Research Article

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Effect of elevation and phenological stages on essential oil composition of Stachys
Stachys’ in uçucu yağ bileşimine yükseklik ve fenolojik aşamalarının etkisi

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Abstract

Objective: Determine the best elevation and phenological stages effects on essential oil content and composition in Stachys obtusicrena.

Materials and methods: Three phenological stages (vegetative, full flowering and seeding) and three elevation ranges (2500–2700, 2700–2900 and over 2900 m), shoots of plants collected from Kallar mountain as natural habitats from Chaharmahal and Bakhtiari Province. Composition of essential oil detected by GC/MS.

Results: The maximum amount of secondary metabolites of β-ocimene, methyl chavicol, germacrene-D, 1,8 cineole and phthalate had measured in vegetative stage and various of elevation ranges. The most essential oil content (0.145%) was obtained in 2700–2900 m and full flowering stage. The most components in full flowering were β-eudesmol, menthol and γ-eudesmol. The most germacrene-D, levomenol, β-caryophyllene, β-sesquiphellandrene, α-eudesmol and delta-cadinene was achieved in seeding stage.

Conclusion: Elevation and phenological stages had significant effect on essential oil of Stachys. The most of delta-3-carene, cis-β-ocimene, β-caryophyllene and levomenol obtained in plants in loam silty sandy texture. It seems that this texture had more capacity to hold of water and nutrients and then made better growth and essential oil production.

Keywords: Stachys obtusicrena; Kallar Mountain; Essential oil composition; Chaharmahal and Bakhtiarı Province.

Özet

Amaç: Stachys obtusicrena'da esansiyel yağ içeriği ve kompozisyonu üzerindeki en yüksek yüksekliğe ve fenolojik aşamalarının etkilerini belirlemiştir.

Gerçek ve Yöntem: 3 fenolojik aşama (vejetatif, tam çiçeklenme ve ekim) ve 3 yüksekleme aralığı (2500–2700, 2700–2900 ve 2900 metreden yüksek), Kallah dağından Chaharmahal ve Bakhtiarı eyaletlerinin doğal yaşam alanları olarak toplanan bitki sürgünleri. GC / MS tarafından tespit edilen uçucu yağ bileşimi.

Bulgar: β-ocimene, metil chavicol, germakren-D, 1,8 sineol ve fitalatın ikiçil met abolilertleri, vejetatif evrede ve çeşitli yüksekleme aralıklarında ölçülmüştür. En önemli yağ içeriği (% 0.145) 2700–2900 m ve tam çiçek açma aşamasında elde edilmiştir. Tam çiçek açmadan en çok bileşenleri β-Eudesmol, Mentol ve γ-Eudesmol idi. Germacrene-D, Levomenol, β-Thin, β-caryophyllene, β-sesquiphellandrene, α-Eudesmol ve delta-Cadinin çoğunun ekim aşamasında elde edildi.

Sonuç: Yükseklik ve fenolojik evreler Stachys esansiyel yağı üzerinde önemli etkiye sahiptir. Delta-3-karen, cis-β-ocimene, β-karyofililen ve Levomenolden en çok, bitkilerde türlü kumanı plaka kumuda elde edilmiştir. Öyle görünüyor ki, bu doku, su ve besin maddeleri tutma kapasitesine sahiptir ve daha sonra daha iyı büyümeye ve uçucu yağ üretimini yapıtı.

Anahtar kelimeler: Stachys obtusicrena; Kallar Dağı; Uçucu yağ bileşimi; Chaharmahal ve Bakhtıarı il.
Introduction

*a Stachys* L. (*Lamiaceae, Lamioideae*) with about 300 species worldwide [1] is the largest genus of the subfamily Lamioideae and among the largest genera of the entire Lamiaceae. The genus was consumed primarily as teas and that this was due to essential oil content and composition. Some species of the genus are also consumed in Europe and China as a carbohydrate source [2]. These plants are scattered in most parts of the globe, but most of its records in the Mediterranean region is well-known plants such as mint, lavender, lemon balm, oregano, sage, thyme, savory, basil, marjoram in this family [3]. The constant characteristics of the dark, irregular flower and cup is always two lobes. The use of indigenous medicinal and wild plants in natural habitats as well as ecological compatibility are able to synthesize the active ingredients secondary environmental stress, in prevention and treatment of diseases to be effective. Regarding identification of secondary phytochemistry in medicinal plants, little research has been done. In recent years with the proliferation of chemical plant chemical compounds most abundant species of *Stachys* been well-known, but proper planning and knowledge in order to harvest the conversion of active ingredients of the compounds in the medicinal value [4], industrial and materials consumption by changing the value of at least maintain soil and water is not carried out [5]. The aim of cultivation of medicinal plants is obtain the final product of secondary metabolites (active pharmaceutical ingredients). Therefore, factors affecting growth and yield and quality are important [6]. Medicinal and aromatic Lamiaceae high ecological flexibility due to diverse climates as one of the important plant genetic resources are downy spikes of very fragrant perennial shrub plant. It is often in the range of mountain springs and aqueducts are influenced by drainage water, is growing [6]. Elevation of their habitats in mountainous areas, between 2300 and 3000 m (and sometimes higher) above sea level. The main habitat of this plant in Iran, are relatively wet spots and areas near rivers, springs around the mountainous provinces of Chaharmahal and Bakhtiari, Lorestan, Isfahan, Tehran, Yazd, Markazi, Fars, Kohgiloyeh and Boyerahmad. *Stachys* comprises annual, as well as, perennial herbs and subshrubs, preferring alpine and subalpine habitats and growth in ecologically divergent localities, such as rocks, mountain steppes, and banks of streams or forests. Phytochemical studies in *Stachys* species have shown the presence of polyphenols, including flavonoids and tannins [7], phenolic acids [8], sesquiterpenes and hydrocarbons [9] and phenylethanoid glycosides [10]. This genus has *Stachys obtusicrena* are common in sub–nival areas but are equally present in the alpine zone, hemicryptophyte and endemic in Zagros of Iran [11] and represented by 34 species [12, 13]. Pharmacological studies have confirmed that extract of plants belonging to the genus *Stachys* exert significant antimicrobial activity, effective in genital tumors, sclerosis of the spleen, inflammatory tumors and cancerous ulcers, anti-inflammatory, antitoxic, antihepatitic and hypotensive activity. Total phenolic content in *Stachys* is more than genus of *Salvia, Scutellaria* and *Satureja* [14–16].

A GC/FID and GC/MS analysis has been carried out of the essential oil composition of *Stachys obtusicrena* originating from the several levels of growth and altitude in Chaharmahal and Bakhtiari Province, and the results are
presented in this research paper. The aim of this research was to determine the best elevation and phenological stages effects on essential oil content and composition in Stachys obtusirena.

Materials and methods

To study the essential oil content and composition in Stachys, a Randomized Complete Design was done in April–July 2014. Plant material of the Stachys obtusirena (Figure 1) was collected from natural populations from Kallar Mountains in the Chaharmahal and Bakhtiari Province, Islamic Republic of Iran (Figure 2). Treatments were three phenological stages (vegetative, full flowering and seeding) and three elevation ranges (2500–2700 m, 2700–2900 m and over 2900 m). Voucher
Table 3: Chemical composition (%) of the essential oils of *Stachys obtusicrena*.

| Compound                  | RI   | 2500–2700 m × vegetative stage | 2700–2900 m × vegetative stage | Over 2900 m × vegetative stage | 2500–2700 m × full flowering stage | 2700–2900 m × full flowering stage | Over 2900 m × full flowering stage | 2500–2700 m × seeding stage | 2700–2900 m × seeding stage | Over 2900 m × seeding stage |
|---------------------------|------|---------------------------------|---------------------------------|-------------------------------|-----------------------------------|-----------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| α-Thujene                 | 928  | 0.12                            | 0.06                            | 0                             | 0.06                              | 0.06                              | 0.24                          | 0.24                          | 0.05                          | 0.26                          |
| α-Pinene                  | 938  | 0.79                            | 0.42                            | 0.2                            | 0.04                              | 0.82                              | 0.82                          | 1.14                          | 1.1                           | 0.2                           |
| Sabine                   | 971  | 0.37                            | 0.3                             | 0.08                           | 0.05                              | 0.44                              | 0.44                          | 1.49                          | 0.23                          | 2.44                          |
| β-Thujene                 | 972  | 4.73                            | 4.48                            | 1.09                           | 0                                  | 3.69                              | 3                             | 5.01                          | 2.65                          | 5.51                          |
| β-Pinene                 | 978  | 0.18                            | 0.15                            | 0                              | 0.08                              | 0.38                              | 0.4                           | 0.58                          | 0.6                           | 0.14                          |
| β-Myrcene                | 986  | 0.15                            | 0.19                            | 0                              | 0                                  | 0.1                               | 0.04                          | 0.29                          | 0.11                          | 0.32                          |
| α-Phellandrene           | 1002 | 0.36                            | 0.31                            | 0                              | 0.04                              | 0.17                              | 0.44                          | 0.2                           | 0.39                          |                               |
| δ-3-Carene              | 1009 | 0.99                            | 0.81                            | 0.24                           | 0.1                               | 0.26                              | 0.27                          | 1.02                          | 0.19                          | 0.84                          |
| α-Terpine                | 1013 | 0.05                            | 0.05                            | 0                              | 0                                  | 0.04                              | 0.04                          | 0.18                          | 0                             | 0.12                          |
| 1,8-Cineole              | 1021 | 0.4                             | 0.88                            | 0.18                           | 0.3                               | 0.87                              | 0                             | 0                             | 0                             | 0                             |
| cis-β-Ocimene            | 1039 | 1.05                            | 1.86                            | 0.27                           | 0.27                              | 0.8                               | 0.83                          | 1.65                          | 0.91                          | 0.65                          |
| trans-β-Ocimene          | 1052 | 0.29                            | 0.72                            | 0                              | 0.03                              | 0.13                              | 0.12                          | 0                             | 0.1                           | 0.09                          |
| γ-Terpinene              | 1060 | 0.14                            | 0.18                            | 0                              | 0.06                              | 0.13                              | 0.13                          | 0.43                          | 0.09                          | 0.34                          |
| cis-Sabinehydrate        | 1077 | 0.04                            | 0.09                            | 0                              | 0.03                              | 0.05                              | 0.06                          | 0                             | 0                             | 0.15                          |
| α-Terpinolene            | 1083 | 0.16                            | 0.03                            | 0                              | 0.03                              | 0.08                              | 0.3                           | 0.09                          | 0.26                          |                               |
| Linalool                  | 1099 | 0.12                            | 0.37                            | 0                              | 0.18                              | 0.51                              | 0.62                          | 0.37                          | 0.38                          | 0.65                          |
| Menthol                  | 1143 | 0.06                            | 0.17                            | 0                              | 0.58                              | 0.1                               | 0.11                          | 0.46                          | 0                             | 0.11                          |
| Terpinene-4-ol           | 1167 | 0.25                            | 0.6                             | 0                              | 0.42                              | 1.29                              | 1.41                          | 0.72                          | 0.89                          | 2.15                          |
| α-Terpineol              | 1190 | 0.25                            | 0.6                             | 0                              | 0.12                              | 0.36                              | 0.38                          | 0.34                          | 0.15                          | 0.52                          |
| Methyl Chavicol          | 1267 | 3.6                             | 10.55                           | 0.94                           | 0.39                              | 3.39                              | 3.56                          | 0                             | 0                             | 1.57                          |
| α-Copaene                | 1355 | 0.24                            | 0.46                            | 0.14                           | 0.24                              | 0.53                              | 0.61                          | 0.88                          | 0.31                          | 0.92                          |
| β-Bourbonene             | 1383 | 0.04                            | 0.11                            | 0                              | 0.13                              | 0.15                              | 0.91                          | 0.18                          | 0.6                           |                               |
| β-Cubebene               | 1392 | 0.05                            | 0.1                             | 0                              | 0.09                              | 0.1                               | 0                             | 0                             | 0                             |                               |
| β-Elemene                | 1395 | 0.15                            | 0.25                            | 0                              | 0.26                              | 0.33                              | 0.35                          | 0.75                          | 0.4                           | 0.76                          |
| β-Caryophyllene          | 1420 | 1.62                            | 3.16                            | 1.05                           | 0.52                              | 2.78                              | 3.13                          | 4.7                           | 1.72                          | 2.29                          |
| α-Bergamotene            | 1416 | 0.3                             | 0.63                            | 0.16                           | 0.57                              | 0.45                              | 0.6                           | 0.95                          | 0.24                          | 0.61                          |
| β-Sesquiphellandrene     | 1447 | 0.7                             | 1.47                            | 0.35                           | 1.14                              | 0.99                              | 1.27                          | 2.62                          | 0.79                          | 1.69                          |
| Germacrene-D             | 1480 | 4.08                            | 6.28                            | 2.36                           | 1.33                              | 6.29                              | 6.87                          | 15.25                         | 7.62                          | 12.6                          |
| Bicyclogermacrene        | 1495 | 0.39                            | 0.61                            | 0.38                           | 0.23                              | 0.32                              | 0.33                          | 0.83                          | 0.24                          | 0.82                          |
| β-Isobolene              | 1508 | 0.21                            | 0.53                            | 0.17                           | 0.37                              | 0.48                              | 0.55                          | 1.02                          | 0.43                          | 0.71                          |
| δ-Cadinene              | 1524 | 0.68                            | 1.16                            | 0.5                             | 0.94                              | 1.05                              | 1.31                          | 2.07                          | 1.05                          | 1.63                          |
| Germacrene-B             | 1555 | 0.25                            | 0.67                            | 0.29                           | 0.43                              | 0.53                              | 0                              | 0.79                          | 0                             | 0.73                          |
| Spathulenol              | 1570 | 0.28                            | 1.08                            | 0.66                           | 0.97                              | 1.03                              | 0.91                          | 0.67                          | 2.05                          | 0.87                          |
| Caryophyllene oxide      | 1580 | 0.27                            | 1.54                            | 1.02                           | 0.75                              | 1.74                              | 1.63                          | 1.94                          | 1.61                          | 1.23                          |
| γ-Eudesmol               | 1604 | 5.14                            | 9.47                            | 5.95                           | 0                                  | 19.55                             | 18.59                         | 14.29                         | 5.45                          | 19.65                         |
| β-Eudesmol              | 1624 | 0.84                            | 2.21                            | 1.48                           | 7.6                               | 4.44                              | 3.66                          | 3.66                          | 1.22                          | 3.88                          |
| α-Eudesmol              | 1671 | 0.8                             | 1.71                            | 1.19                           | 2.36                              | 2.77                              | 2.72                          | 2.51                          | 4.62                          | 2.62                          |
specimens (20074-TUH) of the species have been deposited in the Herbarium of the Center of Agricultural and Natural Resources of Chaharmahal and Bakhtiari Province, Shahrekord, Iran. Plant species were identified by Mozaffarian [17]. The soil characteristics and climatic properties of sampling zones listed in Tables 1 and 2. Ground GC analysis was done on an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column. The carrier gas was helium at a flow of 0.8 mL/min. Initial column temperature was 60°C and programmed to increase at 4°C/min to 280°C. The split ratio was 40:1. The injector temperature was set at 300°C. The purity of helium gas was 99.99% and 0.1 mL samples were injected manually in the split mode. GC–MS analyses were carried out on a Thermo Finnigan Trace 2000 GC/MS system equipped with a HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 120°C for 5 min and then programmed to reach 280°C at a rate of 10°C/min. Detector temperature was 260°C and injector temperature was 260°C. The compositions of the essential oil were identified by comparison of their retention indices relative to a series of n-alkanes (C7-C24), retention times and mass spectra with those of authentic samples in Wiley library [18]. Ground samples of air-dried aerial parts (50 g) were submitted to hydro-distillation for 2.5 h using a Clevenger type apparatus. The yield (w/w) of the obtained essential oils ranged from 0.041% to 0.145%, based on dry weight (Table 3).

All data were subjected to ANOVA using the statistical computer package SAS and treatment means separated using L.S.D’s multiple range test at p < 0.05 level.

### Results and discussion

Forty compounds, which accounted for 28.95%–90.1% of the total composition of the oils, are reported in Table 3. Diterpenic content of the taxa of *Stachys* is phenolic compounds [19]. The main components in this research were phthalate, β-thujene, germacrene-D and γ-eudesmol (76.63%–8.41% of essential oil content). The least of the components were α-terpinene, cis-sabinene hydrate, menthol, β-bourbonene and β-cubebene (0%–2.01%). The highest amount of phthalate content was obtained from over 2900 m and vegetative stage (67.23%), whereas the lowest value of phthalate was observed at over 2900 m in reproductive and seeding stages (0.16%). Phthalate is one of the principal components of *Stachys* essential oil. Phthalate ranged from 67.23% to 0.16% in different phenological levels and elevations. The most
Table 4: Analysis of variation of elevation and phenological stages effects on essential oil and essential oil components of *Stachys*.

| S.O.V D.F | Essential oil content | α- Thujene | α- Pinene | α- Sabinene | β- Myrcene | β- Phellandrene | α- Carene | α- Terpinene | α- Thujene | 1,8- Cineole | cis-β- Ocimene | trans-β- Ocimene | γ- Terpinene | cis- | α- |
|-----------|----------------------|------------|-----------|-------------|------------|----------------|-----------|--------------|------------|--------------|----------------|----------------|-------------|-----|-----|
| T         | 8                    | 0.03^{b}  | 0.02^{b} | 0.5^{b}    | 1.89^{a}   | 0.143^{b}    | 0.04^{b} | 0.08^{a}    | 0.43^{b}   | 0.011^{b}   | 10.3^{b}       | 0.39^{b}        | 2.11^{b}    | 0.15 | 0.054^{b} |
| E         | 18                   | 0.0002    | 0.0008   | 0.002      | 0.001      | 0.002         | 0.0003   | 0.0007      | 0.0001    | 0.00002     | 0.00008        | 0.002          | 0.011       | 0.007 | 0.009 |
| C.V       | 11.4                 | 9.8       | 7.9      | 5.55       | 16         | 14.1          | 4.1      | 0.1          | 9.3        | 0.27         | 15.4           | 4.1            | 5.3         | 7.6  | 12.9 |
| 0.0001    | 0.00001              | 0.0003    | 0.001    | 0.001      | 0.001      | 0.001         | 0.001    | 0.001       | 0.001     | 0.001        | 0.001          | 0.001          | 0.001       | 0.001 |
| 0.23^{b}  | 0.28^{b}             | 0.006^{m} | 0.19^{b} | 4.85^{a}   | 0.17^{b}   | 1.31^{b}      | 61.1^{b} | 0.16        | 0.19^{b}  | 0.67^{b}     | 0.26^{m}       | 0.69^{b}       | 0.86^{a}    | 0.11  |
| 0.011     | 0.007                | 0.003     | 0.01     | 0.011      | 0.01       | 0.011         | 0.011    | 0.011       | 0.011     | 0.011        | 0.007          | 0.011          | 0.011       | 0.11  |
| 11.9      | 13.6                 | 16.6      | 17.6     | 4.5        | 11.1       | 8.6           | 1.5      | 12.8        | 11.2      | 9.1          | 10.1           | 11.1           | 8.9         |

Linalool | Menthol | Terpinene-4-ol | α-Terpineol | Methyl chavicol | γ-Eudesmol | β-Eudesmol | α-Eudesmol | α-Bisabolol | Levomenol | Phthalate |
|----------|---------|----------------|-------------|-----------------|------------|------------|------------|-------------|-----------|-----------|
| 0.0001   | 0.001   | 0.00005       | 0.001       | 0.004           | 0.001      | 0.001      | 0.001      | 0.001       | 0.002     | 0.0001    |
| 0.147^{b} | 0.13^{b} | 0.086^{a}    | 1.32^{b}    | 32.9^{a}       | 161.3^{b}  | 13.2^{b}   | 3.64^{b}   | 5.2^{b}    | 20.2^{b}  | 10.5^{b}  |
| 0.01     | 0.007   | 0.009         | 0.01        | 0.008          | 0.009      | 0.011      | 0.011      | 0.003       | 0.01      | 0.01      |
| 1.9      | 8.12    | 5.9           | 4.7         | 11.6            | 1.45       | 0.89       | 3.2        | 4.45        | 10.9      | 2.87      |

ns, Non significant; *significant at the 5% level of probability; **significant at the 1% level of probability.
| Traits          | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    | 16    | 17    | 18    | 19    | 20    |
|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Essential oil content (1) | -0.1  | 0.2   | -0.1  | 0.1   | 0.1   | 0.1   | 0.1   | 0.2   | 0.2   | 0.1   | 0.2   | 0.3   | 0.1   | 0.7   | 0.6   | 0.1   | 0.3   | 0.1   | 0.3   |
| α-Thujene (2)   | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.5b  | 0.1   | 0.2   | 0.3   | 0.1   | 0.3   | 0.1   | 0.3   | 0.1   | 0.3   | 0.1   | 0.3   | 0.1   | 0.3   |
| α-Pinene (3)    | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| Sabine (4)      | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| β-Pine (5)      | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| β-Mycene (6)    | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| α-Phellandrene (7) | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| δ-3-Carene (8)  | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| α-Terpinene (9) | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| β-Tuene (10)    | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| 1,8-Cineole (11) | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| cis-β-Ocimene (12) | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| trans-β-Ocimene (13) | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| γ-Terpinene (14) | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| cis-Sabine (15)  | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| α-Terpine (16)   | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| 1,8-Cineole (17) | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| Menthol (18)     | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
| Terpinene-4-ol (19) | -0.1  | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   | 0.2   | 0.3   | 0.1   |
Table 5 (continued)

|       | 21  | 22  | 23  | 24  | 25  | 26  | 27  | 28  | 29  | 30  | 31  | 32  | 33  | 34  | 35  | 36  | 37  | 38  | 39  | 40  | 41  |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| α-Terpineol (20) | -0.1 | 0.8b | 0.5a | 0.1 | 0.7b | 0.3 | 0.4a | 0.6b | 0.3 | 0.5a | 0.6b | 0.2 | 0.2 | 0.4a | 0.8b | 0.2 | 0.5b | -0.2 | 0.6a | -0.6b |
| Methyl Chavicol (21) | - | -0.1 | -0.2 | 0.5b | 0.2 | 0.2 | 0.1 | -0.1 | -0.1 | -0.1 | 0.1 | 0.1 | 0.1 | -0.2 | -0.3 | 0.9b | -0.5b | 0.1 |
| α-Copaene (22) | - | - | 0.9b | 0.1 | 0.9b | 0.7b | 0.8b | 0.8b | 0.8b | 0.9b | 0.9b | 0.6b | -0.1 | 0.6b | 0.8b | 0.2 | 0.2 | -0.1 | 0.6b | -0.6b |
| β-Bourbonene (23) | - | - | - | -0.1 | 0.9b | 0.7b | 0.7b | 0.9b | 0.9b | 0.8b | 0.9b | 0.9b | 0.6b | -0.1 | 0.5b | 0.5b | 0.1 | 0.2 | -0.2 | 0.6b | -0.4b |
| β-Cubebene (24) | - | - | - | - | 0.6b | 0.7b | 0.8b | 0.8b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.5b | 0.5b | 0.2 | 0.4a | -0.1 | 0.7b | -0.7b |
| β-Elemene (25) | - | - | - | - | - | 0.6b | 0.7b | 0.8b | 0.8b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.5b | 0.5b | 0.2 | 0.4a | -0.1 | 0.7b | -0.7b |
| β-Caryophyllene (26) | - | - | - | - | 0.6b | 0.9b | 0.7b | 0.8b | 0.8b | 0.7b | 0.9b | 0.9b | 0.7b | -0.1 | 0.5b | 0.4a | 0.4a | 0.1 | 0.1 | 0.4a | -0.6b |
| α-Bergamotene (27) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.7b | 0.9b | 0.9b | 0.7b | -0.1 | 0.5b | 0.4a | 0.4a | 0.1 | 0.1 | 0.4a | -0.6b |
| β-Sesquiphellandrene (28) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| Germacrene-D (29) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| Bicyclogermacrene (30) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| β-Bisabolene (31) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| δ-Cadinene (32) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| Germacrene-B (33) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| Spathulenol (34) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| Caryophyllene oxide (35) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| γ-Eudesmol (36) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| β-Eudesmol (37) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| α-Eudesmol (38) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| α-Bisabolol (39) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |
| Levomenol (40) | - | - | - | - | - | 0.6b | 0.7b | 0.9b | 0.9b | 0.5b | 0.1 | 0.6b | 0.6b | -0.1 | 0.3 | -0.1 | 0.7b | -0.6b | 0.1 | 0.2 | -0.2 |

ns, Non significant; *significant at the 5% level of probability; **significant at the 1% level of probability.
of essential oil content was obtained at elevation of 2700–2900 m and reproductive stage (Table 3). Elevation of over 2900 m and vegetative stage treatment, recorded the most of phthalate content than other elevations and phenological stages. At higher elevation, phthalate was very little. The most components presented in higher elevation were germacrene-D, γ-eudesmol and levomenol. The roles of elevation and phenological stages are important for made high yields and essential oil. By increasing the elevation, the percentage of clay in soil decreased and then the amount of water and mineral nutrition in the soil diminished. Also at higher points of elevation, the lower temperatures resulted in stress to the plants. Elevations and phenological stages had significant differences on essential oil content and composition in Stachys (Table 4). There were significant differences between measured essential oil compositions. There was negative correlation between the percentage of essential oil and components contain of α-thujene, sabinene, β-myrcene, α-phellandrene, delta-3-carene, β-thujene, 1,8-cineole, trans-β-ocimene, γ-terpinene, cis-sabinenehydrate, α-terpinolone, methyl chavicol, bicycle germacrene and phthalate. The only correlation between percentage of essential oil content and phthalate was negative significantly. Correlation coefficients between measured essential oil compositions are listed in Table 5. Most of the major constituents of the essential oil of Stachys species were germacrene D, β-thujene, methyl chavicol, β-caryophyllene, γ-eudesmol, β-eudesmol, α-eudesmol, α-bisabolol and levomenol. The most amount of phthalate was obtained from elevation of 2500–2700 m in vegetative stage. After 2700–2900 elevation, the main essential oils were germacrene-D, β-thujene, methyl chavicol, β-caryophyllene, γ-eudesmol, β-eudesmol, α-eudesmol, α-bisabolol and levomenol. The results of the present study indicates that essential oil components of Stachys obtusicrena can be varied with environmental conditions, its phenological stage and elevation. This study provides some useful information about the efficacy of elevations and phenological stages on essential oil content and composition of Stachys obtusicrena.

**Conclusion**

Results obtained from our study showed that the essential oils composition of Stachys obtusicrena was significantly affected by differences of elevations and several of the phenological stages. The main components of the essential oil of Stachys were phthalate, germacrene D, β-thujene, methyl chavicol, β-caryophyllene, γ-eudesmol, β-eudesmol, α-eudesmol, α-bisabolol and levomenol. The effects of wind, daily temperatures and cloudiness were different. On the other hand, the amount of evaporation and mean temperature decreased with an altitudinal increasing and made the vegetative phases of plant were shortened. It seems that, with variation in altitude, morphological and physiological characters were changed. All these changes in relation to altitude, effected on plant life. The depth of soils in high altitude, decreased, whereas lower ones became humid. A decrease of soil moisture depends on soil texture, essential oil content and therefore, many of the essential oil components were decreased [1, 4, 19, 27, 28].

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