# Phytochemical Diversity of *Eremostachys laciniata* Bunge Populations in Iran

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## Abstract

**Background:** ***Eremostachys laciniata*** **is known as "Chelle-Daghi" in Iran and its rhizomes are used as an emollient to relieve rheumatoid arthritis.**

**Objective:** **A phytochemical study was performed on 15 populations of** ***Eremostachys laciniata*** **to qualify the phytochemical variations.**

**Methods:** **Plants collected from their natural habitats and dried rhizomes of** ***E. laciniata*** **were milled and then Soxhlet-extracted by MeOH, and then total phenols were determined calorimetrically by the Folin-ciocalteau reagent and iridoid glycosides were measured by TLC method.**

**Results:** **The results of MeOH extracts showed that the highest amount with 14.85 gr/plant was obtained in the Areshtanab population. The populations had a significant effect on total phenolic concentrations ($P < 0.001$) and the Areshtanab with 0.281 mg GAE/g DW had higher phenolic levels than other populations. The results of TLC of iridoids showed that 15 populations had about 11 iridoids components. Cluster analysis by using Ward's method detected that the studied populations of** ***E. laciniata*** **were separated into three different groups.**

**Conclusion:** **In summary, higher values of the total phenols and iridoids in the Areshtanab, Malek-Kian, and Til populations were considered to indicate higher levels of phytochemical heterogeneity and significant diversity between populations, which could be used to domesticate and develop the breeding programs.**

**Keywords:** ***Eremostachys laciniata*** **Bunge, Iridoids, Phytochemical diversity, Total phenol**

## Introduction

The growing trend of increase in world population suggests that the world population in the first half of 21 century will exceed 10 billion people. Therefore, beside the food security, the efforts to provide health care will be one of the most important problems and concerns facing the international community [1, 2]. Plants are a rich source of secondary metabolites that have medicinal and aromatic properties. According to some estimates, at least 100,000 such secondary metabolites are now known to occur in 50,000 plant species and 4,000 new secondary metabolites are being discovered every year from a variety of plant species [3].

*Eremostachys laciniata* (L) Bunge (family: Lamiaceae alt. Labiatae; sub-family: Lamioideae) is a perennial herb with thick roots and pale-purple or white flowers. It is one of 15 species of *Eremostachys* grown in Iran and in other countries in the Middle-East, Western Asia, and the Caucasus [4, 5]. This genus has 60 species worldwide and five endemic species in Iran including *E. pulvinaris, E. codonocalyx, E. azerbayjanica, E. adenantha,* and *E. hyoscyamoides* [5].

In traditional medicine, the *E. laciniata* species is known in Iran as "Chelle-Daghi" and its rhizomes are used as an emollient to relieve rheumatoid arthritis [6]. Moreover, a decoction of the rhizomes and flowers of *E. laciniata* are traditionally used to treat allergies, headaches, and liver disease [7]. Antioxidant [8], antibacterial [9], antidepressant [10], anti-inflammatory [11] and analgesic [12] properties have been documented for *E. laciniata*.

Previous phytochemical study on *Eremostachys* genus established the presence of alkaloids, coumarins, flavonoids [13] and mono- and diterpene glycosides [14, 15], two ferulic acid derivatives [16], three phenylethanoid glycosides [17] and highly oxygenated flavonoid named loasifolin [18]. Phytochemical investigations have also reported the isolation of iridoids, phenylethanoid, and flavonoid derivatives from the aerial parts [19] and some iridoid and furanolabdane diterpene glycosides from the rhizomes of this medicinal species. Phytochemical studies also revealed the presence of various monoterpenes and sesquiterpenes among its essential oils [20].

Iridoids, the main secondary metabolites of this species, are secondary metabolites of terrestrial and marine flora and fauna and are found in a large number of plant families usually as glycosides. Structurally, they are cyclopentano pyran monoterpenoids and biogenetically and chemotaxonomically they provide a structural link between terpenes and alkaloids [21, 22]. Iridoids are present in a number of folk medicinal plants used as bitter tonics, sedatives, antipyretics, cough medicines, remedies for wounds, and skin disorders and as hypotensives. This fact encouraged to investigate the bioactivities of these phytochemicals. Intensive study of their bioactivity revealed that these compounds exhibit a wide range of bioactivity: cardiovascular, antihepatotoxic, choleretic, hypoglycemic and hypolipidemic, antiinflammatory, antispasmodic, antitumor, antiviral, immunomodulator and purgative activities [22, 23, 24, 25]. Chemotaxonomically,

they are useful as markers of several genus in various plant families, such as, aucubin of *Plantago* (Plantaginaceae) asperuloside of *Galium* (Rubiaceae) and aucuboside (aucubin) and harpagide of *Scrophularia* (Scrophulariaceae) [23].

The aims of this study were: (i) quantifying phytochemical variation among 15 Iranian populations of *E. laciniata*, using TLC and total phenol analysis; and (ii) establishing phytochemical relationship among the populations.

## Materials and Methods

**Plant material:** The rhizomes of 15 populations of *Eremostachys laciniata* (L) Bunge, were collected from different parts of Iran in 2013-2014 (Table 1). The plants were identified by Flora Iranica [26]. A voucher specimen (TUM-ADE 0204) for this collection has been retained in the herbarium of the School of Pharmacy, Tabriz University of Medical science, Iran.

**Extraction and isolation:** The dried and ground rhizomes of *E. laciniata* (100 g) were milled and then Soxhlet-extracted, successively, with dichloromethane (DCM) and MeOH. The DCM extract was discarded and MeOH extract was concentrated by using a rotary evaporator at a maximum temperature of 45 °C [6].

**Total Phenol:** Total phenols in *E. laciniata* MeOH extract were determined calorimetrically at 725 nm (Human Crop, Xma-2000) with the Folin-ciocalteau reagent as previously done by Gutfinger [27].

**Thin layer chromatography (TLC):** Thin layer chromatography of iridoid glycosides containing fraction was performed using silica gel plate 60 $F_{254}$ (Merck, Germany). The solvent system was chloroform- methanol-water (60:40:4), where the sprayed reagent was anisaldehide-sulphuric acid, followed by heating at 110ºC for 5 min [28].

**Table 1- Collection Sites for *Eremostachys laciniata***

| Site | Altitude (m asl[+]) | Latitude (N) | Longitude (E) | Mean annual temp. (℃) | Mean annual rainfall (mm) |
|---|---|---|---|---|---|
| Ajabshir | 1,387 | 37º 40’ | 45º 51’ | 15.1 | 236.4 |
| Areshtanab | 1,967 | 37º 54’ | 46º 42’ | 13.5 | 222.5 |
| Chelle-Khaneh | 1,365 | 38º 15’ | 46º 01’ | 14.5 | 245.1 |
| Heydarabad | 1,449 | 38º 17’ | 45º 24’ | 16.2 | 341.8 |
| Ilkhchi | 1,426 | 37º 55’ | 46º 00’ | 15.1 | 236.4 |
| Iranagh | 1,986 | 37º 55’ | 46º 35’ | 13.5 | 222.5 |
| Kaleybar | 1,617 | 38º 45’ | 46º 58’ | 13.6 | 367.2 |
| Malek-Kian | 1,607 | 38º 03’ | 46º 32’ | 12.5 | 299.7 |
| Marand | 1,468 | 38º 24’ | 45º 44’ | 13.2 | 444.3 |
| Meshanagh | 1,634 | 38º 13’ | 45º 34’ | 16.2 | 341.8 |
| Mianeh | 1,811 | 37º 36’ | 48º 04’ | 15.3 | 337.4 |
| Sad-Ammand | 1,369 | 38º 11’ | 46º 08’ | 14.5 | 245.1 |
| Sarab | 2,122 | 38º 02’ | 47º 41’ | 10.2 | 276.4 |
| Til | 1,686 | 38º 16’ | 45º 29’ | 16.2 | 341.8 |
| Zglojeh | 1,949 | 37º 48’ | 46º 54’ | 10.5 | 421.5 |

[+] m asl: Meter after sea level

All data were subjected to statistical analysis (one-way ANOVA) by using MSTAT-C software. Differences between the treatments were performed by Duncan's Multiple Range Test (DMRT) at 1% probability level. The Ward's method was used for cluster analysis [29].

## Results

**Total phenolic compounds:** The results of MeOH extracts of roots of *E. laciniata* showed that the highest amount of extract was in the Areshtanab population (Table 3).

Average total phenolic concentrations for 15 Eremostachys populations were determined by the Folin–ciocalteau assay and are presented in table 3. Populations had a statistically significant effect on total phenolic concentrations ($P < 0.001$) by ANOVA analysis (Table 2). Mean comparison with DMRT showed that Areshtanab (0.281 mg GAE/g DW) had higher phenolic levels than other populations.

**Table 2- ANOVA of total phenols in 15 studied populations**

| Source of variation | Degrees of freedom | Mean of squares (Total phenols) |
|---|---|---|
| Populations | 14 | 0.0192 ** |
| Error | 30 | 0.00002 |
| CV% | | 2.46 |

**-Significant at 1% probability level

**Table 3- Mean comparison of extract and total phenols in 15 studied populations**

| Populations | Extract in 100 g plant sample (gr) | Phenols in 1 gr extract (mg GAE/g DW) | Total phenols in 100 gr plant sample (mg GAE/g DW) |
|---|---|---|---|
| Ajabshir | 5.31 | 0.012 | 0.065 i |
| Areshtanab | 14.85 | 0.019 | 0.281 a |
| Chelle-Khane | 13.70 | 0.016 | 0.220 d |
| Heydarabad | 2.87 | 0.014 | 0.041 j |
| Ilkhchi | 6.97 | 0.010 | 0.068 i |
| Iranagh | 7.69 | 0.008 | 0.060 i |
| Kaleybar | 8.85 | 0.016 | 0.139 g |
| Malek-Kian | 13.35 | 0.019 | 0.258 b |
| Marand | 12.73 | 0.020 | 0.251 b |
| Meshanagh | 8.45 | 0.024 | 0.204 e |
| Mianeh | 9.65 | 0.017 | 0.163 f |
| Sad-Ammand | 11.82 | 0.013 | 0.158 f |
| Sarab | 10.21 | 0.021 | 0.213 de |
| Til | 12.48 | 0.019 | 0.236 c |
| Zglojeh | 10.63 | 0.012 | 0.128 h |

Means with the same letter(s) aren't significantly different at P≤0.01 by DMRT

**Iridoids**: The results of TLC of iridoids (Figure 1 and Table 4) showed that, corresponding to $R_f$, 15 populations had about 11 iridoids components. The Malek-Kian, Mianeh, Chelle-Khane, Sarab, Meshanagh, Til, and Kaleybar showed all 11 iridoids components but other population had not all components. The Iranagh had the lowest components. Wagner and Bladt [28] explained that iridoids color in the TLC was brown (light to dark), Blue, Purple, and gray and results of this study showed the same color (Figure 1).

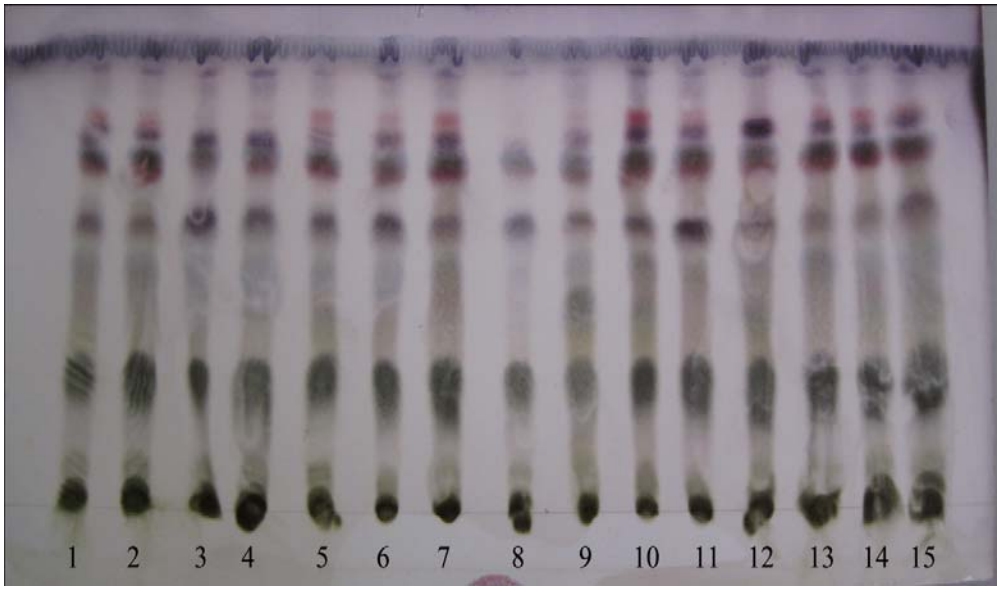


**Figure 1- Columns of iridoids TLC. 1, Malek-Kian; 2, Mianeh; 3, Zglojeh; 4, Ilkhchi; 5, Chelle-Khane; 6, Heydarabad; 7, Sarab; 8, Iranagh;, 9, Sad-Ammand; 10, Meshanagh; 11, Areshtanab; 12, Ajabshir; 13, Til; 14, Kaleybar; 15, Marand**

**Table 3- Color, presence and quantity of the spots on the TLC chromatogram of *Eremostachys laciniata* roots**

| Color | Gray | Black | Purple | Gray | Brown | Gray | Purple | Brown | Blue | Purple | Blue |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Rf** | **0.01** | **0.30** | **0.46** | **0.53** | **0.61** | **0.66** | **0.74** | **0.76** | **0.8** | **0.85** | **0.95** |
| Malek-Kian | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 |
| Mianeh | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 2 |
| Zglojeh | 1 | 2 | 0 | 1 | 2 | 1 | 0 | 1 | 2 | 0 | 2 |
| Ilkhchi | 1 | 2 | 0 | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 2 |
| Chelle-Khane | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| Heydarabad | 1 | 2 | 0 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 2 |
| Sarab | 1 | 2 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 2 |
| Iranagh | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| Sad-Ammand | 1 | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 1 |
| Meshanagh | 1 | 2 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| Areshtanab | 1 | 2 | 1 | 1 | 2 | 2 | 1 | 2 | 1 | 1 | 1 |
| Ajabshir | 1 | 2 | 0 | 2 | 1 | 2 | 1 | 2 | 2 | 0 | 2 |
| Til | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 | 2 |
| Kaleybar | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |
| Marand | 1 | 2 | 0 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |

**Cluster analysis:** A dendrogram was obtained from the cluster analysis by using Ward's method. This method detected that the studied populations of *E. laciniata* were separated into three different groups (Figure 2). The Til, Areshtanab, Malek-Kian, Chelle-Khaneh, and Marand were placed in the first group. This group had the higher levels of iridoids and total phenols. The second group consisted of the Zglojeh, Iranagh, Sad-Ammand, Mianeh, Sarab, and Meshanagh populations. The Ilkhchi, Ajabshir, and Heydarabad populations were placed in the third group.

Results showed that among the populations, the Areshtanab, Malek-Kian, and Til populations can be distinguished from other populations by some phytochemical features that agreed with morphological [36] and cytological diversities [38], which could be used for domestication and development of breeding programs.

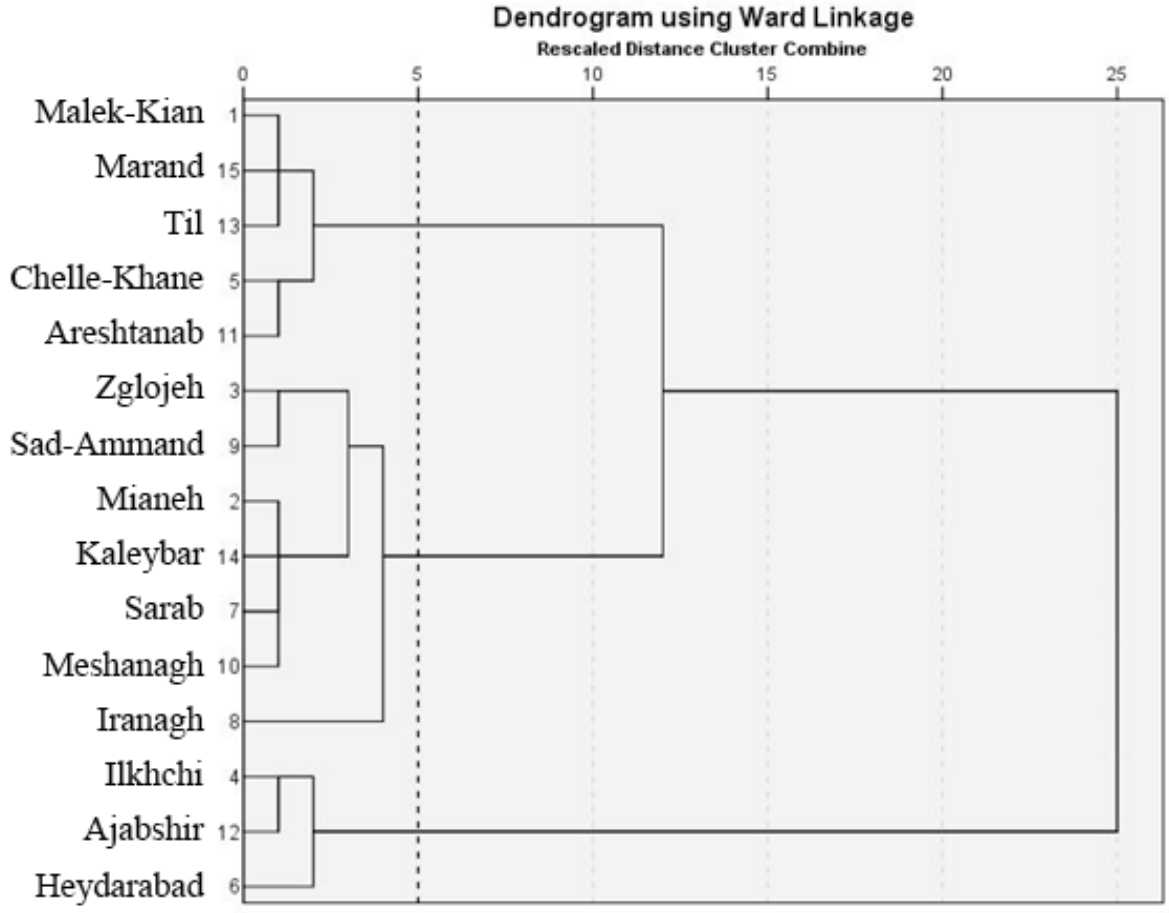


**Figure 2- A dendrogram of cluster analysis by Ward's method**

## Discussion

Cultivar is known to impact phenolic concentrations in a variety of plants, and the effect of genotype has been particularly well studied in fruits and vegetables [30, 31, 32]. The effect of cultivar on the phenolic composition and antioxidant properties of 15 different basil varieties was determined by Folin-ciocalteau reagent. Cultivar had a statistically significant effect on total phenolic levels and anthocyanin concentrations that could be used in breeding programs [33]. The phytochemical study of *Astragalus* showed existence of some secondary metabolites including phenols and flavonoids in this species. Therefore, total phenols were determined by Folin-ciocalteau reagent [34]. In the *Stachys* genus, the results of qualitative study of phytochemical compounds showed the presence of secondary compounds such as tannin, saponin, flavonoid, alkaloid, terpenoid, glycoside and steroid. In the quantitative study obtained the average amounts of total phenol 74.56±4.343 mg GAE g-1 DW [35].

Morphological study of these 15 populations showed that the Til, Areshtanab and Malek-Kian populations had higher root yield with 220, 171, and 164 gr/plant, respectively [36]. Therefore, considering the extract amount and root yield, it can be concluded that the Til, Areshtanab, and Malek-Kian populations had higher root and extract yield and iridoids and were usable in breeding programs.

Phytochemical investigation of a plant known as Tashnehdary (*Onosma chlorotricum*) was performed by TLC method and components $R_f$ was measured. The results confirms the antioxidant activity of some components in the lipophilic extract of *Onosma chlorotricum* root and consequently may confirm its effectiveness in some processes such as wound healing which antioxidant reactions are helpful [37].

## Conclusion

In summary, higher values of the total phenols and iridoids in the Areshtanab, Malek-Kian, and Til populations were considered to indicate the higher levels of phytochemical heterogeneity and significant diversity between populations, which could be used for domestication and development of breeding programs for this species.

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