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Phytochemical Responses of *Stachys inflata* Benth. to Environmental Factors in Semi-Arid Rangelands

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ABSTRACT

Aims: The *Stachys inflata* Benth. is a plant from the Lamiaceae family, widely used as a medicinal species with a significant presence in the northern rangelands of West Azerbaijan Province, Iran. There is a lack of data on the phytochemical compounds of various populations in semi-arid rangelands, and the effective factors influencing domestication and breeding approaches are not well understood. Therefore, this research aimed to examine the changes in the phytochemical characteristics of this plant under the soil and topographic environmental factors.

Materials & Methods: Soil sampling was carried out to measure various soil characteristics such as acidity (pH), electrical conductivity (EC), organic carbon (OC), saturation moisture (SM), lime (TNV), clay, silt, sand, phosphorus (P), calcium (Ca), sodium (Na), potassium (K), and magnesium (Mg). In addition, topographical factors, including slope, direction, and elevation, were also measured. The impact of environmental factors on the phytochemical traits of *S. inflata* was determined using the redundancy detrended analysis (RDA) method.

Findings: Revealed 179 major phytochemical compounds across eight populations, with site 5 being the richest, containing 60 identified compounds. From sites 1 to 8, the amounts of phytochemical compounds were 7, 28, 9, 38, 60, 24, 11, and 40, respectively. The RDA results revealed that the phytochemical characteristics of *S. inflata* are influenced by both soil and topographical factors. Approximately 84.02% of the variation in phytochemical compounds is attributed to the selected soil and topographical factors, as determined by two axes, indicating a notably high reliability of the association. Determining soil in two axes and topography in the first axis demonstrates that soil has a greater effect on the phytochemical compounds of sites. An increase in sand content was associated with higher percentages of alpha-cadinol, geraniol, trans-caryophyllene, trans-anethole, and orjunol compounds. In contrast, the levels of phenol, flavonoid, and antioxidant indices decreased. On the other hand, an increase in calcium, sodium cations, and electrical conductivity contributed to higher percentages of thymol, spathulenol, caryophyllene, and prolol dibenzene furan compounds. Moreover, as altitude increased and on southern slopes, there was an increase in lime content, organic carbon, silt, clay, saturated moisture, and pH levels, leading to higher amounts of phenol, flavonoid, and antioxidant indicators in the compounds. However, the impact of these parameters varied.

Conclusion: Overall, Soil had relatively greater effects than topography. Therefore, to produce a rich extract, regarding soil, stressful environments are a suitable selection. Additionally, for domestication, it is necessary to have soil with higher cation levels and a lower slope and elevation. Site 5, with its richer compounds, can serve as a pilot site for collecting seeds for artificial seeding plantations.

Keywords: Medicinal Plant; Extract; Secondary Compounds; Stachys inflata Benth; Plant Population.

CITATION LINKS

[1] Barbosa Filho ... [2] Bertol I., Santos... [3] Pedrotti F. ... [4] Zhang Q., ZhouZ., ZhaoW., Hu ... [5] Sheidai Karkaj ... [6] Bernáth J. Production ecology of ... [7] Lukas B., Schmiderer C., Novak J ... [8] Younessi Hamzekhanlu M., Sanjari ... [9] Formisano C., Delfine S., Olivie ... [10] Singh R. Chem... [11] Ramak P., Karimian V. Effects of ... [12] Younessi-Hamzekhanlu M., Abdipou ... [13] Amelie ... [14] Omidbeigi R. ... [15] Mozaffarian V. ... [16] Pashova S., Karcheva ... [17] Lindqvist C., ... [18] Mohammadi H., Aghaee A., Pormoha ... [19] Aboukhalid K., Al Faiz C., Douai ... [20] Aldibekova A., ... [21] Santos P.V.L., ... [22] Sadeghi S., Mohammadi M., Salehi ... [23] Ashrafzadeh ... [24] Dehghani Bidgoli R. Essential oi ... [25] Morteza Semnani K., Saeedi M., M ... [26] Ullah I., Adnan M., Begum S., Na ... [27] Odisho S. .... [28] Benjamin M.A.Z., Ng S.Y. Saikim... [29] Boulding J.R. Description and sa ... [30] Carter M.R., Gregorich E.G. Soil ... [31] Gee G.W., Or D. Particle-size an ... [32] Hill M.O., Gauch H.G. Detrended... [33] Jongman R.H.G., ... [34] Motamedi J., Sheidai Karkaj E.,... [35] Lepš J., Šmilauer ... [36] Li X., Li Y., Chen A., Gao M., S ... [37] Figueiredo A.C., Barroso J.G., P ... [38] El Keltawi N.E., Croteau R. Sali ... [39] Ozturk A., Unlukara A., Ipek A., ... [40] Imani Dizjikan ... [41] Alimohammadi ... [42] Eman E. A. .. [43] Baczek K., ... [44] Jafari N., Naderi P.A., Ebrahimz ... [45] Carey D.B.,... [46] Demasi S... [47] Alibakhshi M., Mahdavi S.K.h., M ... [48] Simon J.E., Reiss Bubenheim D.,... [49] Rhizopoulou S.... [50] Salehi M., Kolvandi R. Investiga ... [51] Mohammadi-Rad Z., Sheidai-Karkaj ...

Introduction

The establishment of special conditions in vegetation communities can play a vital role in determining the production of specific secondary metabolites and compounds in plant organs [1,2]. The quality of the soil, in terms of its physical and chemical properties, as well as topography, and various elements, affects plant growth and development of secondary materials by influencing the physiological activity of plants [3, 4, 5]. Environmental factors initially affect the primary metabolism of plants and subsequently affect secondary metabolism. These effects can be observed through changes in plant traits, organ performance, metabolite production per unit of dry weight, and the composition ratio of secondary metabolites [6].

The chemical composition of essential oils of various herb species can vary greatly depending on environmental conditions and geographical location, resulting in variations in their medicinal properties and biological activity [7,8]. Therefore, a plant species in various populations may have different essential oil compositions due to changes in environmental conditions. The potential effects of various metabolic compounds under the influence of environmental factors highlight the importance of understanding the impact of environmental factors on essential oil variations [9].

The study of secondary metabolites, particularly essential oils, provides valuable insights for taxonomists, pharmacologists, and phytochemists in resolving taxonomic issues [10].

Many people have recently been interested in using natural plant compounds. Consequently, different researchers have isolated these compounds from plants for various medicinal purposes [11].The Lamiaceae family includes the valuable genus *Stachys,* which comprises approximately 300 species distributed worldwide. This plant family is highly valued in the pharmaceutical and traditional medicine industries because of its numerous medicinal properties [12]. The genus *Stachys* is most commonly found in Europe, North America, Central Asia, Southeast Asia, and the Middle East [13]. There are about 34 *Stachys* species in Iran [14]. A notable species is *S. schtschegleevii*, commonly known as *S. inflata* Benth. It has various characteristics, including sedative, anticonvulsant, and antiseptic properties, which relieve gout and joint pain, and treat pus from boils [15]. Many *Stachys* species are associated with a rich history in traditional medicine across various cultures, and their extracts and essential oils are rich in non-volatile and volatile compounds. Because of their complex profile of bioactive substances, *Stachys* members are considered to possess an extensive spectrum of therapeutic properties, including antioxidant, anti-inflammatory, analgesic, antibacterial, cytotoxic, and wound-healing effects, as well as benefits for memory enhancement, lipid profile regulation, blood glucose control, and weight management [16]. The *S. inflata* species is a perennial shrub and is often found in rocky areas and mountain steppes [17].

S. *inflata* is known for its essential oils, flavonoids, phenolic acids, and terpenoids, which contribute to its medicinal properties. Few studies have explicitly investigated the relationship between environmental factors and phytochemical metabolites of the S. *inflata* species; however, some literature is available for other Stachys species, as well as similar species, and complex relationships among environmental factors.

In a study investigating the impacts of different irrigation regimes and water deficit on *the* chemical properties of S. schtschegleevii, the results showed that water deficit had adverse effects on the

shoot dry biomass, relative water content, and photosynthetic pigments of the exposed plants. The essential oil (EO) content under water deficit showed an increasing trend. Water deficit notably increased total phenol content, proline, H_2O_2, and malondialdehyde contents [18].

Aboukhalid et al. [19] found a significant relationship between environmental factors and essential oil components in *Origanum compactum* Benth. species in Morocco, distinguishing two groups based on areas with humid weather and semi-arid regions. Light, clayey, and sandy soils, as well as those with gravel, may influence the composition and phytochemical content of the rare species *Fraxinus sogdiana* Bunge. [20]. essential oil production in *Psidium acutangulum* Mart ex DC. exhibits a moderate correlation with insolation, whereas its main constituents, caryophyllene oxide and E-caryophyllene, exhibit a moderate correlation with humidity and insolation [21]. Variation in soil properties, including the texture, pH, and topographical factors such as altitude, slope, and aspect, determines the phytochemical

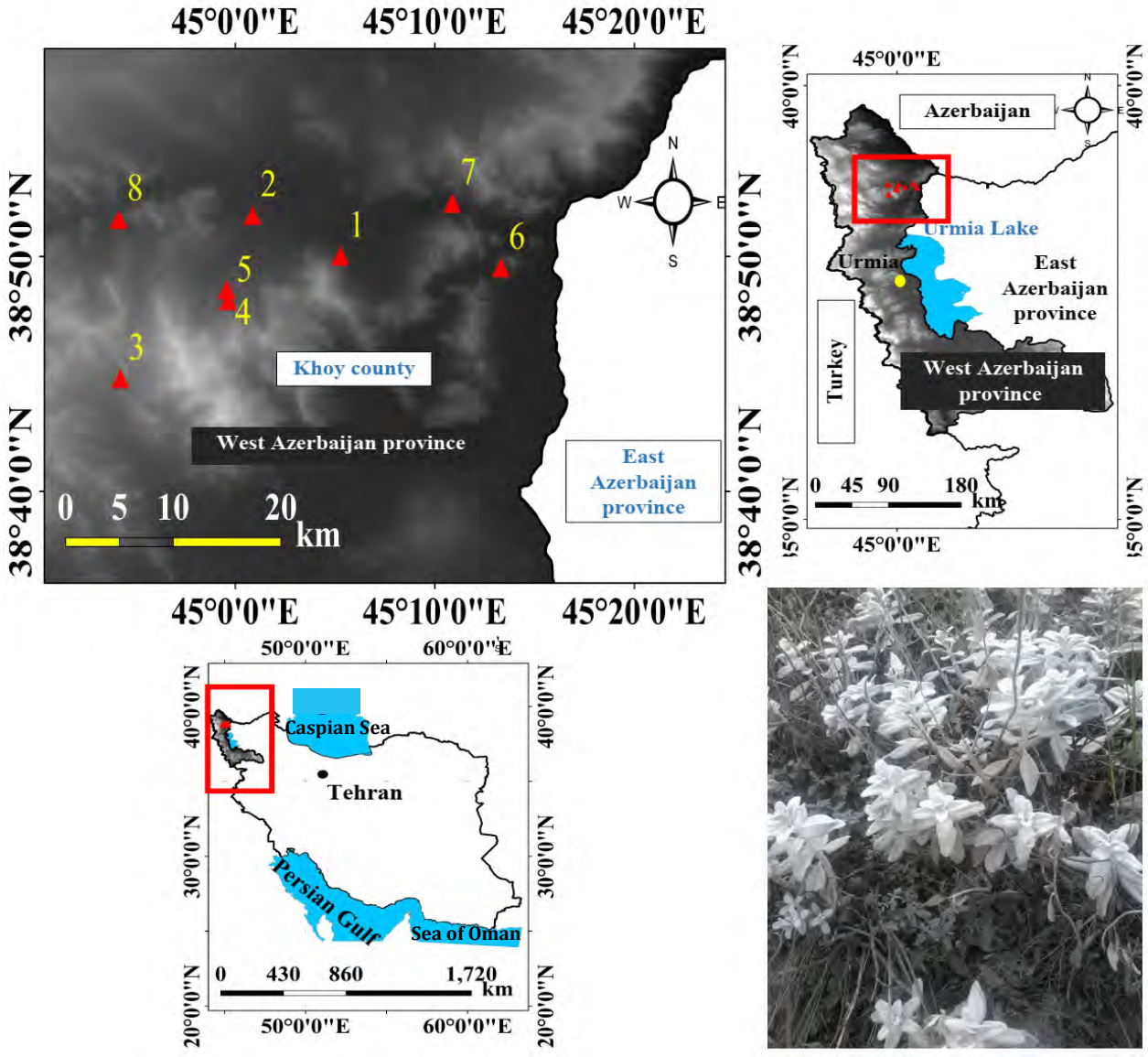

Figure 1) Study area, sampling locations, and *S. inflata*.

composition of wild medicinal plants in the Zagros Mountains. Soil pH and altitude were the main factors influencing the alteration of secondary metabolites. For instance, flavonoids and terpenes are observed at higher concentrations at higher altitudes and in soils with slightly acidic pH [22]. Ashrafzadeh et al. [23] investigated the phytochemical characteristics of *Clematis ispahanica* in two arid and semi-arid regions. Their findings indicate that plants in semi-arid climates produce higher levels of secondary metabolites, with the highest concentrations observed during the flowering stage. A positive correlation was also found between phytochemical characteristics and soil properties (pH, EC, and total Nitrogen). Dehghani Bidgoli et al. [24] demonstrated that significant differences exist in the chemical composition, antioxidant properties, nutrients, and specific morphological and physiological characteristics of *Allium jesdianum*, depending on the planting date. Their results also indicated that the best time to plant *A. jesdianum* is November, and plants planted in this month will have the highest quantitative and qualitative yield. Morteza Semnani et al. [25] identified the main compounds in *S. inflata* Benth. plants as hexadecanoic acid, germacrene D, alpha-pinene, and Bicyclo(2.2.2)octane. Ullah et al. [26] examined the influence of ecological factors on the phytochemical compounds of *Apteranthes tuberculata* (N.E.Br.) Meve and Liede observed higher levels of photosynthetic pigments, saponins, crude growers, crude oils, and crude soils in valley areas of the Khyber Region of Pakistan, suggesting a correlation with rainfall and vegetation abundance.

Given the potential of *Stachys inflata* as a natural health-protective dietary component and its perishable nature, systematic research is needed on the effects of environmental factors on its phytochemical content and biological potential. A limited but growing body of literature exists on the relationship between phytochemical compounds in *S. inflata* and various environmental factors. To date, no research has investigated the influence of soil and topography on a small scale. The majority of research has been conducted on other species and at a large spatial scale. Additionally, the research has mainly focused on the effects of seasonal variation and harvesting time, as well as water stress and drought conditions, on photochemical compounds in *Stachys inflata*. Original studies on the joint effects of topography and soil are lacking. To approach the domestication process and planting of *S. inflata* in croplands and greenhouses, it is necessary to conduct experiments in spatially fine-scale areas with controlled climate variables. This study, therefore, aimed to provide basic information on the initial domestication of *S. inflata* and to establish a suitable population with higher phytochemical compounds.

Our study aimed to address this gap by focusing on the effects of environmental factors, specifically soil and topography, on the phytochemical compounds of *S. inflata* Benth. in the northern rangelands of West Azerbaijan Province in Iran, aiming to determine the impact of different environmental factors on material composition. Specifically, we tested the hypothesis that phytochemical compounds at various sites differ (H1) and also investigated whether the phytochemical compounds of the studied species are related to soil and topography factors (H2).

Materials & Methods

Study Area and Selection of Sampling Sites

This study was conducted in the northern part of West Azerbaijan Province, specifically in Chaipareh and Khoy counties, Iran (Figure 1). These areas are located geographically from 44° 49.56' to 45° 16.95' east longitude and

from 38° 44.74' to 39° 1.01' North latitude. Based on the nearest climatology station in Khoy, the average annual precipitation was 283.94 mm with a maximum temperature of 34.4°C and a minimum temperature of 4.6°C. According to the Emberger Bioclimatic Classification System, the climate is semi-arid. The elevation gradient ranges between 1100 and 1700 m.

To select sampling sites from rangelands, we referred to ecological maps of rangeland types related to the sheets of Khoy and other literature [27]. Additionally, we conducted field visits to the study area in the northern part of West Azerbaijan Province in July 2021. Eight locations in Khoy and Chaipareh Counties were selected as study sites, and historical grazing with sheep and goat was a common land- use in the rangeland. These locations were selected because the *S. inflata* Benth. species, which is a dominant species in the rangelands, represents a significant portion of the region in terms of climate, topography, and soil conditions. Eight sites were selected within the study area, each with distinct attributes, including topography, vegetation, and soil. The specific properties of these sites are summarized in Table 1.

Plant Sampling for Phytochemical Analysis

To conduct a phytochemical analysis of the *S. inflata* Benth. species, we collected 1 kg of leaves and young branches from the base of the plant at each site during the flowering period in July. The samples were harvested at the same time in the morning, between 9:00 a.m. and 11:00 a.m., to ensure consistency in metabolite content. After transporting the plants to a suitable location in the laboratory of the University of Tabriz, we washed them and spread the plant material on a cloth in the shade. We then dried them in the shade using a standard shade-drying method, as slow drying prevents any chemical reactions in the plant material [28]. Therefore, samples were dried at room temperature, between 22°C and 25°C, with humidity levels of 20%-25%, for two weeks. Subsequently, the dried organs were cut into small pieces and ground into powder using an electric blender. The samples were then packed in paper bags and transferred to the laboratory for analysis.

Collecting Soil Samples and Recording Topographical Factors

We collected soil samples at five randomly selected points in each site, ranging in depth from 0 to 20 cm [29]. After the soil samples were taken to the laboratory, they were spread on a clean sheet of paper to air-dry in the shade. Finally, for laboratory analysis according to standard protocols [30], the dried soil samples were sieved through a 2 mm screen [31].

Electrical conductivity (EC) was measured in the water extract of the saturated paste, and pH was measured in both the water and soil solutions. The percentage of saturated moisture was measured using a weighting method, which equals the weight of water required to saturate the pore space divided by the weight of the dry soil. The percentage of soil organic carbon was determined using the Walkley-Black chromic acid wet oxidation method. The Olsen method, based on the spectrometric procedure, was used to measure phosphorus. The hydrometer method was used to determine the particle size distribution of sand, silt, and clay. The flame photometric determination method was used to measure sodium, Potassium, Calcium, and magnesium cations. The Titration method was used to calculate the calcium carbonate content (TNV). Topographical factors, including elevation, slope percentage, and aspect azimuth, were documented. The coordinates and elevation of the selected sites were recorded using a Garmin OREGON 550 GPS device. Slope and aspect azimuth measurements were obtained with a Sunto Clinometer.

Table 1) Ecological factors of sampling sites.

Site	1	2	3	4	5	6	7	8
Area (ha)	2.11	1.28	2.02	1.19	1.06	1.45	1	1
Elevation (m)	1134	1162	1680	1563	1334	1039	1004	1278
Aspect	Northeast	South	North	Northwest	Southwest	Southeast	North	Southwest
Slope (%)	5	6.3	11.1	10.9	3.9	8.9	8.5	11.2
Canopy Stachys	4. 35	4.05	4.25	4.75	7.15	8.9	14	6.8
Bare Soil (%)	11.1	22.7	7.94	7.5	19.1	6.47	12.32	7.59
Soil Gravel (%)	29	35	31	29	32	39	47	37

Essential Oil Extraction and GC-MS Analysis

The aerial portions of the plant underwent Hydrodistillation for a duration of 2.5 h using a Clevenger-type apparatus to facilitate the extraction of Essential oil. The extracted Essential oils were dried over sodium sulfate and stored in sealed glass vials at 4 °C until chemical analysis was performed.

Gas chromatography (GC) analysis of the extracted oils was done by an Agilent 7890A Network GC system equipped with a Flame Ionization Detector (FID). Also, the extracted oils were analyzed using GC–MS with the same Agilent 7890A Network GC system and conditions, coupled with an Agilent 5975C Network equipped with a Triple-Axis Detector. The constituents of the oils were identified by comparing their retention times and mass spectral patterns with those of existing data or the Wiley-Blackwell library. The C8-C20 series of alkanes was analyzed under identical conditions in the GC-MS to determine the retention indices of the compounds. The percentage of each compound was calculated based on the corresponding GC peak areas, which were used for quantification.

Statistical Analysis

To use multivariate ordination methods, firstly, the average values of phytochemical components for each site were organized into an n×m matrix (primary matrix), where the rows represent the sites and the columns correspond to the attributes. Next, the physico-chemical properties of the soil and the topographic characteristics of each site were compiled into another n×p matrix (secondary matrix), with the rows representing the sites and the columns containing the soil's physical and chemical properties as well as topographic features. In the second step, detrended correspondence analysis (DCA) was applied to the phytochemical components (response data) to select the appropriate ordination method [32]. According to the length of the gradient, which was less than 3, the redundancy analysis (RDA) method was used as a linear method to illustrate the correlation between the primary and secondary matrices [33]. A Redundancy Analysis (RDA) is a multivariate statistical technique used to explore the relationship between two sets of variables: response variables and predictor variables. The results of RDA can be visualized in a biplot ordination plot, where both variables, such as explanatory and response, are displayed in the space of coordinate components. Each variable is represented by arrows emanating from the center of the biplot, whose direction and length indicate the strength and direction of the association [34]. All statistical analyses were performed using CANOCO software version 5.

Findings

Table 2 presents the outcomes of the GC-MS analysis conducted on the key components of *S. inflata* Benth at all eight sites.

Table 2) Key components of *Stachys inflata* Benth. in all eight sites detected by GC-MS (mean± standard deviation).

Row	Compounds	RT	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
1	Cyclohexane (CAS); Hexanaphthene	2.3							8.94±0.6	
2	Heptane, 3-methyl- (CAS); 3-Meth	2.16	16.6±1.8						2.09±0.04	
3	Cyclopentane, propyl-	2.63						0.6±0.01		
4	Cyclopentane, 1-ethyl-3-methyl	3.62							1.98±0.02	
5	Octane	3.78	55.03±2	25±1.05				48.1±2.1	5.41±1.01	
6	Piperazine, 1-nitroso-; N-Nitros	4.45		0.88±0.02						
7	Decane	5.09			11.52±1.2					
8	Benzene, 1,4-dimethyl- (CAS); p	5.2		1.15±0.01						
9	4-Pyrrol3-[1-(2-carboxy-1-phenyl	5.39					1.22±0.2			
10	(2,4,5-Trichlorophenoxy)acetic acid	5.47					1.91±0.3			
11	p-Xylene	5.65		1.31±0.02						
12	Nonane, 3,7-dimethyl- (CAS); 3,7	5.98					1.2±0.05			
13	2,2,6,6-Tetramethylheptane	6.82					1.5±0.1			
14	Benzene, 1-methyl-4-(1-methyleth	8.14		1.86±0.05						
15	1-Isopropyl-2-(methoxymethoxy) benzene	8.28				0.47±0.04				
16	Cyclopentane, 3-hexyl-1,1-dimeth	8.37					0.71±0.07			
17	Pyridine, pentafluoro- (CAS); Pe	8.62								1.34±0.2
18	Cyclopropanecarboxamide, N-cyclo	8.66					2.01±0.2			
19	Cyclohexene, 3-butyl-1-trimethyl	8.67								2.84±0.02
20	1,4-Cyclohexadiene, 1-methyl-4-isopropyl-	8.74		1.14±0.04						
21	Dodecane (CAS); n-Dodecane; Ba 5...	8.77			8.17±0.9					
22	7,9-Dimethylhexadecane	8.99			1.66±0.08					
23	Undecane	9.4			15.02±1.05					
24	1,6-Octadien-3-ol, 3,7-dimethyl	9.46		1.46±0.2						
25	Linalool; 1,6-Octadien-3-ol, 3,7	9.65		0.99±0.06						
26	5-(Trifluoromethyl)-4H-1,2,4-triazole-3(2H)-thione	10.09								0.43±0.03
27	11,15-Dimethylheptatriacontane	10.55					1.03±0.1			
28	3-Cyclohexene-1-methanol, .alpha	11.1		3.73±0.02						
29	Linalyl propionate; 1,6-Octadien	11.38								0.04±0.01
.030	2-Butene-1,4-diol, 2,3-dibromo-	11.74					0.69±0.1			
31	2-Cyclohexen-1-one, 2-methyl-5-(2-methyloxiranyl)	12.38		0.93+.02						
32	Geraniol	12.48		7.52±1.01						
33	d-glycero-beta-d-galacto-2-nonulopyranosonic acid	12.67					0.6±0.06			
34	Thymol	13.04							6.27±0.85	
35	Phenol, 5-methyl-2-(1-methylethy	13.1						0.5±0.01	61.3±2.51	
36	3,9-Epoxypregnan-14-ol-20-one, 3,11,18-triacetoxy-	13.28					0.74±0.1			
37	Trans-Anethole	13.37		10.2±0.95						
38	Benzene, 1-methoxy-4-(1-propenyl	13.56		13.98±1.3						
39	3,5-Dipiperonylidene-1-propyl-4-.	13.85					0.520.04			
40	2-Propyn-1-amine, N-2-propynyl-	14.48					0.57±0.05			
41	Androst-5-en-17-one, 3,16-bis	14.68					0.65±0.07			
42	2,6-Octadien-1-ol, 3,7-dimethyl	15.41		5.10±0.9						
43	Geranyl acetate; 2,6-Octadien-1-...	15.63								0.42±0.01

Table 2 Continued) Key components of *Stachys inflata* Benth. in all eight sites detected by GC-MS (mean± standard deviation).

Row	Compounds	RT	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
44	Geranyl propionate; 2,6-Octadien	15.66		0.65±0.05						
45	Tetradecane; n-Tetradecane	15.78								0.54±0.02
46	Trans-Caryophyllene; Bicyclo[7.2	16.18		0.75±0.05						
47	TMS-11-OH-propyl-8-tetrahydrocan	16.51					0.85±0.05			
48	Benzene, 1,3-bis(3-phenoxyphenox	16.75					1.18±0.07			
49	2,3-Dibromo-5-[4(a)-formylcycloh.	17.02					0.7±0.05			
50	4-Methyl-5-phenyl-2-thiazolamine...	19.36								0.62±0.06
51	Cinoxacin	19.31								0.69±0.07
52	Spathulenol	20.01							3.27±0.09	
53	Caryophyllene oxide	20.14							3.79±0.2	
54	Alpha.-Cadinol	21.52		3.86±0.8						
55	Bis Isopropylidiene Of 3.Beta.,2	21.33					0.87±0.05			
56	2-Acetoxycyclopentanone 2,4-dini...	21.59				0.43±0.1				
57	Bicyclo[4.4.0]dec-1-ene, 2-isopr	21.81							1.03±0.04	
58	Androst-5-en-7-one, 3,17-bis(ace	22.29					0.53±0.04			
59	Silane, [[(17.beta.)-3-methoxyes...	23.16					0.78±0.04			
60	Propylthiophosphonic acid, o-iso	23.45					0.6±0.06			
61	Butanoic acid, heptafluoro-, 2-p...	23.53					0.67±0.04			
62	1H-Isoindole-1,3(2H)-dione, 2-bu	24.44					0.53±0.08			
63	2-Methoxy-5-amino-4,6-diphenylpy.	25.1			1.67±0.04					
64	2-Myristynoyl-glycinaide	25.18					1.04±0.1			
65	Cyclobarbital	25.36					0.66±0.1			
66	Cyclohexane-1,3-dione, 2-allylam.	25.4					0.59±0.09			
67	2-Methylthiobenzofuran	25.47							1±0.1	
68	Eugenol	25.54		0.44±0.05						
69	1-Methyl-3-phenylindole; 1-Methy	25.57					0.69±0.02			
70	2-(N-Methylpyrrolyl) thienoate	25.63					0.89±0.03			
71	Anthracene, 9-ethyl-9,10-dihydro	25.73					0.51±0.05			
72	Methanol, [4-(1,1-dimethylethyl)...	26.1					2.08±0.04			
73	6H-phenanthro[9,8-gh] quinolin-6-one	26.22					1.07±0.05			
74	Benzenamine, 4-(2-phenylethenyl)...	26.53					0.66±0.04			

Table 2 Continued) Key components of *Stachys inflata* Benth. in all eight sites detected by GC-MS (mean± standard deviation).

Row	Compounds	RT	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
75	1-(4-chlorophenyl)-1H-tetrazole	26.71					6.31±1.03			
76	Silane, [(3,3-dimethyl-1-cyclohexen-1-yl)oxy]trimethyl-	26.78					9.51±1.2			
77	Hexadecanoic acid, methyl ester	26.8		0.840.3±						
78	2-Propanol, 1-[(1-methylethyl) am	26.97					2.54±0.9			
79	2,3,6,8-tetramethyl-5,7-dioxo-th...	27.13								0.96±0.2
80	Benzeneethanamine, 2-fluoro-4,5	27.23			1.06±0.04					
81	5-Methyl-2-phenylindolizine	27.4					2.29±0.8			
82	4-Acetoxy-1-phenyl-2	27.49					1.53±0.8			
83	2,4-Dimethylaniline-6-sulfonic Acid	27.69					4.63±0.9			
84	Pyridine, 1,2,3,6-tetrahydro-1-m	28.04					2.67±0.5			
85	Indole-2-one, 2,3-dihydro-N-hydr	28.27					3.37±0.7			
86	1H-Indole, 1-methyl-2-phenyl-; I	28.41					1.01±0.1			
87	8-Methylisothiazolo[4,5-c]-2,1,3.	28.56					1.77±0.1			
88	Hexadecanoic acid, trimethylsily	28.62		0.95±0.1				6.5±0.4		3.04±0.9
89	5-Nitrobenzofuran-2-Carboxylic Acid	28.66					2.27±0.2			
90	2-Benzyl-6-methylpyridine-3-carb	28.8					1.77±0.05			
91	Anthracene, 9-ethyl-9,10-dihydro	28.92					3.17±0.9			
92	Pyrrolidine, 1,5-dimethyl-3,3-di	28.94		1.86±0.1						
93	9,12-Octadecadienoic acid (Z,Z)-...	29.38		1.53±0.2				0.68±0.05		
94	9-Octadecenoic acid, methyl este.	29.4		2.09±0.25						
95	Methyl (2S)-2-Hydroxy-2-[(2R,3R,...	29.49				0.79±0.15				
96	6-Octadecenoic acid, methyl este	29.53								0.44±0.15
97	cis-2-((E)-1-Butenyl)-1-methoxy-...	29.6				0.45±0.05				
98	2-Pentadecanol	29.69						0.65±0.05		
99	Thiocarbamic acid, N, N-dimethyl	29.83				0.59±0.03	1±0.04			
100	Octadecanoic acid, methyl ester	29.84						1.12±0.05		
101	2,6,10,14-Tetramethylpentadecan	29.88				0.71±0.06				
102	Diethyl 2,2'-(1,1'-binaphthalene	30.06					0.9±0.04			
103	10,13-Octadecadienoic acid, meth...	30.19						0.8±0.08		

Table 2 Continued) Key components of *Stachys inflata* Benth. in all eight sites detected by GC-MS (mean± standard deviation).

Row	Compounds	RT	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
104	2-Methyl-7-phenylindole; 2-Methy	30.19					1.32±0.9			
405	1,2-Bis(trimethylsilyl)benzene	30.27					1.46±0.03			
106	Nonadecanoic acid (CAS); n-Nonad...	30.31				0.61±0.22				
107	8,11-Octadecadienoic acid, methy	30.32						1.17±0.05		
108	L-Valine, N-cyclopentylcarbonyl	30.34								1.63±0.04
109	3,cis-(1,1-dimethylethyl)-4,cis-...	30.37				0.83±0.05				
110	1-trimethylsiloxy-1-(3,4-di(trim..	30.49						0.6±0.05		
111	3-[4'-(2"-Dimethylaminoethoxy)ph..	30.53								0.85±0.08
112	Karbutilate	30.54				0.44±0.05				
113	Methanesulfonanilide, 4'-(1-hydr...	30.59				0.4±0.03				
114	Silane, [(3,7,11,15-tetramethyl	30.65		0.63±0.1						
115	Hexahydro-1-methyl-2H-azepine-2	30.67						1.01±0.12		
116	3-Heptanone, 6-(dimethylamino)-4	30.75	15.54±1.03	7.98±1.02	49.79±3.1	37.62±2.1		18.2±1.8		43.35±2.3
117	Butanal (CAS); n-Butanal; Butyra...	30.98						2.1±1.02		
118	3-Dodecanone (CAS); Ethyl nonyl ...	31.6								0.85±0.02
119	1H-Indole-2,3-dione, 1-(tert-but	31.26					2.55±0.2			
120	1-(2-trimethylsiloxy-1,1-dideute	31.36					1.06±0.03			
121	Pyridostigmine Bromide; Pyridini	31.42								2.35±1.2
122	1-Benzopyrylium, 2-phenyl-; Flav...	31.44					0.65±0.02			
123	2,6-Diethoxytetrahydropyran	31.54								0.41±0.03
124	t-Amyl-t-butyl-p-benzoquinone	31.97					0.64±0.08			
125	Bis(2-ethylhexyl) phthalate	32.39			2.79±0.9					
126	Silicic acid, diethyl bis(trimet	32.48					0.84±0.05			
127	9-Octadecenamide, (Z)- (CAS); OL...	34.09								4.58±0.9
128	Oxirane, 2-ethyl-3-propyl-; 2-Et...	34.12				0.64±0.04				
129	Pentacosane (CAS); n-Pentacosane	34.68				0.82±0.05				
130	Nonadecane, 1-chloro-; 1-Chloron...34.69	34.69								0.52±0.05
131	Urea, 1-butyl-3-(propylsulfonyl)...	35.99						1.54±0.04		
132	Hexanedioic acid, bis(2-ethoxyet...	36.01				0.48±0.04				
133	N-Isopropoxy-2-carbomethoxyazeti...	36.02								0.57±0.08
134	3,Trans-(1,1-dimethylethyl)-4,ci...	36.23				0.45±0.06				

Table 2 Continued) Key components of *Stachys inflata* Benth. in all eight sites detected by GC-MS (mean± standard deviation).

Row	Compounds	RT	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
135	Butanal, 2-ethyl- (CAS); 2-Ethyl..	36.23						1.55±0.4		
136	Eicosane	36.23						4.85±0.91		0.94±0.09
137	1,2-Benzenedicarboxylic acid, bi	37.01	2.82±0.02			2.8±1.01				
138	Di-n-octyl phthalate	37.01						3.01±1.09		
139	Di-(2-ethylhexyl)phthalate	37.02								2.56±0.9
140	2-(4'-nitro-3'-thienyl)pyrimidine	37.25				0.48±1.02		1.33±1.03		
141	4,14-Bis(Hydroxymethyl)-[2.2] Met...	38.4						0.77±0.05		
142	N, N-Dimethylthiocarbamic acid, 3	38.4		1.13±0.05						
143	trans-2-Ethoxy-.beta.-methyl-.be	38.4				1.36±0.04				
144	5.Beta.-Pregnane-3.Alpha,20.Bet...	38.42								0.79±0.02
145	2-Butanone, 4-[2-(1-methylethyl)...	38.6	3.09+0.5							
146	1-butylthio-1,3,5-trimethyl-7-me	38.6						1.08±0.06		
147	Heptanoic acid, heptyl ester	38.62								1.77±0.1
148	7-Amino-1,4-dimethylpyrimido[4,5	38.7				1.13±0.2				
149	13-Methylhentriacontane	38.9								0.51±0.02
150	1H-Indole, 5-methyl-2-phenyl-	39.05				3.74±0.9				
151	1H-Indole, 2-methyl-3-phenyl- (C	39.48				1.85±0.15				
152	N-Methyldeacetylcolchicine; Benz...	39.55								0.58±0.12
153	Heneicosane, 3-methyl-; 3-Methyl...	40.07				0.84±0.1				
154	Pyrido[2,3-d]pyrimidine, 4-pheny	40.7				0.52±0.03				
155	1-(4-Amino-furazan-3-yl)-5-morph	40.88				2.66±0.9				
156	Benzoxazole, 2-[2-(4-morpholyl)e...	40.88						0.85±0.02		
157	syn-2-(Phenylmethyl)-3-hydroxy-3..	40.88								1.27±0.12
158	2-Ethylacridine	41.03				0.46±0.03	2.89±0.2			0.63±0.2
159	1,3,5,7-Tetraethylbicyclo[3.3.1]...	41.09				0.62±0.05				
160	3,5-Dimethyl-2,6-bis(trimethylsi...	41.17								0.93±0.02
161	2-Propen-1-one, 3-[4-(1-methylet...	41.47								0.61±0.5
162	4-Dehydroxy-N-(4,5-methylenediox...	41.52								0.57±0.3
163	trans-3,4-Dihydro-2,2-dimethyl-6	41.59				1.08±0.4				
164	N-Hydroxy-N-Dimethyl-20. Alpha.-M	41.76						1.21±0.5		

Table 2 Continued) Key components of *Stachys inflata* Benth. in all eight sites detected by GC-MS (mean± standard deviation).

Row	Compounds	RT	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
165	1,1,1,3,5,5,5-Heptamethyltrisilo	41.77	2.82±1			3.02±0.9	0.89±0.05	0.57±0.05		5.1±1.02
166	1,3-dimethyl-4-azaphenanthrene	42				1.83±0.2	1.55±.09			
167	1,2-Bis(trimethylsilyl)benzene	42.06				1.67±0.2	3.29±0.5			0.97±0.06
168	9-Methyl-11-(1-Cyano-2-Methoxy-2...	42.09				0.64±0.04				
169	2-(Acetoxymethyl)-3-(methoxycarb.	42.21				1.83±0.03	2.22±0.1			
170	7-Amino-1,4-dimethylpyrimido[4,5	42.21								1.08±.08
171	Silane, 1,4-phenylenebis[trimeth...	42.6				4.28±1.01				0.57±0.02
172	2,4-Cyclohexadien-1-one, 3,5-bis...	42.8								1.6±0.2
183	Ethane, 1-(4,4,4-trifluoro-1,3-d...	43.31				1.01±.2				
174	Silane, trimethyl[5-methyl-2-(1-...	43.34								3.77 ±0.5
175	pyrrolo[2,3-b]dibenzofuran	43.82				1.31±0.08	0.96±0.05			
176	Niralin; Benzenamine, 4-(methyls	44		1.48±0.5						
177	N-Methyl-1-adamantaneacetamide; ...	44.65								0.48±0.08
178	Trimethyl[4-(2-methyl-4-oxo-2-pe...	45.05				0.49±0.08				
179	Benzene, 1,4-Bis (Trimethylsily)-	45.13				1.36±0.04				1.96±0.12

According to the findings presented in Table 1, seven compounds were identified in the extract of *S. inflata* Benth. plant at site 1, with the highest value belonging to the octane compound at 55.03%. At site 2, 28 compounds were identified, of which octane accounted for the most significant percentage, at 25%. Site 3 had nine identified compounds, with 3-heptanone and 6-(dimethylamino)-4 having the highest percentage of compounds at 49.79%. At site 4, 38 compounds were identified, among which 3-heptanone and 6-(dimethylamino)-4 had the highest percentage at 37.62%. Site 5 had 60 identified compounds, of which 3,3-Dimethyl-1-oxa-3-silacyclohe accounted for the highest percentage at 9.51%. In site 6, 24 compounds were identified, with the octane compound having the highest percentage (48.11%) among the available compounds. Site 7 identified 11 compounds, with the phenol 5-methyl-2-(1-methylethy) compound having the highest percentage at 61.3% among the existing compounds. Lastly, site 8 identified 40 compounds, with 3-heptanone and 6-(dimethylamino)-4 having the highest percentage (43.35%) among the existing compounds.

Detrended Correspondence Analysis (DCA) was initially employed to identify an appro-

priate response model for the response matrix of phytochemical compound data. DCA is a straightforward multivariate technique used to arrange response matrices and sites along environmental gradients indirectly. The gradient length of the first DCA axis serves as an indicator of beta diversity (the extent of species turnover) along independent gradients (ordination axes). A gradient length below 3 suggests a linear response, while a length greater than 4 indicates an unimodal reaction [35]. In this study, the gradient length of the first DCA axis was 1.68, which is shorter than the threshold of 3.0 (Table 3).

Table 3) The results of Detrended Correspondence Analysis (DCA) based on two axes.

Axis	Gradient Length	Eigenvalues	Explained Variation (Cumulative)
1	1.68	0.2936	45.96
2	0.57	0.0338	51.25
3	0.51	0.0018	51.54
4	0.44	0.0009	51.68

Therefore, RDA was conducted as a linear method to determine how Environmental factors affected the phytochemical compound data (Table 4). The sum of all canonical eigenvalues indicated the total variance explained by Environmental parameters in the dataset and was 0.99 for the phytochemical compounds. For the variance of the compounds–environment relationship, the first and second canonical axes explained 54.02% and 30% of the total variation, respectively, indicating that the reliability of the association is notably high.

Table 4) Results of Redundancy Analysis (RDA) on environmental data and plant phytochemical compounds.

Axis	Eigenvalues	Explained Variation (Cumulative)
1	0.5402	54.02
2	0.3000	84.02
3	0.0975	93.59
4	0.0616	99.75

The biplot obtained from RDA is shown in Figure 2. The results revealed a clear relationship between plant phytochemical compounds (represented by blue solid lines) and environmental parameters (represented by red solid lines). Furthermore, it illustrates the distribution of the eight sampling sites (black solid circles) in space based on the coordination of chemical compounds and environmental factors.

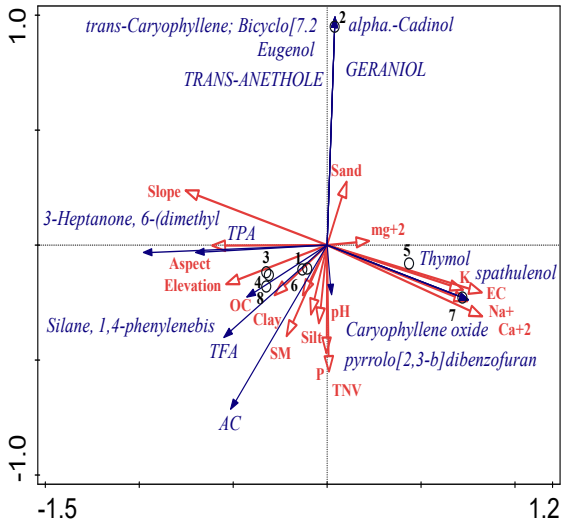

Figure 2) Biplot of redundancy analysis (RDA) showing the association between phytochemical compounds and environmental variables. The arrow length indicates the importance of the variable in the model, and the arrow orientation indicates the direction in which the variable increases.

The biplot of RDA illustrates the intensity of the relationships between the dependent and independent variables. A larger vector length and a smaller angle between variables indicate a higher correlation. The vectors of thymol, spathulenol, and caryophyllene oxide have a high relation with cations and EC. This means that an increase in cations and EC increases the levels of the mentioned compounds. Conversely, thymol, spathulenol, and caryophyllene oxide have a negative correlation with the slope, aspect, and elevation.

Various factors, including elevation, organic

carbon, clay and silt, pH, TNV, and saturated moisture percentage, positively influence Silane, 1,4-phenylenebis, TFC, and AC. The sand and magnesium have adverse effects on these compounds. However, the elevation axis has the most significant impact on this composition. The axes of alpha-cadinol, geraniol, eugenol, trans-caryophyllene, bicyclo [7.2], and Trans-anethole have a positive relationship with sand and Mg axes. These compounds were not correlated with height, organic carbon, clay, and saturated moisture. The composition of 3-heptanone and 6-(dimethyl) has a positive relationship with the slope, aspect, and elevation, and a negative relationship with cations and EC. The aspect parameter has the maximum positive effect on the compound compared with the other two parameters. In contrast, 3-heptanone and 6 6-dimethyl are incompatible with Mg. Therefore, an increase in Mg decreases the percentage of these compounds. The composition of pyrrolo[2,3-b]dibenzofuran exhibits a positive correlation with pH, silt, phosphorus (P), and the percentage of lime (TNV). An increase in these parameters increases the percentage of the compound. Among these parameters, TNV had the most significant effect on the composition. On the other hand, pyrrolo[2,3-b]dibenzofuran exhibits a negative correlation with the sand parameter.

The results indicate that higher elevation, percentage of saturated moisture, clay, and percentage of organic carbon lead to higher amounts of AC (antioxidant) and TFA (phenolic) compounds in the composition of 1,4-phenylenebis. Among these factors, the height axis has the most significant positive effect on the amount of antioxidant and phenolic compounds in the composition. Consequently, the first axis is primarily associated with soil and topography factors, while soil properties largely determine the second axis. This indicates that phytochemical characteristics are jointly controlled by soil and topography.

Discussion

Different research studies have shown that environmental factors affect various characteristics of medicinal plants, including growth, the synthesis of secondary metabolites, as well as genetic factors and substance accumulation [36]. In line with this, the results of the current research indicated a positive correlation between certain soil factors—namely sodium, calcium, and electrical conductivity—and the compounds thymol, spathulenol, and caryophyllene oxide. In contrast, the slope parameter exhibits a negative correlation with these compounds, indicating that steeper slopes are associated with lower concentrations of these compounds. Figueiredo et al. [37] emphasized the importance of soil elements, such as potassium and calcium, for the efficient production of plant compounds. In contrast, soil acidity was found to influence the synthesis of volatile compounds. Similarly, El Keltawi and Croteau [38] reported that saline environments not only inhibit plant growth but also suppress the production of essential oils in the genus *Lamium* species. Ozturk et al. [39] further observed that salt stress reduces essential oil yields in lemon balm plants.

The compound of silane, 1,4-phenylenebis, is positively related to soil clay. One of the main reasons for this finding can be attributed to the distinctive properties of soil clay, which promote various processes such as adsorption, retention, and stability of chemical compounds. Additionally, soil clay provides a favorable environment for the growth, living, and activity of soil microorganisms. Some of these microorganisms can play a role in the decomposition of organic materials and the

production of phytochemical compounds in plants, as demonstrated by Imani Dizjikan et al. [40], who also identified a positive correlation between soil silt and clay content and compounds such as sabinene, 1,8-Cineole, and Alpha-thujone. These findings suggest that an increase in silt and clay content enhances the concentrations of these compounds.

The critical environmental factors, such as altitude, saturated soil moisture, clay content, and organic carbon, significantly influenced the compound of silane, 1,4-phenylenebis. These elements lead to an increase in the amount of this compound as well as antioxidant and phenolic index values. Conversely, higher sand and magnesium amounts in the soil reduce the composition of silane, 1,4-phenylenebis, which his a result that may be related to the increase in moisture, by providing a suitable condition for microbial activity. Similarly, clay particles enhance the soil's ability to retain nutrients. The increase in organic carbon can accelerate the biochemical and metabolic activities of microorganisms. Therefore, environmental factors play a crucial role in the metabolic process that leads to variation in the formation of secondary compounds in the soil. Following this, the results of Alimohammadi et al [41] demonstrate that altitude and phenological stages have a significant impact on the essential oil of the Stachys species. This finding also contradicts the results of Eman et al. [42], who reported that sand has a positive effect on the essential oil content of *Thymus vulgaris*. Similarly, the findings of Baczek et al. [43] indicated that soil depth significantly decreases at high altitudes. Conversely, lower-altitude areas exhibit higher humidity. This change in soil moisture amount depends on soil texture and finally leads to a decline in various essential oil components. These findings highlight the significant impact of environmental factors, such as altitude, on altering soil characteristics and, consequently, the secondary metabolites of plants. Jafari et al. [44] found a negative association between elevation and phenolic compounds in leaf extracts, and also a positive relationship between elevation and fig plant metabolites. Carey and Wink [45] found that alkaloid content in *Lupinus argenteus* Pursh. decreases with increasing altitude, while Demasi et al. [46] reported that lower latitudes enhance the production of volatile compounds and essential fatty acids in *Lavandula angustifolia* Mill. Similarly, Alibakhshi et al. [47] noted higher essential oil content in *S. inflata* at lower altitudes.

Soil moisture amounts were also positively associated with silane, 1,4-phenylenebis, phenolic compounds, and antioxidant indices. This relationship may be associated with the concept that higher soil moisture promotes nutrient availability for plants, reduces environmental disturbances, and enhances the activity of soil microorganisms. Overall, sufficient soil moisture provides a suitable environment for plant growth, resulting in higher production of important secondary metabolites, such as phenols and antioxidants.

In contrast, reduced soil humidity has been shown to increase basil essential oil production [48]. Rhizopoulou and Diamantoglou [49] similarly found that lower soil moisture enhances the production of major essential oils in *Origanum majorana* L. Salehi and Kholvandi [50] conducted a study on morphological and phytochemical variations in different populations of *S. inflata* Benth. in Hamedan Province, Iran, reported significant differences in phenolic and flavonoid compound concentrations. They attributed this variation to transient environmental factors, including soil type, climatic conditions, and various

stressors. Additionally, Mohammadirad [51] examined the variability of phytochemical characteristics and morphological traits in the species *Stachys inflata* Benth. concerning environmental factors. They demonstrated that as the amount of sand increases, the percentage of phytochemical compounds in this species, including alpha-cadinol, geraniol, trans-caryophyllene, trans-anethole, and eugenol, also increases. In contrast, the levels of phenol, flavonoid, and antioxidant indices decrease. The percentage of thymol, spathulenol, caryophyllene acid, and proulo dibenzofuran compounds increases with increasing calcium, sodium cations, and electrical conductivity. With increasing altitude, the percentage of lime, organic carbon, silt, clay, saturation moisture, and pH, as well as placement on southern slopes, increases the levels of phenol, flavonoid, and antioxidant indices present in the compounds. However, the extent of the impact of these parameters varies. Ashrafzadeh et al. [23] concluded that heavier soil texture, a semi-arid climate, and the flowering stage provide more favorable conditions for the production of secondary metabolites in C. *ispahanica*.

Conclusion

The analysis of *S. inflata* Benth. extract using GC/MS across eight populations from their natural habitats identified 179 major compounds. Multivariate statistical analysis (RDA) revealed that the phytochemical compounds of *St. inflata* are significantly influenced by both soil and topographical factors. The findings revealed a positive association between specific soil parameters, including sodium, calcium, and electrical conductivity, and increased concentrations of compounds such as thymol, spathulenol, and caryophyllene oxide. This relationship suggests that soils with higher amounts of these factors present a more suitable condition for the production of these valuable compounds. Such findings have significant implications for establishing cultivation conditions and soil management strategies to enhance the yield of these compounds. Conversely, a higher slope is a limiting factor, leading to a significant decline in the amount of these compounds. This implies the vital role of topography for the suitable growth of the species, as lower slopes can lead to the preservation and accumulation of phytochemicals. Furthermore, our research revealed that certain compounds, such as silane and 1,4-phenylenebis, are profoundly influenced by soil factors. All soil properties, such as elevation, saturated soil moisture, clay content, and organic carbon, increased the concentrations of these compounds. Importantly, these factors also demonstrate a positive association with higher antioxidant and phenolic indices, underscoring the important role of these environmental variables in promoting the medicinal properties of the study species. These findings show the importance of soils with suitable clay texture, high organic content, and sufficient moisture for the production of these valuable compounds. In addition, a significant increase in sand content was associated with higher concentrations of compounds such as alpha-cadinol and geraniol, while simultaneously reducing the amounts of phenols, flavonoids, and overall antioxidant indices. This contrasts with the positive relationship observed between higher calcium and sodium cations and electrical conductivity, as well as an increase in thymol and caryophyllene. Elevation and south-facing slopes also showed a significant impact, with higher elevations and southern exposures correlating with greater concentrations of phenols, flavonoids, and antioxidant indices. These results powerfully illustrate that *S. inflata* Benth. is notably well-adapted to its

natural environment, particularly under stressful conditions, thereby highlighting its significant potential for extract production. Based on these results, we suggest expanding phytochemical analyses to include chemotypes found in other stress-prone environments, especially those influenced by climatic variables and soil nutrient deficiencies. Future studies should focus on investigating the influence of extreme climatic events and nutrient limitations in greater detail to understand better their impact on the phytochemical profiles of this resilient species. Overall, for producing a rich extract, stressful environments are a suitable selection. Additionally, for domestication, a soil with higher cations and a lower slope and elevation is required. Site 5, with its richer compounds, can serve as a pilot site for collecting seeds for artificial seeding plantations.

In this study, we investigated two hypotheses. The first hypothesis posited that phytochemical compounds differ across various regions. Based on the obtained results, which indicated variations in the chemical compounds of the species across different sites, this hypothesis is supported. The second hypothesis suggested a relationship between the phytochemical compounds of the studied species and soil and topographical factors. Our findings also support this hypothesis, as our results demonstrate that phytochemical compounds vary across different sites due to prevailing environmental conditions, including topography and soil characteristics.

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References

1. Barbosa Filho V.M., Waczuk E.P., Kamdem J.P., Abolaji A.O., Lacerda S.R., da Costa J.G.M., de Menezes I.R.A., Boligon A.A., Athayde M.L., da Rocha J.B.T. Phytochemical constituents, antioxidant activity, cytotoxicity and osmotic fragility effects of Caju (*Anacardium microcarpum* Ducke). Ind. Crop Prod. 2014; 55(1): 280–288.
2. Bertol I., Santos M.P., Barbosa M.A. The role of native vegetation and the absence of human degradation in soil fertility and production of vegetables. Agric. Ecosyst. Environ. 2004; 102(3): 389-394.
3. Pedrotti F. The quality of the soil, in terms of its physical and chemical properties, directly affects plant growth and development by influencing the resistance exerted on root growth. Soil Sci. 2001; 166(6): 387–394.
4. Zhang Q., Zhou Z., Zhao W., Huang G., Liu G., Li X., Wu J. Effect of slope position on leaf and fine root C, N and P stoichiometry and rhizosphere soil properties in *Tectona grandis* plantations. J. For. Res. 2023; 34(6): 1997-2009.
5. Sheidai Karkaj E., Sepehry A., Barani H., Motamedi J., Shahbazi F. Establishing a suitable soil quality index for semi-arid rangeland ecosystems in northwest of Iran. J. Soil Sci. Plant Nutr. 2019; 19(3): 648–658.
6. Bernáth J. Production ecology of secondary plant products. Herbs, spices, and medicinal plants. Rec. Adv. Bot.Hort Pharm. 1986; 1(1): 185–234.
7. Lukas B., Schmiderer C., Novak J. Essential oil diversity of European *Origanum vulgare* L. (Lamiaceae). Phytochemistry. 2015; 119(1): 32–40.
8. Younessi Hamzekhanlu M., Sanjari S., Dejahang A., Sheidai Karkaj E., Nojadeh M.S., Gönenç T.M., Ozturk M. Evaluation of essential oil from different *Artemisia fragrans* Willd. Populations: Chemical composition, antioxidant, and antibacterial activity. J. Essent. Oil-Bear. Plant. 2020; 23(6): 1218–1236.

9. Formisano C., Delfine S., Oliviero F., Tenore G.C., Rigano D., Senatore F. Correlation among environmental factors, chemical composition, and antioxidative properties of essential oil and extracts of chamomile (*Matricaria chamomilla* L.) collected in Molise (South-central Italy). Ind. Crop. Prod. 2015; 63(1): 256–263.
10. Singh R. Chemotaxonomy: a tool for plant classification. J. Med. Plant. Stud. 2016;4(2) 90–93.
11. Ramak P., Karimian V. Effects of planting times on the chemical compositions, antioxidant activity, and nutritional properties of *Allium jesdianum* Boiss. & *Buhse leaves* in Lorestan Province. ECOPERSIA 2023;11(3): 175–185.
12. Younessi-Hamzekhanlu M., Abdipour M., Dejahang A., Sabzi-Nojadeh M., Amani M. Herbals used in western Iran as food and for health treatments. In Biodiversity, conservation and sustainability in Asia: Volume 1: prospects and challenges in West Asia and the Caucasus. Cham: Springer International Publishing. 2021; 547–599.
13. Amelie P., Naseri S., Rahmani A., Kalirad G., Ahadiyeh H. Medicinal plants of Kerman Province. IJMAPR. 2013; 20(1): 532-487.
14. Omidbeigi R. Production and processing of medicinal plants, Volume 1, Astan Quds Razavi Publications. 2009;347p.
15. Mozaffarian V. *Dictionary of Iranian plants*. Publications of the Institute of Contemporary Culture, Tehran. 1996; 522 p.
16. Pashova S., Karcheva-Bahchevanska D., Ivanov K., Ivanova S. Genus Stachys—Phytochemistry, Traditional Medicinal Uses, and Future Perspectives. Molecules. 2024; 29(22): 5345.
17. Lindqvist C., Albert V.A. Origin of the Hawaiian endemic mints within North American *Stachys* (Lamiaceae). Am. J. Bot. 2002; 89(10): 1709-1724.
18. Mohammadi H., Aghaee A., Pormohammad P., Ghorbanpour M., Hazrati S. Physiological reaction and chemical composition of *Stachys schtschegleevii* Sosn. essential oil under water deficit. Acta Sci. Pol. Hortorum Cultus. 2022; 21(2): 103–114.
19. Aboukhalid K., Al Faiz C., Douaik A., Bakha M., Kursa K., Agacka Mołdoch M., Machon N., Tomi F., Lamiri A. Influence of environmental factors on essential oil variability in *Origanum compactum* Benth growing wild in Morocco. Chem. Biodivers. 2017; 14(9): e1700158.
20. Aldibekova A., Kurmanbayeva M., Aksoy A., Permitina V., Dimeyeva L., Zverev N. Anatomical structure and phytochemical composition of a rare species *Fraxinus sogdiana* Bunge (Oleaceae) growing in different soils in Kazakhstan. Diversity. 2023; 15(6): 769.
21. Santos P.V.L., Ellen de Nazaré S., de Sousa Barroso A., Mourão R.H.V., Setzer W.N., da Silva J.K., do Nascimento W.M.O., da Costa J.S., Figueiredo P.L.B. Chemometric analysis of the seasonal variation in the essential oil composition of Psidium acutangulum Mart. ex-DC. growing in the Brazilian Amazon. Biochem. Syst. Ecol. 2022; 105: 104528.
22. Sadeghi S., Mohammadi M., Salehi A. Influence of soil and topographical factors on the phytochemical composition of wild medicinal plants in the Zagros Mountains. J. Plant Ecol. 2018; 47(2): 121-134.
23. Ashrafzadeh M., Niknahad – Gharmakher H., Saharkhiz MJ., Ghorbani Nohoji M. Effects of Phenological stages and ecological factors on secondary metabolites of *Clematis ispahanica* Boiss. ECOPERSIA 2023; 11(2): 93–104.
24. Dehghani Bidgoli R. Essential oil composition and antioxidant properties of *Artemisia sieberi* Besser in two enclosure and grazed sites at three phenological stages. ECOPERSIA 2019; 7(1): 13–20.
25. Morteza Semnani K., Saeedi M., Mahdavi M.R., Rahimi F. Antimicrobial effects of methanolic extracts of some species of *Stachys* and *Phlomis*. J. Mazandaran Univ. Med. Sci. 2007; 17(57): 57-66.
26. Ullah I., Adnan M., Begum S., Nazir R., Javed T., Aziz M.A. Effects of ecological factors on phytochemical and nutritional composition of *Caralluma tuberculata* N.E.Br. (*Apteranthes tuberculata* (N.E.Br.) Meve & Liede). Biocem. Syst. Ecol. 2022; 105: 104518.
27. Odisho S. Ecological regions of Iran, vegetation Types of the Khoy-Salmas area. Publications of the Research Institute of Forest and Rangelands. 1997; 60 p.
28. Benjamin M.A.Z., Ng S.Y. Saikim F.H. Rusdi N.A. The effects of drying techniques on phytochemical contents and biological activities on selected bamboo leaves. Molecules. 2022; 27(19): 6458.
29. Boulding J.R. Description and sampling of contaminated soils: a field guide. CRC Press. 2017.
30. Carter M.R., Gregorich E.G. Soil sampling and methods of analysis. Canadian Society of Soil Science. CRC Press, 2008; Taylor and Francis Group Boca Raton, FL.
31. Gee G.W., Or D. Particle-size analysis. Methods of soil analysis: Part 4 Physical methods 2002; 5: 255–293.
32. Hill M.O., Gauch H.G. Detrended correspondence analysis: an improved ordination technique. Vegetatio.1980; 42(1): 47–58.
33. Jongman R.H.G., Ter Braak C.J.F., Van Tongeren

O.F.R. Data analysis in community and landscape ecology, Cambridge University Press, Cambridge, England. 1995.
34. Motamedi J., Sheidai Karkaj E., Zarei Barenji M.Z. The association pattern of structural and biomass traits of *Capparis spinosa* L. with topographic and soil factors. Acta Ecol. Sin. 2020; 40(2): 145-152.
35. Lepš J., Šmilauer P. *Multivariate analysis of ecological data using CANOCO.* Cambridge: Cambridge University Press. 2003.
36. Li X., Li Y., Chen A., Gao M., Slette I.J., Piao S. The impact of the 2009/2010 drought on vegetation growth and terrestrial carbon balance in Southwest China. Agric. For. Meteorol. 2019; 269(1): 239–248.
37. Figueiredo A.C., Barroso J.G., Pedro L.G., Scheffer J.J. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour. Frag. J. 2008; 23(4): 213–226.
38. El Keltawi N.E., Croteau R. Salinity depression of growth and essential oil formation in spearmint and marjoram and its reversal by foliar applied cytokinin. Phytochemistry. 1987; 26(5): 1333–1334.
39. Ozturk A., Unlukara A., Ipek A., Gurbuz B. Effects of salt stress and water deficit on plant growth and essential oil content of lemon balm (*Melissa officinalis* L.). Pak. J. Bot. 2004; 36(4): 787-792.
40. Imani Dizjikan Y., Ebrahimi Gajoti T., Abdi Ghazi Jahan, A. Investigating the pattern of quantitative and qualitative changes in the essential oil of *Thymus pubescens* Boiss. & *Kotschy ex Čelak*. under the influence of the soil of the habitat area in East Azerbaijan. Iran. Biol. J. 2019; 33(1): 257–265.
41. Alimohammadi M., Yadegari M., Shirmardi H. A. Effect of elevation and phenological stages on essential oil composition of *Stachys*. Turk. J. Biochem. 2017; 42(6): 647–656.
42. Eman E. A. Hendawi S.T., El Din E.A., Omer E.A. Effect of soil type and irrigation intervals on plant growth, essential oil yield, and constituents of *Thymus vulgaris* L. plant. Am. -Eurasian J. Agric. Environ. Sci. 2008; 4(4): 443-450.
43. Baczek K., Kosavowska O., Jaroslaw L., Przibil Z. Accumulation of phenolic compounds in the purple betony herb (*Stachys officinalis* L.) originated from cultivation. Herb. Botanica. 2016; 62(2):7–16.
44. Jafari N., Naderi P.A., Ebrahimzadeh M.A. Measurement of total phenol and flavonoid content and investigation of antioxidant properties of fig (*Ficus carica* L.) and large (*Pterocarya fraxinifolia*) leaf extracts by spectrophotometry and high-performance liquid chromatography. Iran. J. Biol. 2014; 25(1): 1–16.
45. Carey D.B., Wink M. Elevational variation of quinolizidine alkaloid contents in a lupine (*Lupinus argenteus* Pursh) of the Rocky Mountains. J. Chem. Ecol. 1994; 20(4): 849–857.
46. Demasi S., Caser M., Lonati M., Cioni P.L., Pistelli L., Najar B., Scariot V. Latitude and altitude influence secondary metabolite production in peripheral alpine populations of the Mediterranean species *Lavandula angustifolia* Mill. Front. Plant Sci. 2018; 9: 387787.
47. Alibakhshi M., Mahdavi S.K.h., Mahmoudi J., Qalichnia H. Phytochemical evaluation of *Stachys inflata* Benth. Essential oils in different habitats of Mazandaran Province. Ecophytochemist. 2013; 2(2): 56–68.
48. Simon J.E., Reiss Bubenheim D., Joly R.J., Charles D.J. Water stress-induced alterations in essential oil content and composition of sweet basil. J. Essent. Oil Res. 1992:4(1); 71–75.
49. Rhizopoulou S., Diamantoglou S. Water stress-induced diurnal variations in leaf water relations, stomatal conductance, soluble sugars, lipids, and essential oil content of *Origanum majorana* L. J. Hortic. Sci. 1991; 66(1): 119-125.
50. Salehi M., Kolvandi R. Investigating the amount of changes in morphological and phytochemical characteristics of different populations of flounder (*Stachys inflata* Benth.) in Hamadan Province. J. Hortic. Sci. 2019; 2(1): 260-247.
51. Mohammadi-Rad Z., Sheidai-Karkaj E., Mofidi-Chelan M., Younessi-Hamzekhanlu M. The relationship between the morphological traits of the medicinal *plant Stachys inflata* Benth and environmental factors. Rangeland. 2023; 17(1): 145–164 (in Persian with English abstract).