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Original Article

Eco-phytochemistry of *Nepeta asterotricha* Rech. f. (Case study: Deh Bala, Yazd)

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Abstract

Ecological study of behavior is essential elements of the renewable natural resources. The study area in this research was Deh Bala where is one of the main habitat of *N. asterotricha* Rech. f. This plant is one of the 34 endemic species of *Nepeta* L. in Iran. In the study area, different parameters were determined such as essential oil, climate factors, phenology, co-dominant plants, morphology, vegetation variation, and physical and chemical properties of the soil. Results have showed that the plant is distributed in regions with pH=7.5±0.1, EC=0.633±0.06 ds/m with silt-loam texture. The average rainfall is 308.7 millimeters and average annual temperature is 12.2 centigrade in 10-years period. Density, frequency, and production were 0.7, 36.67%, and 46.78 (gr), respectively. The plant grows in March, flowering stage occurs in May and seeding happens at mid-June. The main component in flowering stage was 4aα,7β,7aα-nepetalactone (33.9%). 1,8-cineole, and cis-sabinene hydrate are the other main constitutions. Because of the importance of the plant, domestication and breeding program are urgently required.

Keywords: Essential oil, Phenology, Soil properties, *N. asterotricha* Rech. f., Yazd.

Introduction

Because of the importance of aromatic plants and their metabolites on human health, scientists are interested to find new components by dissimilar situation. Therefore, the commercial development of medicinal plants as new source of bioactive products to enhance human health and food preservation is one of the main importances. There are a lot of families and herbs, which are known as medicinal plants. One of the largest and most distinctive families of plants in the world is Lamiaceae. Some of species in Lamiaceae are one of the major sources of culinary, food flavoring, vegetable, and medicinal plants all over the world. Wide ranges of compounds, such as terpenoids, phenolic compounds, and flavonoids, have been isolated from the members of the family [1, 2]. Labiateae family contains of 258 genera and 6970 species. The family, with 46 genera and ca. 420 species and subspecies, have also a great diversity and distribution in the flora of Iran [3-5]. This family is important for their essential oils, and many biological activities. Most species of Lamiaceae have being used in perfumery, confectionary and pharmaceutical preparations. They are also used in different traditional medicinal treatments as herbal remedies and in the food industry as food additives and taste enhancers because of their good odors [6]. *Nepeta* L. with the common Persian name Pune-Say is one of the largest genera in Lamiaceae family. It has more than 280 species that represents a significant diversity in growth forms, pollination biology, floral morphology, and secondary metabolites [7].

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Nepeta is represented in the flora of Iran by 75 species, [4] 34 of which such as *Nepeta asterotricha* Rech. f. are endemic [8]. The oil of *Nepeta* have been used as laxative for the treatment of dysentery, teeth troubles, ear pain, kidney, and liver diseases [9, 10]. It is used as febrifuge, anti-septic, antitussive, diaphoretic, sedative, anti-asthmatic, anti-oxidant [11-15]. *Nepeta* species have anti-bacterial, anti-fungal, anti-viral and anti-inflammatory activity [16]. Several species of *Nepeta* can be used as antispasmodic, diuretic, febrifuge, diaphoretic and for tooth, kidney and liver disease [17]. Interests about plant ecology have increased in desert areas recently [18]. Identifying ecological factors such as soil, topography, climate, and disturbance that influence plant species distribution is essential to conserve, manage and improve rangeland ecosystems [19, 20] and many studies were done in this context. The habitat characteristics of *N. oxyodonta* have been investigated in central Zagros Mountains [21]. The results showed that *N. oxyodonta* usually grows in the mountain regions with the elevation of 2150 to 2800 meters (above sea level) and the slopes of 10% to 75%. This plant can tolerate the EC from 0.37 to 0.85 µ mho/cm. The ecological desires of *N. nuda* were investigated. This species grows in fields, meadows and glades [22]. *N. binaludens* grows in north-facing slopes with more than 50% and 2300-2700 m elevations. Also found that it prefers the average annual rainfall ranging from 350 to 370 mm and temperature of 6 to 7°C and grows in light soils with a neutral pH and low mineral content [23]. There is different research on the chemical composition of *Nepeta*. An investigation on *N. prostrate* Benth. revealed its major component was spathulenol (36.5%), terpinen-4-ol (13.3%), 1,8-cineole (8.8%), myrtenal (6.1%), cis-sabinene hydrate (5.9%), cis-sabinol (5.5%) and γ-terpinene (4.5%) [24]. *N. racemosa* Lam. was explored. It consisted of 4aβ,7α,7aβ-nepetalactone (33.6%), 4aα,7α,7aβ-nepetalactone (25.6%), 4aα,7α,7aα-nepetalactone (24.4%) and 1,8-cineole (9%) as the main component [25]. The main constitutions of *N. satureioides* Boiss. were linalool (23.8%), *(Z,E)*-farnesol (14.7%), linalyl acetate (11.1%), *β*-caryophyllene (6.6%), lavandulol acetate (6.6%) [26]. The present research not only aims to investigate the ecological characteristic but also it explores chemical composition of *N. asterotrich* Rech. f. In other words, this research studies some biotic, abiotic factor for knowing about environmental distinctive and on the other hand phytochemistry of essential oil for their chemical constitution.

Material and Methods

The studied site

The main habitat of the studied plant was selected in Deh Bala, Yazd, Iran. The latitude, longitude, and altitude of the study area is 31° 34' 35" N, 54° 05' 22" E, and 2791 m, respectively. According to 10-year statistics, mean annual temperature is 12.2°C and precipitation is 308.7 mm. The rainfall regime occurs in the cold season in this area.

Soil properties

Samples were performed with three replications from 0-50 cm depth where is the distribution of root growth. According to 2 mm sieve, all samples were passed and subjected for analysis. The chemical analysis of the soil samples were about nitrogen (Kjeldahl), phosphorus (Olsen), potassium (Flame photometric) and its physical properties were measured including humid (Weight), pH (pH meter), electrical conductivity (EC meter) and soil texture (Hydrometric).

Plant parameters

The plant samples were collected from Deh Bala, Taft, Yazd, Iran. The plant was compared with herbarium specimens to be approved. For this purpose sample was identified and deposited in Herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran. Plant samples were collected from the locality during flowering stages, separately. In following phenology, co-dominant plant and vegetation variation (density, frequency, and production) were determined. The study area was divided to three part by using three transect 50 meters apart about 50 meter from each other parallel to the line level. On each transect 10 points with a distance of 5 meters were considered. According to type of vegetation, 1 m^2 plot were used to evaluate the characteristics of the plant. To study the morphological characters, 20 morphological feature of *N. asterotricha*. were examined that were collected from full flowering stage. To investigate on the essential oil in flowering time, some samples were collected from parts of the fresh plant and dried in the shade at room temperature. Then 100 grams of dried sample was powdered. After that,

essential oils were obtained by hydro distillation using a Clevenger apparatus for 3 hours. Resulted oils were dried by anhydrous sodium sulfate and kept in tightly closed vials at 4°C before chemical analyses. GC and GC-MS performed the analyses of quantitative and qualitative the oils.

Gas chromatography-flame ionization detector and Gas chromatography-mass spectrometry analysis (GC & GC/MS)

The GC analyses have been done by using a Thermoquest-Finnigan gas chromatograph equipped with a flame ionization detector (FID). The analyses were performed on a fused silica capillary DB-5 column (60 m×0.25 mm i.d.; film thickness 0.25 µm). The carrier gas was nitrogen at a flow rate of 1.1 ml/min. Planning thermal column was raised from 60 ºC to 250 ºC. The rate was 5 ºC/min and finally held isothermally for 10 min. The split ratio was 1:50, too. The injector and detector (FID) temperatures were set at 250 ºC and 280 ºC, respectively. A Thermoquest-Finnigan Trace GC-MS instrument was same with GC. The injector and detector temperatures were kept at 250 ºC and 300 ºC, respectively. The carrier gas was helium with ionization voltage of 70 eV. Ion source and interface temperatures were 200ºC and 250ºC, respectively. Mass range was from 35 to 456 amu. The oven temperature program was the same as given above for the GC.

Identification and quantification of the essential oil compounds

Calculation of the retention indices under temperature-programmed conditions for n-alkanes (C6–C24) and the oil on a BPX-5 column under the same chromatographic conditions identified the constituents of essential oils. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (NIST, Adams and Wiley 7.0) or with authentic compounds (purchased from Sigma-Aldrich and Merck Co.) and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature. For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors.

Result

Soil Results

The assessment of soil in the studied area showed that low salinity with silt loam as the soil texture. The mean of nutrient elements were nitrogen (0.068%), phosphorus (6.1 mg/kg), and potassium 162 (mg/kg).

Plant Results

Vegetation Variation

According to transect, density, frequency, and production were determined. All results are presented in table 2.

Morphologıcal Properties

N. asterotricha was as a shrub with woody base and multiple branches. The height varied between 14-53 centimeters, that is covered with short fur and tumor secreted. There were lots of fur on both side of leaves and petiole, too. In other words, glandular and eglandular hairs are present on both the upper and lower epidermis in leaves and petioles. The length of petioles were varied between 0.6-1.3 mm. The venation is clear at the leaf and the entire leaf often was rectangle or semi-rectangle that the length and width of leaves fluctuated between 15-30 and 7-10 mm, respectively. The leaves that stay on the top of the stem are smaller than the low one. Inflorescence was raceme. The flowers were arranged verticality with zygomorphic situation. The size of calyx and corolla were 5-6 and 11-13 mm, respectively. Corolla appeared in light purple color. The filament is 14-16 mm long and anther 1-2 mm in length. Its tube was straight and slightly ventricose above. The fruit type was nutlet that were appeared in brown and rounded.

Phenological Stages

The most effective factors on phenological activity are environmental factors specially precipitation. In addition, it is the date of occurrence of phenological stages of studied plant: vegetative growth began in March up to April. Flowering period started in May until mid-June. After this stage, the plant welcome to seeding phase. Seed production started almost from June and it continued until the first half of August. Seeds of the plant have said enough to arrive in early July. In mid-July, the seeds can be separated from the plant and scattered around. Finally, in November the plant started the winter dormancy.

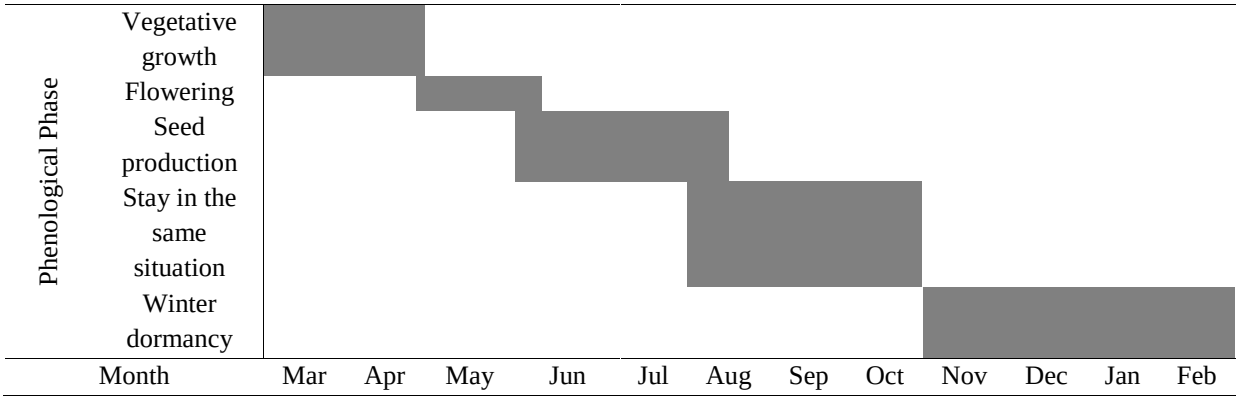
Table1 Mean of soil properties in the studied area

Station	pH	Ec dc/m	Soil moisture	N %	P (mg/kg)	K (mg/kg)	Texture
1	7.4	0.7	48	0.061	5.9	162	Silt loam
2	7.6	0.6	45	0.074	7.3	153	Silt loam
3	7.5	0.6	46	0.069	5.1	171	Silt loam
Mean±Stdev	7.5±0.1	0.633±0.06	46.33±1.53	0.068±0.006	6.1±1.11	162±9	

Table 2 qualitative characteristics of vegetation variation

Variation / Station	Density	Frequency (%)	Production of a base (gr)
1	0.6	30	40.5
2	0.7	40	47.85
3	0.8	40	52
Average	0.7	36.67	46.78

Table 3 Phenology of *Nepeta asterotricha* Rech. f



Isolation of the essential oil

The oil yields of *N. asterotricha* Rech. f. is 2.23%. The phytochemical properties of the oils are present in table 4. As can be seen thirty-one compounds representing 99.7% of the oils were identified. Only the compounds representing least 0.05% of the mixture are given in the table 4. The oil contained 4aα,7β,7aα-nepetalactone (33.9%) as a major component. Furthermore, 1,8-cineole, cis-sabinene hydrate, terpinen-4-ol are the main constitution of the oil.

Co-dominant plant

Table 5 shows the co-dominant plant in the habitat of *N. asterotrich* Rech. f.

Discussion

N. asterotricha Rech. f. is one of the endemic plants in Iran, which is just grown in Yazd [27]. This genus has different pharmacology value such as treatment of dysentery, teeth troubles, ear pain, kidney [9, 10]. Since different situation effect on chemical composition, which have dissimilar efficacies, the present study aim to investigate on *N. asterotricha* Rech. f. in the main habitat. The mean annual rainfall and temperature were about 308.7 mm and 12.2 °C for the habitat, respectively.

The edaphic factor has a high impact on the distribution of plants and its secondary metabolites in desert areas [28]. Soil texture, pH, EC and SAR were more important factors than others [28]. This plant was more abundant on soils with pH=7.5 and Ec=0.633 DS/m.

There are different researches about ecological characteristic of *Nepeta* species such as *N.septemcrenata*, *N.binaludensis* and the soil and topography results are same with this study [23], [29, 30]. The plant was found on silt-loam texture. Nutrients of soil are known as an important factor for growing. On this study area nitrogen (0.068±0.006), phosphorus (6.1±1.1), potassium (162±9) were determined.

Table 4 Composition of essential oil in *N. asterotricha* Rech. f.

	Component	RI	%
1	α-Thujene	926	0.7
2	α-Pinene	934	0.6
3	Sabinene	972	1.2
4	β-Pinene	978	1.2
5	β-Myrcene	989	0.5
6	α-Phellandrene	1005	Tr
7	α-Terpinene	1017	1.7
8	p-Cymene	1024	1.5
9	Limonene	1029	2.8
10	1,8-Cineole	1032	13.2
11	(Z)-β-Ocimene	1035	0.6
12	γ-Terpinene	1058	3.3
13	*Cis*-Sabinene hydrate	1068	12.7
14	*Cis*-Linalool oxide	1072	0.7
15	α-Terpinolene	1089	0.1
16	Linalool	1101	4.8
17	*Cis*-p-Menth-2-en-1-ol	1122	1.2
18	Trans-p-Menth-2-en-1-ol	1140	0.5
19	Rose furan epoxide	1175	0.5
20	Terpinen-4-ol	1179	8.8
21	α-Terpineol	1192	2.0
22	Geranial	1271	0.1
23	Geranyl formate	1302	0.2
24	4aβ,7α,7aα-Nepetalactone	1354	0.1
25	4aα,7α,7aα-Nepetalactone	1363	5.5
26	4aβ,7α,7aβ-Nepetalactone	1378	0.3
27	4aα,7α,7aβ-Nepetalactone	1392	0.1
28	4aα,7β,7aα-Nepetalactone	1401	33.9
29	*Trans*-Caryophyllene	1423	0.1
30	(E)-β-Farnesene	1457	0.7
31	(Z)-α-Bisabolene	1504	0.1
	Monoterpene hydrocarbons		14.2
	Oxygenated monoterpens		84.6
	Sesquiterpene hydrocarbons		0.9
	Total		99.7

tr: Trace (< 0.05%)

Table 5 Co-dominant plant in the habitat of studied species

Acantholimon incomptum Bioss. &Buhse.	*Marrubium vulgare* L.
Acantholimon nigricans Mobayen	*Melicajacque montii* Decne. ex Jacquem
Acanthophyllum laxiusculum Schiman-Czeika	*Melica persica* Kunth.
Achillea wilhelmsii C. Koch	*Phagnalon nitidum* Fres.
Artemisia aucheri Boiss.	*Pimpinella aurea* DC.
Artemisia persica Boiss.	*Plantago ciliate* Desf.
Artemisia sieberi Besser.	*Plantago lanceolate* L.
Astragalus spachianus Boiss. &Buhse.	*Salvia macrosiphon* Boiss.
Berberis integerrima Bunge	*Scutellariam ulticaulis* Boiss.
Carex physodes M. B.	*Silenegyn odioica* Ghazanfar
Cousinia onopordioides Ledeb.	*Stachys inflate* Benth.
Ferula assa-foetida L.	*Stachys obtusicrena* Boiss.
Ficus johannis Bioss.	*Stipa barbata* Desf.
Helichrysum davisianu Rech. f.	*Teucrium polium* L.
Helichrysum leucocephulum Bioss.	*Trifolum pretense* L.
Hyoscyamus pusillus L.	*Verbascum songaricum* Schrenk ex Fisch. &C. A. Mey.
Juncus bufonius L.	*Ziziphora clinopodioides* Lam.

The density and production in the study area was quite good. The vegetative growth of *N. asterotricha* Rech. f. began in March up to April. After that flowering period started in May until mid-June. Then seed production started almost from June and it continued until the first half of August. In mid-July, the seeds can be separated from the plant and scattered around. Finally, in November the leaves and stems remained in the same position. The aerial parts of *N. asterotricha* Rech. f. yielded 2.23% (v/w) of a yellowish oil with an aromatic odor. The identification of the compounds was done by GC and GC/MS. All components categorized in three groups such as monoterpene hydrocarbons (14.2%), oxygenated monoterpens (84.6%), and sesquiterpene hydrocarbons (0.9%). The main constitution in each division was γ-terpinene, 4a*α*,7*β*,7a*α*-nepetalactone, and *(E)-β*-farnesene, respectively. In addition, the oil contained 4aα,7β,7aα-nepetalactone (33.9%), 1,8-cineole (13.2%), and cis-sabinene hydrate (12.7%) as the main components. This result is quite different with other research about *N. asterotricha* in dissimilar region. A research group just studied the chemical composition of aerial parts and the main components were reported to be terpinen-4-ol (22.8%), 1,8-cineole (17.4%) and 4aβ,7α,7aβ-nepetalactone (14.8%) [31]. Another research in Tezerjan were investigated the chemical composition of aerial part of *N. asterotricha* at flowering time [32]. The research group found the main constitution of the aerial part were 1,8-cineole (26.12 %), terpinen-4-ol (14.83 %), and 4aα,7α,7β-nepetalactone (8.65 %). These variances may be because of the geographic factors, climatic, and collection time. In other words, each biotic and abiotic factor can effect on secondary metabolites. Based on the main components in different species of *Nepeta*, it could be said that the main components such as nepetalactone, 1,8-cineole, terpinen-4-ol are same with *N.menthoides, N.crassifolia, N.racemosa* [25], [33,34]. It seems to be important that the results were in contrast with *N. depauperata, N. bracteata, N. macrosiphon, N.nuda* [35-38]. The main components for *N.depauperata* were spathulenol (31.84%) *β*-caryophyllene (12.93%) caryophyllene oxide (10.27%). *N. bracteata* was same as *N. depauperata* in spathulenol (14.0%) caryophyllene oxide (12.3%) as main components. It seems to be interesting that *N. asterotricha* has 5 isomers of nepetalactone together. In other words, it can help for future study about nepetalactone isomers. The results of morphological features were compared with the information published in the Flora of Yazd [39]. There were some differences about the size of length and width of leaves and some more information about filament and anther. According to the outcome, domestication and breeding program are required for this valuable genus. Native plants that have evolved within these regions are perfectly adapted to thrive in the current climate, soil, and environmental conditions. *N. asterotricha* Rech. f. is a native plant in Deh bala that has adapted perfectly to conditions. It not only can be effectively used for rehabilitation but also for it is valuable for having five isomers of nepetalactone together. This is a good situation for studying the pharmacologic efficacies and biological activity in the future.

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