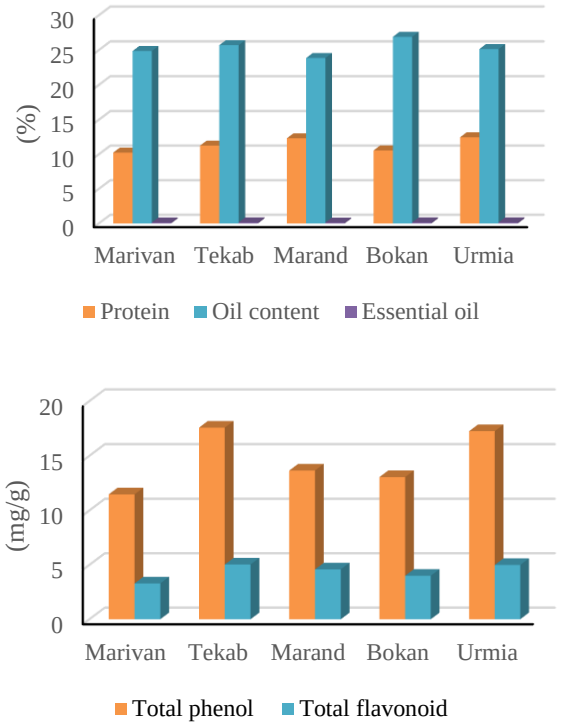

Figure 4. Comparison of different phytochemical properties of *Lallemantia iberica* among studied sites.

Table 4. Radical scavenging activity of *Trigonella foenum graecum* L.

Study area	DPPH (%)	Nitric oxide (%)	Super oxide (%)
Marivan	24.82 [b]	42.32 [a]	57.36 [a]
Tekab	32.48 [a]	48.97 [a]	57.71 [a]
Marand	25.75 [b]	49.67 [a]	57.25 [a]
Bokan	22.19 [b]	41.47 [a]	57.17 [a]
Urmia	25.38 [b]	41.39 [a]	58.14 [a]

Different letters indicate significant differences at P < 5%.

Table 5. Radical scavenging activity of *Nigella sativa* L.

Study area	DPPH (%)	Nitric oxide (%)	Super oxide (%)
Marivan	13.23 [a]	18.47 [b]	36.21 [b]
Tekab	14.81 [a]	29.65 [a]	43.29 [a]
Marand	14.47 [a]	25.00 [a]	40.37 [a]
Bokan	13.16 [a]	20.31 [b]	38.12 [a]
Urmia	12.96 [a]	20.59 [b]	39.61 [a]

Different letters indicate significant differences at P < 5%.

Table 6. Radical scavenging activity of *Lallemantia iberica* L.

Study area	DPPH (%)	Nitric oxide (%)	Super oxide (%)
Marivan	27.95 [b]	25.02 [c]	40.21 [a]
Tekab	40.49 [a]	43.93 [a]	43.54 [a]
Marand	33.41 [b]	32.22 [b]	41.63 [a]
Bokan	39.41 [a]	38.67 [a]	41.44 [a]
Urmia	29.04 [b]	31.31 [b]	36.24 [b]

Different letters indicate significant differences at P < 5%.

CONCLUSIONS

Some phytochemical properties of three types of local medicinal plants of northwest of Iran (*T. foenum graecum* L., *N.sativa* L. and *L.iberica*) were evaluated. According to results the *T. foenum graecum*. grown in Tekab, Marand and Bokan regions showed the highest phytochemical properties, including protein and total phenol contents in compare with *T. foenum graecum* L. grown in other two regions. Comparing of the studied plants in phytochemical properties affected the higher phytochemicals in *N.sativa* than other. The highest content of total phenol and flavonoid content in *L.iberica* were observed in Tekab, followed by Urmia, Marand, Bokan, and Marivan respectively. In terms of DPPH and nitric oxide radical inhibition, *L.iberica* seeds collected from Tekab and Bokan regions gave better results than other regions. The lowest superoxide radical scavenging activity was determined in the seeds of *L. iberica* collected from Urmia region. In comparison with other researches of other countries it can conclude that different climate changes significantly affect the phytochemical composition of medicinal plants that should be attended in medical studies. This limited study is important in terms of providing a source for future pharmacological and phytochemical studies.

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