



The study of environment conditions on the chemical composition of medicinal species *Nepeta Menthoides* of Sabalan

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Abstract

Given the medicinal importance of the *Nepeta menthoides* and to prevent natural extinction of mentioned species, it is necessary to be examined as soon as possible the amount of be at risk and the main reasons for the extinction of species using new methods and also appropriate approaches must be considered in order to develop natural habitats of species. *Nepeta menthoides* species is an herbaceous and perennial plant from the family of Mint that is native to Iran and it is dispersal in northwestern of Iran. Habitat of this species is at Sabalan altitudes in Shabil and Ghotoursoei in Ardabil. In this Lab, the target seeds were collected from selected habitats at the appropriate time at the end of the growing season and in the seed stage. And they were prepared after cleaning in order to planting in the following year under ideal conditions. Plowing, leveling and preparation of three pieces of land were conducted in three parts of the province (Ardabil - Meshkinshahr - Khalkhal) in the autumn of that year and then collected seeds were planted in the center greenhouse and in the separate boxes in March of that year. The plants were transferred to plastic pots in the four leaves stage and were ready for planting. In early spring, the pots were transferred to the target regions and were cultured in a randomized complete block design with three replications. Generally it can be said that the yields of essential oils under normal conditions has been variable between 1.6 to 2.5 percent so that the highest percentage of the area has been Shabil. The number of chemical composition in the stations, Khalkhal, Ardabil and Meshkinshahr have been respectively 45, 39 and 37 and the highest chemical composition has been related to 1 and 8 Cineole with 39.9 percent in Meshkinshahr station.

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Introduction

Medicinal herbs have special material called active ingredients that nowadays, they have different use and these plants based on active ingredients in their tissues are classified into three main groups, including medicinal herbs, spices and aromas (Omid Beighi, 2009). Medicinal herbs are a valuable source of needed medicines of the people in the world. Common active ingredients extracted from plants usually used as a food additive, dye, pesticide, deodorant, etc. These compounds made in the extremities or different tissues medicinal herbs are collectively known secondary metabolites (Venysereh and Tesay, 2004). Medications which are directly supplied from the nature to man such as varieties and geographical native, seeds and fruits are valuable genetic and biochemical models that must be maintained as protectors of consumable drugs of society in the nature of Bank. So putting these models exposed to public expenditure is non-normative. Since wild plants (unlike agricultural plants) grow in a wide range of environmental conditions and gathering them from natural localities due to the lower quality and quantity of product is not responsive to industry and even destroys them, thus mass production of these plants seems wiser at widespread levels (Omid Beighi, 1994). Iran is considered as one of the best parts of the world in terms climate, geographical location and growing of medicinal herbs and in the past it has been the source of production and consumption of medicinal plants, therefore, in addition to the increasing importance of Medicinal herbs worldwide which is rapidly being replaced for many chemical agents, The exports of these plants can also be a major source of foreign exchange earnings for the country. Unfortunately, there is not enough awareness of the importance of this plant despite these resources in our country and many of these plant materials remains without use (Samsam shariat, 2003). Ardabil province, except in the area of Moghan and Qezel Ozan margin in the south of khalkhal, has cool semi-steppe and ultra-cool

climates. And cold winter in this province has effective role as one of the major factors limiting in the selection of compatible species in the green spaces and appearance of the green space is indicated the issue. Besides the above factor, other limiting factors such as Saline soils, calcareous soils, sloping lands and water shortages are which will affect the selection of improved ornamental species for Green space. And they prevent the quantity and quality expansion of the Green space in this province. To solve this problem, it seems to be necessary the identification and study of native ornamental plants of the province in order to use them in green spaces and increase species diversity and also possibility of assigning and decreasing the problems. However, due to their ecological variety in Ardabil (Semi-temperate steppe in Moghan and the margins of ghezel Ozan, cold Semi-steppe in many parts of Ardabil and Ultra-cold semi-steppe at mountainous areas and highlands) and Elevation from sea level difference which is between 20 m in the Aras river margin and in the Moghan region and 4811 m in sabalan peaks and it was extremely high in plant diversity. Which consequently the native medicinal plants are very diverse and each species have different capabilities. The important and role of medicinal plants known to everybody, and over time this plant acquire a better position in society. Pooneh Sai sabalan plant is herbaceous several years and Iran native which is from Lamiaceae family and it is distributed in northwestern Iran. This species grows at altitudes above 2500 of Sabalan in Shabyl and Qotoursuyei in Ardabil (Azimi, 2000). Therefore, with implementing this plan, adaptability of this plant is investigated in three points of Ardabil province with different heights. The evaluation of active substances, amount of changes ingredients in this medication measured at different heights and determines the most appropriate height.

Materials and methods

For the present study, in addition, library studies as analysis of existing resources including flora and articles, results of conducted research in the field of medicinal plants and native essence, also consult with teachers and experts and their distribution across the province and its habitats have been determined and then field and desert operations were carried out in the natural habitats in order to determine the species and with finding location and any necessary notes in terms of slope, elevation and soil. Then seeds were collected from habitats at the appropriate time, at the end of the growing season and the seed stage. And they were ideally prepared after cleaning for planting in the following year. Plowing, leveling and preparation of three pieces of Province land (Ardabil - Meshkinshahr - khalkhal) were conducted in the autumn of that year that in February of the same year collected seeds were planting at separate boxes in the center greenhouse. The plants were transferred to plastic pots at four leaves stage and were ready for planting. Selective pots were transferred to the target regions in a randomized complete block design and were cultured with three replications in the early spring. During the growing season plant samples were collected while evaluating a number of morphological and phonological traits at 50 percent flowering stage. Samples collected as standard in dry place and in Aboureyhan Laboratory in Research Institute of Forests and Rangelands essential oils were conducted by distillation method using steam and separation and its components were identified using Gas Chromatography systems and chromatography gas connected to spectrometry which specifications of the device discussed below.

Specifications of gas chromatograph and chromatography connected to mass spectrometer

Gas chromatography (GC)

Gas chromatography, the model of GC-9 A Shimadzu equipped with (Dector) F.I.D (ionization by flame hydrogen) and data processor Chromatopae C-R3 A column DB-1 Which is non-polar column the length 60 m, internal diameter 0.25 mm and the thickness of the stationary phase is equal to 0.25 micron. Helium

carrier gas and its pressure at the top of the column were adjusted to 2.5 kg per square centimeter. The ratio of Gap 1: 100 was used to dilute the sample. Temperature of injection part 220 ° C and the detector temperature was calculated 250 ° C.

Chromatography Connected to Mass Spectrometry GC/MS

Gas Chromatography; model Varian, connected to a mass spectrometer Saturn II model, column DB-1 with a length of 60 meters, diameter 250 mm, stationary phase thickness of 0.25 mm, column head pressure Psi35 and ionization energy was equivalent to 70 eV. Planning thermal of the column was temperature of 40° to 250 ° C with increasing speed of 4 ° C per minute, injection chamber temperature was 260 degrees.

Location of project locations

The geographical position of Ardabil

Ardabil province is located in North West of Iran, with an area of 17,953 square kilometers; it is allocated about 1.09% of the total area of the country. It is located between longitude of 3' 47° to 55' 48° east and latitudes of 45' 37° to 42' 39° north. It is bordered with Republic of Azerbaijan from the north in length 282.5 km (which Aras and Balha Rivers is flowing in its 159 km). It is limited from the East to Gilan, from the West to East Azarbaijan province and from south to Zanjan province.

Geographical location of Research Station of Khalkhal Kandirag

This region is located at geographical position of 48° 22' 16 "to 48° 23' 03" longitude and 37° 27' 11" to 37° 27' 49" North latitude and altitude of 1550 m above sea level. This forest stand is located at 35 km road Khlkhal- Hashtjin.

Geographical position of Horticultural Research Station of Bijaq (Meshgin shahr)

The station is located at the northeast, Bijaq crossroad located at 10 km Meshkinshahr road to Ardabil and altitude of 1320 m above sea level.

Results

Medicinal plant essential oils

Table 1 analysis results of medicinal plant essential oils *Nepta mentoedes* at medicinal plant research station of Samian (Ardabil) showed that the number of constituent compounds varied from 33 to 47, as the

sample No. 2 (seeds collected from the Shabyl 1) had the highest number in terms of chemical compounds and 1-8 Cineole with an average of 34.5 percent was allocated the highest percentage in this area.

Table 1. Analysis of medicinal plant essential oils of *Nepta mentoedes* at research station medicinal plants of Samian (Ardabil).

Compounds with a high percentage	Studied samples						
	Samian area	Shabil Area 1	Houshang Meydani region	Shabil Area 2	Area between gotour souei and Shabil	gotour souei area	The overall mean
Alfa - pinene	0.69	0.65	-	0.47	1.01	0.80	0.724
Sabinene	1.51	1.60	-	1.36	2.01	2.01	1.698
Beta – pinene	2.97	3.25	-	3.24	3.97	3.15	3.316
1,8 – cineole	35.72	38.72	-	20.43	47.91	29.73	34.5
The number of identified compounds	36	47	-	33	36	44	39

Table (2) analysis results of medicinal plant essential oils *Nepta mentoedes* at medicinal plant research station of Meshkinshahr showed that the number of constituent compounds varied from 23 to 48, as the

sample No. 4 (seeds collected from the Shabyl 2) had the highest number in terms of chemical compounds and 1-8 Cineole with an average of 39.9 percent was allocated the highest percentage in this area.

Table 2. Analysis of medicinal plant essential oils of *Nepta mentoedes* at horticultural research station of (Meshkinshahr).

Compounds with a high percentage	Studied samples						
	Samian area	Shabil Area 1	Houshang Meydani region	Shabil Area 2	Area between gotour souei and Shabil	gotour souei area	The overall mean
Alfa - pinene	0.54	1.02	1.19	1.18	1.18	1.13	1.04
Sabinene	1.44	-	2.44	2.20	2.14	2.18	2.08
Beta – pinene	3.55	-	4.46	5.06	4.90	4.36	4.466
1,8 – cineole	25.52	44.27	37.01	39.58	54.38	38.55	39.9
The number of identified compounds	23	47	41	48	23	41	37

Table (3) analysis results of medicinal plant essential oils *Nepta mentoedes* at medicinal and forest plant research station of kandirag (Khalkhal) showed that the number of constituent compounds varied from 30 to 67, as the

sample No. 6 (seeds collected from the Gotoursoei) had the highest number in terms of chemical compounds and 1-8 Cineole with an average of 30.18 percent was allocated the highest percentage in this area.

Table 3. Analysis results of medicinal plant essential oils *Nepta mentoedes* at medicinal and forest plant research station of kandirag (Khalkhal).

Compounds with a high percentage	Studied samples						
	Samian area	Shabil Area 1	Houshang Meydani region	Shabil Area 2	Area between gotour souei and Shabil	gotour souei area	The overall mean
Alfa - pinene	0.60	1.32	0.56	-	-	0.48	0.74
Sabinene	-	-	-	1.20	-	-	1.20

Beta – pinene	3.03	4.29	2.10	2.03	-	-	2.86
1,8 – cineole	43.23	47.77	25.57	28.01	-	6.32	30.18
The number of identified compounds	50	30	40	36	-	67	45

Discussion

This species observed only in Sabalan hillside the high elevation of 1800 m in provincial level. In this study, species Seed was collected, from 6 points Sabalan heights (from 2,600 to 3,800 meters above sea level). The evaluation results of percent of samples oil in a planted condition at different stations (three points from Ardabil) showed that between the area of study the highest essence percent was related to the Khalkhal (1.177 percent), Ardabil (1.085) and Meshkinshahr (0.82) percent.

Also determine the mean of all samples under planted conditions that samples collected among the Qotoursoui and Shabyl and Hoshang Meydani had the highest value respectively 2.80 and 2.28 percent.

Analysis of variance the performance evaluation data of aerial parts of samples in Samian research station in Ardabil showed that there was significant difference between treatments studied at 5% level. So that the survey of yield average showed that the samples 1 and 6 had the highest value in terms aerial parts.

Also investigate the essential oil percentage of studied samples at Samian research station showed that samples 4 and 5 respectively with 1.34 and 1.28 percent had the highest essential percentage. And the results showed that Ardabil station was located in the second place with 1.08 percent among the studied stations in terms of essential oil percentage.

Analysis of variance data evaluation of aerial parts Performance in samples at research station of Khalkhal Kandirag showed that there was no significant difference between treatments studied so that the survey of yield average showed that the samples 2 and 4 had the highest value in terms of aerial parts.

Although the results related to the analyzes of variance of data obtained from the evaluation of the performance of samples had no significant difference at the Horticultural Research Station of Meshkinshahr but the investigation of yield mean of aerial parts showed that the Sample No. 5 in this station is dedicated to the highest aerial parts.

Combined analysis of performance evaluation aerial parts at three points of Ardabil showed that there was significant difference between different locations and treatments studied at 95 percent level and also the interaction effect of treatment * place with 95 percent in terms of this trait. Due to the interaction between treatment * Place is significant, the highest performance aerial parts was related to samples collected Gotorsouei and Samian, in Ardabil.

Results from analysis of medicinal plant essential oils *Nepta mentoedes* at research station medicinal plant in Samian (Ardabil) showed that the number of constituent compounds varied from 33 to 44, as the sample No. 6 (seeds collected from the Gotorsouei) had the highest number in terms of chemical compounds and 1-8 Cineole with an average of 34.5 percent was allocated the highest percentage in this area.

Results from analysis of medicinal plant essential oils *Nepta mentoedes* at research station Horticultural plants (Meshkinshahr) showed that the number of constituent compounds varied from 23 to 48, as the sample No. 4 (seeds collected from the Gotoursoui) had the highest number in terms of chemical compounds and 1-8 Cineole with an average of 39.9 percent was allocated the highest percentage in this area.

Results from analysis of medicinal plant essential oils *Nepta mentoedes* at research station Medicinal and forest plants of Kandirag (Khalkhal) showed that the

number of constituent compounds varied from 30 to 67, as the sample No. 6 (seeds collected from the Shabil 2) had the highest number in terms of chemical compounds and 1-8 Cineole with an average of 30.18 percent was allocated the highest percentage in this area.

Generally it can be said that the yields of essential oils under normal conditions was

Conclusion

Variable between 1.6 to 2.5 percent, so that the highest percentage was related to the Shabyl area. And the numbers of identified chemical compounds have been between 19 to 20 cases as the investigation showed that Cineole and citronellyl acetate are compounds that had the highest percentage among the identified compounds. As Shabyl area with a height of 2700 meters was allocated the maximum amount in terms of Cineole with the 50.90 percent that respectively Ardabil, Meshkinshahr stations had the highest value in terms of the aerial parts yield and Khalkhal and Samian research station in Ardabil had the highest percentage of essential oils. Reason of the low number of aerial parts at the Khalkhal station can be attributed soil and climatic conditions of the region and studies have shown that the percentage of essential oils goes up in difficult conditions.

The number of chemical composition at the stations, Khalkhal, Ardabil and MeshkinShahr was respectively 45, 39 and 37 and the highest chemical composition was related to 1 and 8 cineole with 39.9 percent and has been Meshkinshahr station.

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