# Guide to Harvesting Heracleum Persicum: How Essential Oil Composition and Phenolic Acid Profile Fluctuate at Different Phenological Stages?

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# Abstract

**BACKGROUN:** Heracleum persicum commonly named Golpar, is a principal native medicinal plant in Iran. Collecting H. persicum at the appropriate growing stage is the key factor to achieve the high phytochemical quality to meet consumer's needs. In the present experiment, the aerial parts of this plant were harvested at up to six different developmental stages during the growing season to determine the phytochemical profiles.

**RESULTS:** Our results indicated that the highest essential oil yield and extract were obtained in the mid-mature seed stage (3.5%),) and floral budding stage (10.40%). In the vegetative stage, limonene (18.05), in floral budding stage, caryophyllene (14.07), anethole (14.55%), and β-bisabolene (12.56%), in the full flowering stage, myristicin (15.02%), and hexyl butyrate (9.05%); in the early development of seeds stage, hexyl butyrate (32.08%), and octyl acetate (11.67%); in the mid-mature seeds stage hexyl butyrate (38.75%), octyl acetate (14.47%); in the late-mature/ripe seeds stage, hexyl butyrate (23.59%), and octyl acetate (10.48%) recorded as the main components. The analysis of phenolic acids demonstrated cinnamic acid, p-coumaric acid, p-hydroxybenzoic acid, ferulic acid, and rosmarinic acid as the main phenolic acids. The highest phenolic acids content was obtained in the floral budding stage (287.40 mg g –1 dry extract). Cinnamic acid was found as the major phenolic compound in the vegetative stage following by floral budding, the full flowering stage, the early development of seeds and late-mature/ripe seeds stages. P-coumaric acid was the most abundant phenolic compounds in the mid-mature seeds stage. The development stage has a significant impact on the content and composition of both essential oil and phenolic acid composition.

**CONCLUSION:** In this regard, the harvest time of H. persicum aerial parts can be selected to achieve the highest secondary metabolites of interest. The results of this study can be used as a guideline for grower to obtain the highest amount of desirable metabolites, beneficial in the food and pharmaceutical industries as well as economic benefits.


# Introduction

Until now, more than 125 species of the genus *Heracleum* have been discovered all around the world. Most species of this genus are distributed in Asia, and ten perennial aromatic species grows in the flora of Iran. *Heracleum persicum* L., commonly known as Persian hogweed simply hogweed, or Golpar, is a polycarpic perennial herbaceous and a flowering shrub that belongs to Apiaceae family. It is originally native to humid mountainous areas of Iran, Iraq and Turkey [1].

This plant is widely distributed and grown in Iran in regions with different ecological conditions. The best growing condition for this plant is moist and fertile areas, especially in the mountains in the northern part of the country, with altitudes from 1500 to more than 3000 m above sea level [1,2].

*H. persicum* L. is a perennial herb that usually grows up to 1.5–2 m. This alternate-leaved plant has an anise-like smell with thick and hollow stem. The leaf blades are elongated, densely haired on the lower side, glabrous on top, and pinnate with blunt-toothed margins. The flowers with five petals and five stamens are small with pale white and lime-green color. The fruits are broadly obovate, with slightly ridged schizocarp [3].

Different parts of this plant have a long reputation as a natural remedy in the Iranian folk medicine. Aromatic fruits of *H. persicum* are extensively used in the daily diet of the general Iranian population as a flavoring agent and spice and the stems are used in making pickles. In addition, in Iranian traditional medicine, its fruits are used as a carminative, anti-inflammatory, digestive aid, antimicrobial tonic and antiepileptic. It is also believed that this plant can work against stomach ailments, flatulence, infections, memory impairment, forgetfulness, vertigo, and stupidity aphrodisiac [3,4,5,6]. According to previous studies, *H. persicum* have shown several biological activities, such as antioxidant [7,8], anti-inflammatory, analgesic [3,9], antidiabetic [10], antihyperlipidemic [9,11], cardioprotective [12], gastroprotective [3], neurological [13], immunomodulatory [14], hepatoprotective [3], antibacterial [15], antifungal [16,17], anticonvulsant [13], and insecticidal properties [18].

Recently, different phytochemical compounds such as tannins, saponins, alkaloids, flavonoids, and furanocoumarins were extracted from different parts of *H. persicum* plants [19]. Among them, the essential oil composition of *H. persicum* fruits has been widely studied, and the results suggests that the fruit can be considered as a suitable source of essential oils and aliphatic ester compounds [1,8,20,21,22,23]. Until now, the majority of studies on *H. persicum* have focused on its essential oil fraction. Limonene, γ-terpinene, anethole, hexyl butyrate, octyl acetate, hexyl-2-methylbutanoate, hexyl isobutyrate were identified as the major constituents of the *H. persicum* essential oil. The amount of essential oils constituents depends on the plant part which essential oil is extracted [1, 4,6,21].

The major source of secondary metabolites in plants of the *Apiaceae* family is in the leaves, stems, flowers, and especially fruits. Secondary metabolites contents are mostly allocated to the essential oils and phenolic compounds, which are more important for industrial use, than other phytochemical compounds. Other favorable compounds with antioxidant properties are mainly used in the food industry, while essential oil is edible and has pharmaceutical properties [24]. Medicinal plants with antioxidant properties can replace synthetic antioxidants, because less costly and more environmentally friendly. In addition to the above mentioned advantages, dietary phenolic antioxidants of *H. persicum* play important roles in delaying the development of chronic diseases such as cardiovascular diseases, inflammatory bowel syndrome, and Alzheimer's diseases [25, 26]. Studies have shown that *Heracleum* species are rich in phenolic compounds and exhibit high biological activities making them worthy medicinal plants to study [8].

Phenological and harvest stages are vital factors that may influence content and biological activities of phenolic compounds in different organs and growing stages of plants [27,28]. Choosing the best-growing stage will help us to harvest the higher amount of essential oil yield as well as other beneficial compounds. Moreover, the amount of phenolic compounds in medicinal plants is affected by genetic variation among different species, even within the same species and also by the maturity of plant organs at harvest time [28, 29, 30]. However, the proper time of harvest to achieve the maximum beneficial compounds of *H. persicum* remains unknown and needs more research work. Until now, there is limited information on phenolic compounds of *H. persicum* in different organs and different growth stages. Thus, it is essential to specify more precisely the time when *H. persicum* plant should be harvested to attain the highest possible quality and quantity of beneficial contents. In this study, we aimed to investigate the distribution pattern of phenolic compounds and essential oil in the *H. persicum*. We also tried to find the best phenological stage that has the high concentration of these components in the aerial parts of *H. persicum* that were collected from the Iranian population at different developmental stages. To the extent of our knowledge, this is the first report aimed to evaluate the phytochemical composition of different growing stages of *H. persicum*, during its biological cycle, which results will be useful to find the best harvest time to reach the high beneficial yield with less harm to the environment.

# Experimental Procedure

### *Plant material:*

The current study took place in the spring and summer of 2018 in the city of Sarab, East Azerbaijan Province, Iran (Location: 37°56'27"N and 47°32'12"E; altitude: 1750 m). The first sampling started 30 days after the onset of the vegetative growth, in spring. All samplings were randomly selected from ten *H. persicum* plants. The harvest was done at different developmental stages (Fig 1). Each sampling was repeated three times and samples weight was 1 kg per replication. The samples were dried in a shade at room temperature (25 °C).

### *Extraction of Essential Oils*

Applying hydro-distillation for three hours, the essential oil were extracted from 100 g dried samples in 600 ml of distilled water in a 2 L flask using Clevenger apparatus extraction technique in three replications (British Pharmacopoeia method) [31]. The obtained essential oil was dried over anhydrous $Na_2SO_4$ and kept at -20 °C until further analysis.

### *Preparation of the Extracts*

In order to prepare the extracts, dried powdered of different samples of *H. persicum* were used. About 30 ml of the Methanol were added to 2 g of samples and put in an ultrasonic bath (Frequency, 100 kHz; power intensity, 160 W; temperature, 35 °C) for 30 min. Then, the extract was filtered, evaporated, and stored at –20 °C until further analysis.

### *Extraction of phenolic acids*

The phenolic acids extraction was performed using our previous work with some modification [28]. Briefly, 10 ml of 80% ethanol was added to 1.0 g of the dried powdered plant, vigorously shaked and centrifuged at a speed of 12000 rpm for 10 min. The collected supernatant was evaporated and stored at –20 °C for further analysis.

### *Analysis of phenolic acids*

In order to analyze the phenolic acid compounds, HPLC (Waters 2695, USA) system equipped with a diode-array detector, a 20 µl loop, and an ODS column (250 mm × 0.46 mm, 5 µm) was operated. The reverse-phase separation was done with gradient elution solvent A [methanol TFA (99.9:0.1, v/v)] and B [water TFA (99.9:0.1, v/v)] at a flow rate of 0.5 ml/min with the following elution gradient: 20% A, at 0 min; 30% A, (from 0 to10 min); 60% A, (from 10 to 30 min); 80% A, (30 to40 min); 100% A, (40 to 45 min); 20% A, (from 45 to 52 min); isocratic, 6 min. The phenolic compounds were detected at 254, 275, and 320 nm, and identified based on the retention time and spike method. Finally, the standard external method was applied to quantify the studied phenolic acids. The results were expressed as mg per g of the extract weight.

### *Gas Chromatography (GC)*

Essential oils analysis was done using a gas chromatography device (model: Agilent 7890 A G). The separation was carried out using the Column MS HP-5 (30 m×0.25 mm, 0.25 µm). The oven temperature program was set at 50 °C (3 min) to 260 °C with a ramp-up of 3 °C/min, and then held for 5 min. The detector and the injector temperature were adjusted at respectively. Nitrogen was used as carrier gas at a rate of 1 ml/min.

### *GC-MS chromatography*

In addition to GC chromatography, a gas chromatography device connected to a mass spectrometer (Model: Agilent 7890 A G Chromatograph and Agilent 5975 c Mass), called GC-MS chromatography, was used to analyze the essential oil. In this experiment GC-MS chromatography was equipped with HP-5 column (30 m×0.25 mm, 0.25µm). According to the planned program, the oven temperature remained constant at 50 for 3 min and remained constant for 5 min after increasing the temperature to 260 with a ramp-up of 3 °C/min. Here again, Nitrogen was utilized as carrier gas at the rate of 1 ml/min. The analysis was performed with scan time (30 m/z), range of analysis (600 m/z), ionization 0/6 s, 70 Electron volt and solvent evaporation rate of 2 minutes. The compounds were then identified by index (ki) and Wiley Library and Nist11.

### *Statistical analysis*

In order to determine the relationship between different phytochemical compounds, variance analysis, comparison of means, principal component analysis (PCA) and cluster analysis were done using the statistical package SAS 9.4. All determinations were conducted in triplicate, and the results were calculated as mean value ± standard error (SE). The variations (standard error of means, SE), and the significances of treatment effects (F-test) were calculated and tested using the General Linear Models procedure of SAS. The mean values of treatments were separated by *Tukey's* test ($P < 0.05$).

## Results And Discussion

### *Content of essential oil and extracts*

Medicinal plants have different capacities to produce essential oil at different phenological stages [32]. To achieve their goal, breeders must consider the proper harvest time to reach the best yield for their target purposes, such as pharmaceutical, food and cosmetic industries applications. For this reason, the essential oil content was studied in different phenological growth stages of *H. persicum*. Statistical analysis of this experiment showed that the essential oil content was significantly different in the various phenological stages of this plant (Fig. 1). The results indicated that the essential oil yield obtained in the vegetative stages, floral budding stage, full flowering stage, early development of seeds stage, mid-mature seeds stage and, final stage or late-mature/ripe seeds stages were 0.45, 0.72, 0.5, 1 and 3.5 and 2.2%, respectively. According to these data, the greatest yield was observed at the mid-development stage of the seeds, while the minimum yield was associated with the vegetative stages (Fig. 2). There is no significant difference in essential oil yield between the vegetative stage and seeds at the early development stage. Other medicinal plants such as the *Apiaceae* family, including *Oliveria decumbens* [27], *Trachyspermum Ammi* [33], and *Echinophora tenuifolia* [34], followed the same pattern and produced different essential oil content at various growth stages that could be due to the interaction between the physiological activities of these plants at various stages of development in their environment. These plants had low essential oil content in early growth stages, while the highest essential oil content was obtained at seed setting and seed phase. Less moisture content and lower activity of some essential enzymes for biosynthesis of specific compounds at seeding stage lead to less essential oil production at early stages of growth [28, 33, 34]. Furthermore, previous studies showed that essential oil content was not the same in different plant organs. Results of the study on *H. persicum* showed 0.41 to 5.23% difference in essential oil content of various organs such as stem and seeds [6]. In our experiment, we observed a low content of the essential oil in vegetative and flowering stages and high essential oil content in seed at setting stage; possibly, *H. persicum* plants spend most of their produced photosynthetic materials manufacturing vegetative organs instead of synthesis of useful biological active compounds for various industries at the early stages of its growth in comparison to later stages.

Figure 3 shows *H. persicum* extracts content in different growing stages. According to our results, there was a significant difference between the extracts content in different phenological stages while the maximum extract content percentage was obtained in the floral budding stage (10.40%) followed by full flowering stage (10.20%). However, with the plant reaching the seed setting stage the extracts percentage decreased, as the lowest percentage of extracts were obtained at mature seeds stage at the rate of 5.10%. Based on the results, there were no significant differences between the flowering and floral budding stages, also between vegetative and mid-mature seeds stages.

### *Chemical compounds of essential oil*

Recent reports suggest that biological features of the *H. persicum* such as anti-inflammatory, anti-pain, antioxidant, and anti-seizure can be attributed to basic essential oil compounds [3]. The analysis of our data at various phenological stages of *H. persicum* indicated significant variations in the type and percentage of essential oil constituents (Table 1). In total, in the vegetative stages, floral budding stage, full flowering stage, early development of seeds stage, mid-mature seeds stage and, final stage or late mature/ripe seeds stage, values of 96.75, 97.02, 96.94, 98.02 and 97.07 and 98.23% of the total essential oil constituent were respectively identified.

Table 1
Essential oil composition of Heracleum persicum at different phenological stages and their mean comparisons.

| No. | Compounds | RI | Vegetative stage | Floral budding | Full flowering | Early development of seeds | Mid-maturation of seeds | Late-mature/ripe of seeds |
|---|---|---|---|---|---|---|---|---|
| 1 | Isopropyl isovalerate | 894 | 0.41 ± 0.03 $^{b}$ | tr | tr | tr | tr | 1.62 ± 0.15 $^{a}$ |
| 2 | α-Pinene | 930 | 1.93 ± 0.05 $^{b}$ | 4.50 ± 0.27 $^{a}$ | 4.89 ± 0.20 $^{a}$ | 2.26 ± 0.33 $^{b}$ | tr | 0.33 ± 0.03 $^{c}$ |
| 3 | Isopropyl 3-methyl-2-butenoate | 946 | tr | tr | tr | tr | tr | 1.18 ± 0.16 |
| 4 | β-Pinene | 985 | 8.67 ± 0.22 $^{a}$ | 1.41 ± 0.09 $^{b}$ | tr | tr | tr | 0.32 ± 0.04 $^{c}$ |
| 5 | Pseudolimonen | 992 | tr | 2.18 ± 0.32 $^{b}$ | 2.98 ± 0.08 $^{a}$ | 0.46 ± 0.05 $^{c}$ | tr | tr |
| 6 | Octanal | 1002 | tr | 0.28 ± 0.04 $^{c}$ | 0.30 ± 0.03 $^{c}$ | tr | 1.23 ± 0.12 $^{a}$ | 0.97 ± 0.05 $^{b}$ |
| 7 | Butyl butyrate | 1005 | tr | tr | tr | 1.82 ± 0.11 $^{b}$ | 4.19 ± 0.74 $^{a}$ | 4.38 ± 0.35 $^{a}$ |
| 8 | *p*-Cymene | 1010 | tr | 1.38 ± 0.06 $^{b}$ | 4.20 ± 0.26 $^{a}$ | tr | tr | tr |
| 9 | Limonene | 1018 | 18.05 ± 1.72 $^{a}$ | 0.48 ± 0.05 $^{b}$ | tr | tr | tr | tr |
| 10 | Hexyl acetate | 1025 | tr | tr | tr | 0.43 ± 0.04 $^{b}$ | 0.92 ± 0.06 $^{a}$ | 1.02 ± 0.12 $^{a}$ |
| 11 | Carene | 1027 | 4.19 ± 0.22 $^{a}$ | 1.16 ± 0.05 $^{c}$ | 2.66 ± 0.19 $^{b}$ | 0.57 ± 0.05 $^{d}$ | tr | tr |
| 12 | γ -Terpinene | 1036 | 1.39 ± 0.12 $^{c}$ | 4.37 ± 0.18 $^{b}$ | 6.31 ± 0.77 $^{a}$ | 1.01 ± 0.20 $^{cd}$ | tr | 0.72 ± 0.10 $^{d}$ |
| 13 | Butyl 2-methylbutanoate | 1044 | tr | tr | tr | 1.05 ± 0.06 $^{a}$ | 0.64 ± 0.14 $^{b}$ | 0.70 ± 0.08 $^{b}$ |
| 14 | 2-Methylbutyl isobutyrate | 1048 | tr | tr | tr | 0.60 ± 0.10 $^{a}$ | 0.81 ± 0.10 $^{a}$ | 0.25 ± 0.02 $^{b}$ |
| 15 | cis-5-Octen-1-ol | 1051 | tr | tr | 0.93 ± 0.03 $^{c}$ | 5.75 ± 0.16 $^{a}$ | 6.03 ± 0.18 $^{a}$ | 4.85 ± 0.24 $^{b}$ |
| 16 | Linalool | 1061 | 0.50 ± 0.10 $^{d}$ | 0.64 ± 0.06 $^{cd}$ | 0.94 ± 0.22 $^{c}$ | 3.72 ± 0.19 $^{a}$ | 0.91 ± 0.10 $^{c}$ | 1.89 ± 0.08 $^{b}$ |
| 17 | Thujone | 1098 | tr | tr | tr | tr | tr | 1.37 ± 0.11 |
| 18 | 4-Methylpentyl isobutyrate | 1109 | tr | tr | 1.97 ± 0.20 $^{c}$ | 4.91 ± 0.24 $^{b}$ | 3.61 ± 0.21 $^{b}$ | 8.21 ± 0.83 $^{a}$ |

Values are given as mean ± SE (n = 3). According to the Tukey's Test application: means of the same column and main variable labeled with the same letters are not significantly different at $p < 0.05$

| No. | Compounds | RI | Vegetative stage | Floral budding | Full flowering | Early development of seeds | Mid-maturation of seeds | Late-mature/ripe of seeds |
|---|---|---|---|---|---|---|---|---|
| 19 | Camphor | 1136 | tr | tr | tr | tr | tr | 1.60 ± 0.14 |
| 20 | Hexyl butyrate | 1183 | 0.92 ± 0.03 e | 8.22 ± 0.27 d | 9.05 ± 0.74 d | 32.08 ± 2.52 b | 38.75 ± 2.89 a | 23.59 ± 1.62 c |
| 21 | Octyl acetate | 1203 | tr | 0.72 ± 0.10 d | 3.33 ± 0.37 c | 11.67 ± 0.92 ab | 14.47 ± 1.66 a | 10.48 ± 1.09 b |
| 22 | Anethole | 1220 | 0.85 ± 0.08 c | 14.55 ± 1.02 a | 8.08 ± 1.05 b | 1.32 ± 0.10 c | 2.04 ± 0.23 c | tr |
| 23 | Hexyl 2-methylbutyrate | 1234 | 0.31 ± 0.08 d | 1.39 ± 0.10 d | 3.22 ± 0.38 c | 5.76 ± 0.64 b | 4.22 ± 0.19 c | 8.01 ± 0.78 a |
| 24 | Hexyl isovalerate | 1240 | tr | 0.33 ± 0.05 b | 1.13 ± 0.08 ab | 2.01 ± 0.72 a | 2.12 ± 0.08 a | 2.45 ± 0.30 a |
| 25 | Octyl Isobutyrate | 1329 | tr | 1.90 ± 0.05 b | 1.45 ± 0.24 b | 4.48 ± 0.51 a | 6.00 ± 0.71 a | 6.26 ± 0.94 a |
| 26 | Hexyl hexanoate | 1369 | tr | 0.57 ± 0.07 d | 0.91 ± 0.04 c | 3.13 ± 0.43 ab | 3.70 ± 0.16 a | 2.64 ± 0.19 ab |
| 27 | Octyl 2-methylbutyrate | 1416 | tr | 0.69 ± 0.03 c | 1.10 ± 0.17 bc | 1.94 ± 0.05 b | 2.18 ± 0.16 b | 5.38 ± 0.67 a |
| 28 | Caryophyllene | 1421 | 14.07 ± 1.45 a | 4.36 ± 0.19 b | 0.54 ± 0.06 c | 0.73 ± 0.14 c | tr | 0.87 ± 0.07 c |
| 29 | Octyl isovalerate | 1442 | tr | | 0.26 ± 0.02 d | 0.37 ± 0.07 c | 0.69 ± 0.05 b | 1.13 ± 0.14 a |
| 30 | α-Curcumene | 1460 | 3.48 ± 0.41 b | 7.63 ± 0.52 a | 1.15 ± 0.05 c | tr | tr | tr |
| 31 | Phenethyl 2-methylbutyrate | 1481 | 1.39 ± 0.10 a | 0.85 ± 0.07 b | 1.27 ± 0.05 a | tr | tr | tr |
| 32 | Myristicin | 1491 | 5.24 ± 0.66 b | 7.31 ± 0.55 b | 15.02 ± 1.30 a | tr | tr | tr |
| 33 | (E)- γ -Bisabolene | 1501 | 2.04 ± 0.69 a | 0.77 ± 0.07 b | tr | tr | tr | tr |
| 34 | 1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene | 1508 | 1.73 ± 0.14 a | 0.20 ± 0.07 c | tr | tr | 0.65 ± 0.11 b | 2.04 ± 0.24 a |
| 35 | β-Bisabolene | 1516 | 8.59 ± 0.57 b | 12.56 ± 0.59 a | 8.26 ± 0.94 b | 1.73 ± 0.15 c | | 0.39 ± 0.09 c |
| 36 | Spatulenol | 1541 | 5.77 ± 0.33 a | 1.02 ± 0.31 b | 0.28 ± 0.04 c | tr | tr | tr |
| 37 | 1-Allyl-2,3,4,5-tetramethoxybenzene | 1568 | 0.69 ± 0.05 b | | 2.98 ± 0.25 a | tr | tr | tr |

Values are given as mean ± SE (n = 3). According to the Tukey's Test application: means of the same column and main variable labeled with the same letters are not significantly different at $p < 0.05$

| No. | Compounds | RI | Vegetative stage | Floral budding | Full flowering | Early development of seeds | Mid-maturation of seeds | Late-mature/ripe of seeds |
|---|---|---|---|---|---|---|---|---|
| 38 | Caryophyllene oxide | 1576 | 6.10 ± 0.25 [a] | 2.47 ± 0.29 [b] | 0.40 ± 0.11 [d] | 0.49 ± 0.09 [d] | tr | 0.77 ± 0.05 [c] |
| 39 | d-Viridiflorol | 1591 | tr | tr | tr | 3.03 ± 0.36 [b] | 1.30 ± 0.09 [c] | 3.76 ± 0.16 [a] |
| 40 | Butylphosphonic acid, hexyl 4-methoxybenzyl ester | 1597 | 0.32 ± 0.06 [d] | 1.87 ± 0.15 [c] | 2.81 ± 0.12 [b] | 3.61 ± 0.43 [a] | 1.37 ± 0.16 [c] | tr |
| 41 | Apiol | 1675 | 1.61 ± 0.04 [c] | 3.05 ± 0.30 [b] | 7.08 ± 0.91 [a] | tr | tr | tr |
| 42 | 1-Tetradecanol | 1681 | 7.06 ± 0.75 [a] | 5.41 ± 0.33 [b] | 2.38 ± 0.50 [c] | 0.66 ± 0.12 [d] | tr | tr |
| 43 | Falcarinol | 2005 | 0.44 ± 0.07 [c] | 2.59 ± 0.16 [a] | 0.66 ± 0.07 [c] | 1.43 ± 0.15 [b] | tr | tr |
| 44 | Manool | 2056 | tr | 0.38 ± 0.04 [b] | tr | 0.98 ± 0.14 [a] | 1.25 ± 0.07 [a] | 1.05 ± 0.10 [a] |
| 45 | trans-Geranylgeraniol | 2201 | 1.01 ± 0.51 [ab] | 1.80 ± 0.10 [a] | 0.41 ± 0.08 [c] | tr | tr | tr |
| | **Total** | | **96.77** | **97.02** | **96.94** | **98.02** | **97.07** | **98.23** |

Values are given as mean ± SE (n = 3). According to the Tukey's Test application: means of the same column and main variable labeled with the same letters are not significantly different at $p < 0.05$

The main constituents of the essential oil in various stages were limonene (18.05%), caryophyllene (14.07%), β-pinene (8.67), β-bisabolene (8.86%), 1-tetradecanol (7.06%), caryophyllene oxide (6.10%), espatulenol (5.77%), myristicin (5.24%), carene (4.19%), α-curcumene (3.48%) at the vegetative stage. Anethole (14.55%), β-bisabolene (12.56%), hexyl butyrate (8.22%), α-curcumene (7.63%), myristicin (7.31%), 1-tetradecanol (5.41%), α-pinene (4.70%), caryophyllene (4.36%), γ-terpinene (4.37%), and apiol (3.05%) were the main constituents of the essential oil in the early flowering stage. In the full flowering stage values were: myristicin (15.02%), hexyl butyrate (9.05), β-bisabolene (8.26%), anethole (8/08%), apiol (7/08%), γ -terpinene (6.31) %), α-pinene (4.89%), p-cymene (4.20%), octyl acetate (3.3%), and hexyl isovalerate (3.22%). During seed development, butyrate (32.08%), octyl acetate (11.67%), hexyl 2-methyl butyrate (5.76%), cis-5-octen-1-ol (5.75%), 4-methyl pentyl isobutyrate (4.91%), octyl isobutyrate (4.48%), and hexyl 4-methoxybenzyl ester (3.61%) were dominant constituent of the essential oil. Lastly, in the phenological stage of immature seeds, hexyl butyrate (38.75), octyl acetate (14.47), hexyl 2-methyl butyrate (4.22), cis-5-octen-1-ol (6.3), and 4-methyl pentyl isobutyrate (3.61%) were measured as the predominant constituent of the essential oil, however, these percentages changed to 23.59, 10.48, 8.01, 4.85, and 8.21% at the seed final maturation stage, respectively (Table 1). Previous studies have shown almost the same compounds of the essential oil in *H. persicum's* plant seeds while the value of these compounds were negligible in the leaf and flower organs [4,6,22,23]. According to the mean comparisons' analysis, there were significant differences between these compounds obtained in the various phenological stages. Figure 3 shows that the essential oil constituent were different in the different phenological stages. In addition, predominant compounds of essential oil changed during different growth stages, which are also presented in the Fig. 3.

The study of essential oil constituents indicated that α-pinene and β-pinene were predominant in the early growth stages. According to the results (Table 1), the highest α-pinene value was observed at the full flowering stage (4.89%), but we did not see the same results at the immature seed stage. Moreover, β-pinene was detected in the vegetative stage and at the time of flower opening. With entering the seed set stage, the value of these two compounds decreased significantly.

Limonene was observed only in the vegetative and floral budding stages, and the highest value was 18.05% in the vegetative stage. In line with these results, other researchers reported the existence of this compound in the vegetative organs of *H. persicum* [6], while it was rarely seen in the seeds. In the few cases that limonene was observed in seeds, the value was not significant.

P-cymene was identified in the floral budding and full flowering stage, however its maximum value (4.2%) was observed in the full flowering stage. γ-terpinene was detected in the full flowering stage and the floral budding stage (6.31 and 4.37%, respectively). In a study on the Ajowan plant, the amount of γ-terpinene in the early stages of growth was low, and arrived to its maximum at flowering stage, then subsequently decreased, which could be due to the adsorption of pollinators [33]. α-curcumene was observed only in the early growth stages of *H. persicum* and the maximum amount was related to the floral budding stage (7.63%). Caryophyllene and myristicin were the predominant compounds in the early growth period which were quantified by the amount of 14.07 and 15.02%, respectively. β-bisabolene was the predominant compound that was detected in the floral budding stage and as the plant age increased, its value decreased.

Anethole was the predominant compounds in the floral budding stage and full flowering stage, and the highest value was obtained in the floral budding stage (14.55%); prior to flowering its amount was not significant, and after that it was greatly reduced. Apiol and 1-tetradecanol were the other compounds that observed in the first three stages of plant vegetative stages. As shown in Table 1, the maximum values of apiol (7.06%) and 1-tetradecanol (7.66%) belonged to the full flowering stage and the vegetative stages, respectively. Caryophyllene oxide was the compounds that declined with the increase in plant age, and the highest value was obtained in the vegetative stage at the rate of 6.10%.

The highest value of hexyl butyrate and octyl acetate, that are the most predominant and important compounds in *H. persicum*, were observed in the immature seeds stage and in the mid-mature seeds stage, by 38.75% and 14.47%, respectively. These beneficial compounds significantly increased to the seedling stage. In agreement with our results, previous studies reported these two compounds as the predominant compound in the seeds of *H. persicum* [3,6,22].

Among all the compounds, Hexyl 2-methylbutyrate, octyl Isobutyrate, and 4-methylpentyl isobutyrate content were increased with the increase in plant age, so that at the end of the phonological stages, they were the dominant compounds.

The essential oils were classified according to their chemical formula to eight groups (Fig. 4). Based on the results, the main percentage of the essential oil was aliphatic esters, which value was 3.35% in the vegetative stage and finally hit 83.67% in the immature seeds stage or in the mid-mature seeds stage. Therefore, by increasing the age of the plant and seeds formation, the value of this group of compounds was increased (Fig. 4). The main components of aliphatic esters group were reported as hexyl butyrate, octyl acetate, octyl butyrate, and hexyl 2-methylbutyrate, which the amounts of them were different in the phenological stage of growth. In the present study, these compounds were observed mostly in the last three phenological post-flowering stages (Fig. 4); other researchers also found more aliphatic compounds in the generative organs, such as in seeds of *H. persicum* [1, 6, 9,22,23,35].

The second and large group of the constituent compounds was the monoterpene hydrocarbons which their value in the early stage of the vegetative period was 34.23%, and with increasing the age of the plant and the seed maturation, its value reduced, and finally this compound was not found in the mid-mature seed stage (Fig. 4). Several compounds of approximately 40 compounds of the monoterpene hydrocarbons group have been reported by various researchers in the *H. persicum.* In the present study, most of these compounds were observed in the flowering stage and prior to it, and when the plant entered into the seed set stage, the value of these compounds was significantly reduced (Fig. 4). The most important compounds of this group were limonene, β-pinene, α-pinene, and γ-terpinene. Other studies also exhibited these compounds are predominant compounds in the *H. persicum* [6, 22, 36]. Similar to the present study, other researchers reported high amount of these compounds in flowers and leaves, in comparison to seeds [6, 36].

Sesquiterpene hydrocarbons was one of the other groups of the constituent compounds of which its amount was high at the beginning of the phenological growth time; the highest value was observed at the beginning stage of flowering (21.16%) and its value decreased with increasing plant age. Oxygenated sesquiterpene was another group of the constituent compounds. Analysis of our data showed 25.94% of sesquiterpene hydrocarbons at the vegetative stage, and after this phenological stage, this amount significantly reduced (Fig. 4). The most important compounds of this group were caryophyllene espatulenol, and β-bisabolene which also reported as predominant compounds in the *H. persicum* in other studies [8, 37].

The other compound evaluated in this experiment was phenylpropenes, which amount at the full flowering stage was 23.10%, and after passing this phenological stage it was significantly reduced (Fig. 4). Anethole and myristicin were two important compounds related to phenylpropenes group. In a study on *H. persicum* plants, researchers found three phenylpropenes compounds (Anethole, myristicin and estragole) that were usually predominant in the vegetative and flower organs that match the results of the present study [1, 6, 8].

Changes in essential oil compounds in different phenological stages of *H. persicum* growth are probably due to the fact that the production of essential oil and aromatic compounds are under the control of physiological, biochemical and metabolic mechanisms dependent on age and growth stages of plant. Besides, these changes related to terpene biosynthesis as well as its accumulation in the secretory organs [38]. The change in the essential oil ingredients is also influenced by factors such as the age and the development stage of medicinal plants.

### *Phenolic acids in different phenological stages*

Phenolic compounds such as phenolic acids are one of the most critical compounds in medicinal plants and have paramount importance due to the high biological activity and their function as antioxidants, anti-inflammatory, anticancer, and anti-Alzheimer [25, 39].

In this study, we investigated the phenolic acids content in different phenological stages of *H. persicum.* For this purpose, ten phenolic acid compounds were evaluated in the methanolic extract, harvested at different growth stages, using HPLC. According to the obtained results, there were 8, 9, 9, 10, 8 different phenolic compounds at the vegetative stages, floral budding stage, full flowering stage, early development of seeds stage, mid-mature seeds stage and, final stage or late-mature/ripe seeds stage, respectively (Table 2).

The methanolic extract of the floral budding stage had the highest amount of phenolic acids (287.4 mg/g dry extract) following by early seed development (259.77 mg/g dry extract), full flowering stage (153.69 mg/g dry extract), vegetative stage (72.95 mg/g dry extract), and mid-mature seed stage (46.21 mg/g dry extract). On the other hand, the mature seed extract contained the minimum amount of phenolic acids (24.63 mg/g dry extract) (Table 2).

Cinnamic acid, p-coumaric acid, p-hydroxybenzoic acid, ferulic acid, and rosmarinic acid are predominant phenolic acids in *H. persicum*. Table 2 shows the different phenolic compounds at different morphological stages of *H. persicum* plants. The predominant phenolic acids were, in the vegetative stage, cinnamic acid (16.10 mg/g extract), p-hydroxybenzoic acid (14.37 mg/g extract), p-coumaric acid (13.43 mg /g extract), and rosmarinic acid (13.33 mg/g extract); in the floral budding stage, cinnamic acid (225.25 mg/g extract), p-coumaric acid (24.05 mg/g extract), and ferulic acid (12.03 mg/g extract); in the full flowering stage, cinnamic acid (56.42 mg/g extract), p-coumaric acid (39.22 mg/g extract), p-hydroxybenzoic acid (16.77 mg/g extract); in the early stage of seed development, cinnamic acid (218.64 mg/g extract), p-coumaric acid (11.91 mg/g extract), and ferulic acid (6.46 mg/g extract); in the mid-mature seeds stage p-coumaric acid (10.02 mg /g dry extract), ferulic acid (8.77 mg/g extract), and cinnamic acid (8.04 mg/g extract); in the final stage or late-mature/ripe seeds stage, cinnamic acid (8.69 mg/g extract), Gallic acid (6.49 mg/g extract), and rosmarinic acid (3.57 mg/g extract).

Cinnamic acid was recorded as the predominant compounds of phenolic acid in all growth stages. However, its maximum value (218.64 mg/g of dried extract) was in the early stages of flowering, and its lowest value (8.04 mg/g of dried extract) was in the mid- mature stage. Cinnamic acid is a natural aromatic phenolic acid whose long - term - consumption is associated with low toxicity to humans and is used in flavorings, artificial color, and some specific medications. The frequent use of cinnamic acid is as a precursor for the methyl cinnamate, ethyl cinnamate, and benzyl cinnamate production used in the perfume industry. It is also a precursor for the artificial sweetener aspartame. Cinnamic acid is one of the phenolic acids

with several biological and medicinal properties, as well as other economic and industrial values [25, 40]. According to our results, *H. persicum* is considered as one of the plants with a rich source of cinnamic acid, especially in the floral budding stage and early development of seeds stage; however, as the plant entered the mature seed stage, we observed the significant reduction in its value.

P-coumaric acid was another beneficial predominant phenolic acid we explored in this experiment. P-coumaric acid amount was increased during the plant growth to 39.22 mg/g at the full flowering stage and then decreased to the lowest amount in the seed maturing stage (1.67 mg/g extract). Studies showed p-coumaric acid has antioxidant properties, which is reported to reduce the stomach cancer by suppressing nitrous amines, and has anti-tumor and anti-mutagenesis activities [25,41]. Ferulic acid is one of the other critical phenolic compounds that researchers have been proven its antioxidant properties [25]. This compound was found at all growth stages of the *H. persicum* plant, and the highest and lowest amount were recorded for the full flowering stage and the seed mature stage with values 15.8 and 2.39 mg/g, respectively.

P-hydroxybenzoic acid is noted as the basis for the preparation of its esters, named parabens, and are employed as preservatives in cosmetics and some ophthalmic solutions. This material was one of the other predominant phenolic acids in the *H. persicum* observed in all phenological stages in this experiment. According to the obtained results, the maximum value of 16.77 mg/g of dry extract was obtained at the flowering stage, while the seed maturing stage had the lowest amount (0.76%). The amount of this phenolic acid decreased significantly by entering the seed to the sowing stage. Rosmarinic acid is an ester of caffeic acid called 3,4-dihydroxy phenyl lactic acid [25,42]. This compound has many pharmaceutical properties such as antimicrobial, anti-rheumatism, and anticancer and was present in all phenological stages of the *H. persicum.* The highest (13.33 mg/g of dry extract) and the lowest amounts (3.57 mg/g of dry extract) of this compound were obtained in the vegetative stage and the immature seed stage, respectively. Therefore, it can be concluded that *H. persicum* can be considered a plant rich in phenolic acids, which content was different in different phenological stages. Phenolic compounds were at the lowest rate in the floral budding stage while the maximum content was recorded at the seed mature stage. An increase in phenolic acid levels during flowering stage recommended a higher expression level of the phenylalanilammiase lyase enzyme [43, 44], and it is also an indication of enzyme activity reduction with plant maturation. These changes that occur in the process of primary metabolites adsorption are a result of starch synthesis in the middle stages of seed maturation, and this phenomenon can affect the biosynthesis of phenolic acids [45].

### *Cluster analysis, and principal component analysis (PCA)*

Another goal of this study was to monitor the differences and similarities between different phenological stages in order to find the consequences of different harvesting time on identified phytochemical compounds in *H. persicum*. To reach the above-mentioned goals, we used the principal components analysis (PCA) and hierarchical cluster analysis (HCA) methods. The results of the main components analyses are shown in Table 3. Based on these results, three components had highest eigenvalue, reporting 89.45% of the total variance. The relative variance for the first, second, and third components was 55.52%, 23.28%, and 10.65%, respectively. In the first component, the compounds of butyl butyrate, carene, 4-methyl pentyl isobutyrate, hexyl butyrate, cis-5-octen-1-ol, octyl acetate, hexyl 2-methyl butyrate, octyl isobutyrate, β-bisabolene, 1-tetradecicanol, p-hydroxybenzoic, rosmarinic acid, and essential oil content had the highest loading factor. Differently, in the second and third components, m-coumaric acid and gallic acid compounds had the most top loading factor, respectively (Table 3).

Table 2
Contents of phenolic acid compounds (mg/g dried extract) of Heracleum persicum at different phenological stages

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Phenological stage | GA | PHBA | VA | CaA | PCA | FA | MCA | CiA | RA | SA | **Total** |
| Vegetative | 1.34 ± 0.5c | 14.37 ± 0.15b | 0.46 ± 0.02bc | 0.00 ± 0.00c | 13.43 ± 0.07c | 9.92 ± 0.16c | 0.00 ± 0.00c | 16.10 ± 0.23c | 13.33 ± 0.36a | 3.64 ± 0.15a | **72.59** |
| Floral budding | 1.89 ± 0.12c | 8.58 ± 0.53c | 0.57 ± 0.00b | 0.00 ± 0.00c | 24.05 ± 0.3b | 12.03 ± 0.25b | 4.90 ± 0.06b | 225.25 ± 5.30a | 9.18 ± 0.06b | 0.95 ± 0.1b | **287.40** |
| Full flowering | 3.98 ± 0.12b | 16.77 ± 0.67a | 8.25 ± 0.21a | 0.00 ± 0.00c | 39.22 ± 1.04a | 15.80 ± 0.35a | 6.35 ± 0.21a | 56.42 ± 2.54b | 6.25 ± 0.55c | 0.65 ± 0.03b | **153.69** |
| Early development | 1.28 ± 0.12c | 3.61 ± 0.32d | 0.61 ± 0.11b | 5.61 ± 0.3a | 11.91 ± 0.63 cd | 6.46 ± 0.25c | 5.90 ± 0.35a | 218.64 ± 5.69a | 4.82 ± 0.45 cd | 0.93 ± 0.05b | **259.77** |
| Mid-maturation | 1.95 ± 0.03c | 1.78 ± 0.01e | 0.00 ± 0.00c | 3.04 ± 0.26b | 10.02 ± 0.86d | 8.77 ± 0.45c | 6.62 ± 0.16a | 8.04 ± 0.26c | 5.99 ± 0.52c | 0.00 ± 0.00c | **46.21** |
| Late-mature/ripe | 6.49 ± 0.28a | 0.76 ± 0.08e | 0.00 ± 0.00c | 0.00 ± 0.00c | 1.67 ± 0.13e | 2.39 ± 0.12d | 0.00 ± 0.00c | 8.69 ± 0.35c | 3.57 ± 0.14d | 1.06 ± 0.15b | **24.63** |
| Significance | ** | ** | ** | ** | ** | ** | ** | ** | ** | ** | |
| Values are given as mean ± SE (n = 3). According to the *Tukey's* Test application: means of the same column and main variable labeled with the same letters are not significantly different at $p < 0.05$<br>GA: Gallic acid; PHBA: p-Hydroxybenzoic acid; VA: Vanillic acid; CaA: Caffeic acid; PCA: p-Coumaric acid; FA: Ferulic acid; MCA: m-Coumaric acid; CiA: Cinnamic acid; RA: Rosmarinic acid; SA: Salicylic acid | | | | | | | | | | | |

Table 3
Principal component analysis of main phytochemical compounds for different phenological stages of Heracleum persicum medicinal plants.

| Number of compounds | Phytochemical compounds | Principal component (PC) | | |
|---|---|---|---|---|
| | | PC1 | PC2 | PC3 |
| $x_1$ | a-Pinene | -0.76 | 0.59 | -0.02 |
| $x_2$ | β-Pinene | -0.61 | -0.78 | 0.00 |
| $x_3$ | Butyl butyrate | 0.95 | -0.15 | 0.13 |
| $x_4$ | Limonene | -0.56 | -0.79 | 0.02 |
| $x_5$ | Carene | -0.87 | -0.29 | 0.17 |
| $x_6$ | γ-Terpinene | -0.68 | 0.68 | 0.25 |
| $x_7$ | 4-Methylpentyl isobutyrate | 0.89 | -0.01 | 0.37 |
| $x_8$ | Hexyl butyrate | 0.92 | 0.15 | -0.32 |
| $x_9$ | cis-5-Octen-1-ol | 0.97 | 0.02 | -0.16 |
| $x_{10}$ | Anethole | -0.57 | 0.60 | -0.19 |
| $x_{11}$ | Octyl acetate | 0.96 | 0.09 | -0.15 |
| $x_{12}$ | Hexyl 2-methylbutyrate | 0.89 | 0.14 | 0.32 |
| $x_{13}$ | Octyl Isobutyrate | 0.98 | 0.08 | 0.01 |
| $x_{14}$ | a-Curcumene | -0.72 | -0.02 | -0.27 |
| $x_{15}$ | Caryophyllene | -0.67 | -0.74 | -0.05 |
| $x_{16}$ | Myristicin | -0.77 | 0.53 | 0.32 |
| $x_{17}$ | β-Bisabolene | -0.94 | 0.14 | -0.06 |
| $x_{18}$ | Apiol | -0.69 | 0.62 | 0.34 |
| $x_{19}$ | Caryophyllene oxide | -0.69 | -0.72 | -0.04 |
| $x_{20}$ | 1-Tetradecanol | -0.91 | -0.36 | -0.12 |
| $x_{21}$ | Gallic acid | 0.36 | 0.14 | 0.89 |
| $x_{22}$ | p-Hydroxybenzoic acid | -0.91 | 0.13 | 0.21 |
| $x_{23}$ | Vanillic acid | -0.42 | 0.68 | 0.43 |
| $x_{24}$ | Caffeic acid | 0.55 | 0.10 | -0.66 |
| $x_{25}$ | p-Coumaric acid | -0.69 | 0.70 | 0.07 |
| $x_{26}$ | Ferulic acid | -0.77 | 0.53 | -0.09 |
| $x_{27}$ | m-Coumaric acid | 0.08 | 0.80 | -0.52 |
| $x_{28}$ | Cinnamic acid | -0.17 | 0.37 | -0.62 |
| $x_{29}$ | Rosmarinic acid | -0.81 | -0.51 | -0.21 |
| $x_{30}$ | Salicylic acid | -0.57 | -0.77 | 0.11 |
| $x_{31}$ | Essential oil content | 0.81 | -0.06 | -0.06 |
| $x_{32}$ | Extraction content | -0.65 | 0.30 | -0.61 |
| Eigenvalue | | 17.77 | 7.45 | 3.41 |
| Relative variance (%) | | 55.52 | 23.28 | 10.65 |
| Cumulative variance (%) | | 55.52 | 78.80 | 89.45 |

Since 78.80% of the total variance was allocated to the first and second components, they were used for the biplot diagram obtained from the PCA. Different phenological stages were divided into three distinct groups which shown in the Fig. 5. The biplot analysis showed the vegetative stage (S1), based on the first and second components alone and with high distance with other phenological stages. It was strongly correlated with the compounds of carene, rosmarinic acid, anethole, caryophyllene, β-pinene, limonene, and salicylic acid. The phenological stages of floral budding (S2) and full flowering (S3) were segregated in one group according to the first and second components which have a high correlation with β-bisabolene, p - hydroxybenzoic acid, anethole, p - coumaric acid, γ - terpinene, apiol, a-pinene, myristicin, ferulic acid, extraction content, cinnamic acid and, vanillic acid compounds. On the other hand, other phenological stages, including early seed development, mid-mature seed, and mature seed, were grouped together and have a strong correlation with butyl butyrate, 4-methyl pentyl isobutyrate, hexyl 2-methyl butyrate, hexyl butyrate, octyl acetate, cis-5-octen-1-ol, octyl isobutyrate, caffeic acid, gallic acid, and essential oil content. In research on the *Ferulago angulata*, using principal component analysis, the first two components reported as 76% of the total variance [28]. Results of this study also showed that the measured phytochemical compounds

were affected by different treatments. Moreover, they showed that for the first component, eight compounds had a highest loading factor). Esmaeili et al. (2018) showed the first two components accounted for 95% of the total variance using the principal component analysis based on phytochemical compounds of *Oliveria decumbens* [27]. They showed that 12 and 7 compounds accounted for the highest factor loading in the first and second components, respectively. Other researchers also used principal component analysis to identify the dominant compounds in the useful components in other medicinal plants like *Satureja pilosa* [46].

Considering the higher extract content quantity or quality in the general floral budding and full flowering stage, it is possible to point the suitability of harvest in these stages for producing a higher extract content. The final growth stage also had higher essential oil percentage, indicating the effect of different phenological stages on the essential oil percentage quality. So we can pinpoint the phytochemical compounds extracted from the plant strongly depend on the plant organ and phenological stage [34, 47, 48].

In a study to investigate the phenological effects on phytochemical compounds at different populations of the *Tithonia diversifolia*, the cluster and principal component analyses were used and the results showed that phytochemical compounds were differed in various phenological stages [49].

Classified phenological stages based on all the studied characteristics in this experiment using cluster analysis method are shown in Fig. 6. Based on the dendrogram obtained by cluster analysis, different phenological stages were segregated into four separate groups. Primary growth stages consisted of the vegetative stage (S1) and floral budding stage (S2) and they were more similar to each other in terms of characteristics. The middle phenological stages, including full flowering (S3) and early seed development (S4), were placed in a separate group, and the mid-mature stage of seed (S5) and matured seed (S6), were individually grouped. Hazrati et al. (2019) used the cluster analysis method to classify the target treatments based on identified phytochemical compounds [28]. The results of the cluster analysis based on different phenological stages showed that two primary and intermediate stages of physiological processes were highly correlated with each other but were strongly influenced by the plant physiological stage while were separately placed in a separate group. Based on the dendrogram obtained from the cluster analysis, it was observed that the *H. persicum* was affected by the genetic and the environment in the final stages of phenology more than the primary and middle stages. Pretti et al. (2018) stated that principal component analysis and cluster analyses can be used to determine the optimum phenological stage and to achieve the best yield of phenolic acids and essential oil compounds from plants [49]. This research illustrated in the vegetative phase and in the beginning of the generative phase; the rate of phenolic acids was high. The current research confirmed that the different phenological stages had affected the phenolic acids contents and essential oil compounds of *H. persicum*. However, the mechanisms in which these changes have been made will require further studies.

# Conclusion

*H. persicum* is one of the most important aromatic medicinal plants in Iran that has valuable phytochemicals compounds, which make it a precious medicinal plant to grow in other parts of the world. The results of this study clearly indicated that the yield and quality of the phytochemical composition of the *H. persicum* were significantly changed during the growth period and strongly depends on various phenological stages. The highest yield of essential oil was obtained in the intermediate stage of seed mature, and the highest content of extract was captured in the floral budding stage. Limonene, myristicin, and anethole were predominant compounds in vegetative and flowering stages, and the best stage for achieving the maximum value of hexyl butyrate and octyl acetate content was the seed formation stage. The results of the present study indicated the *H. persicum* was a rich source of phenolic acids, of which cinnamic acid was one of the predominant compounds in all growing stages, and its highest value was obtained in the floral budding stage. Finally, it can be concluded that to reach the maximum essential oil content and composition of aliphatic esters compounds, harvest at the seed set stage, especially in the mid-mature seeds stage is desirable. In addition, harvesting the *H. persicum* at the flowering stage, especially at the floral budding stage, leads to maximum content of extract yield and phenolic acid compounds.

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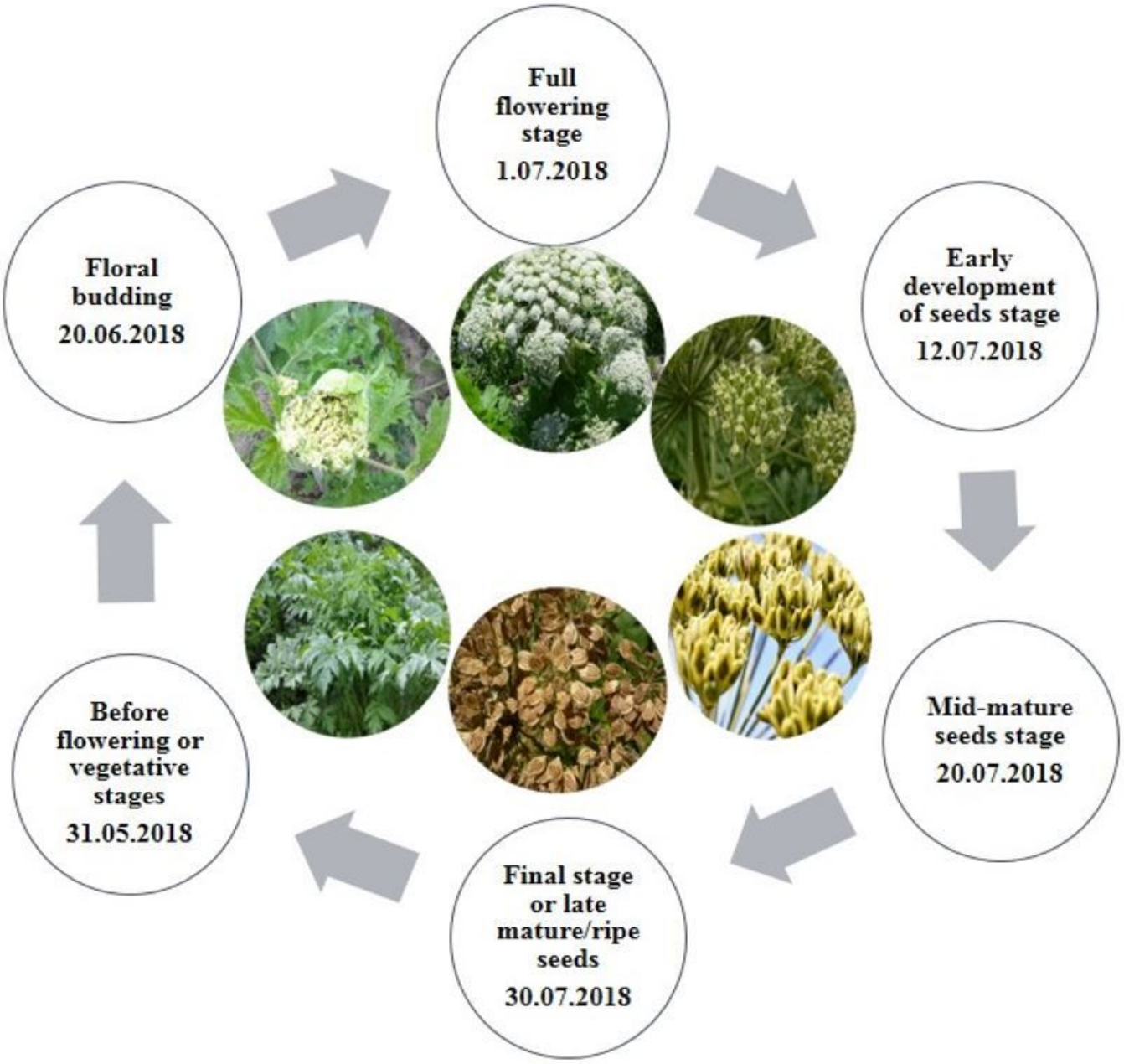
# Figures




## Figure 1

Heracleum persicum at different phenological stages

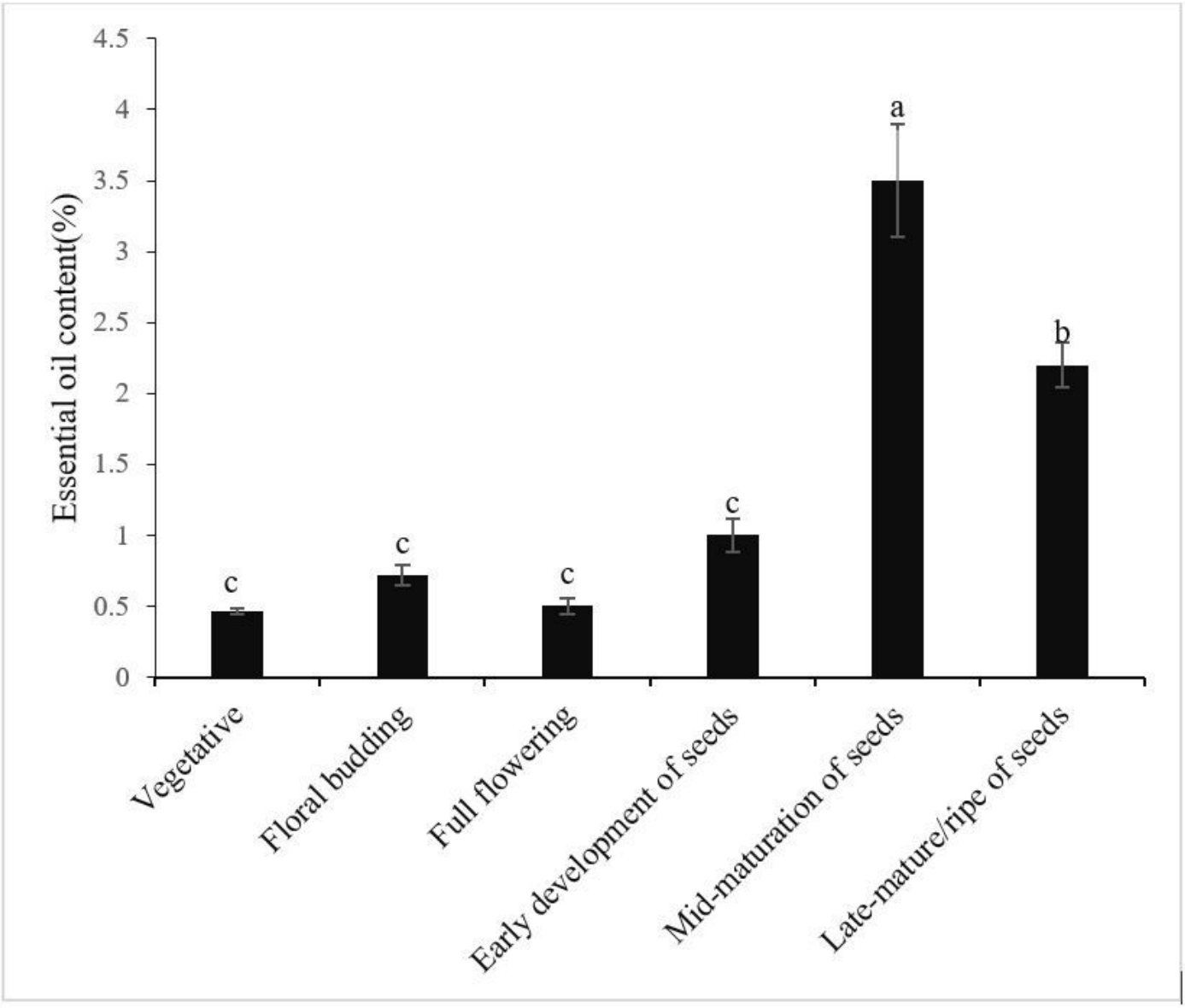



**Figure 2**

Changes in essential oil content (% w/w) of Heracleum persicum at different phenological stages. Essential oil content with different subscripts were significantly different at $p < 0.05$ (Tukey's test)

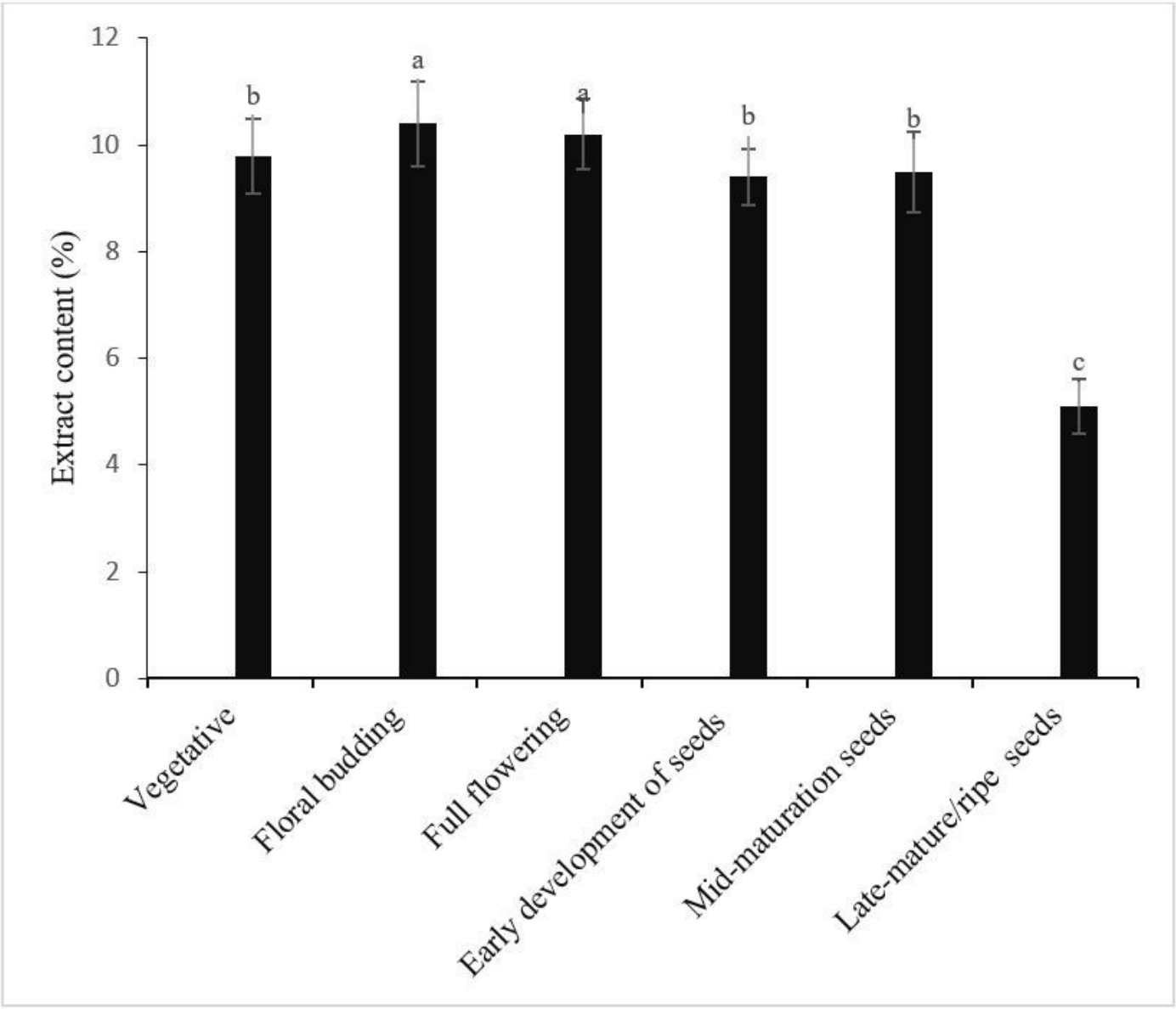
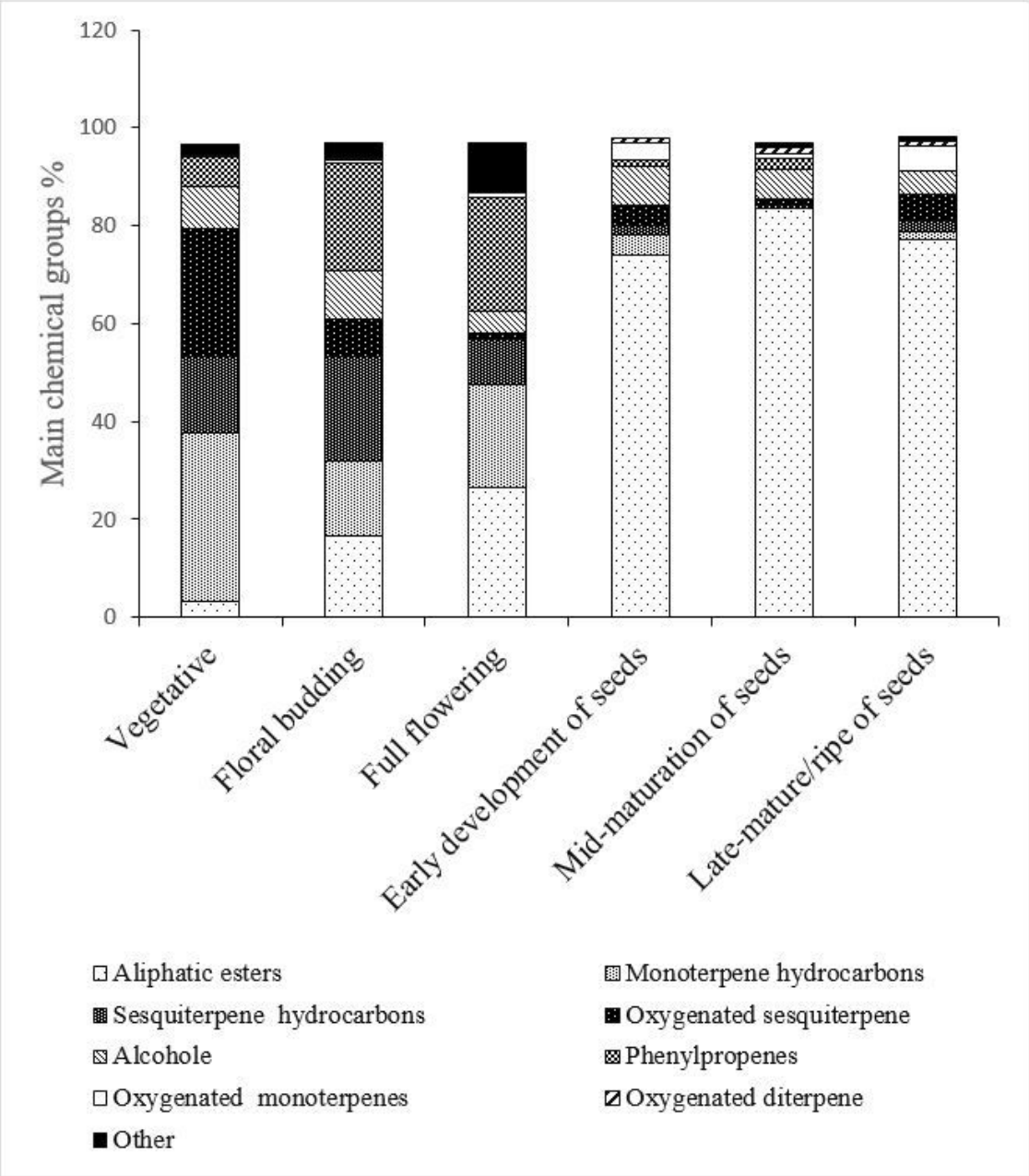


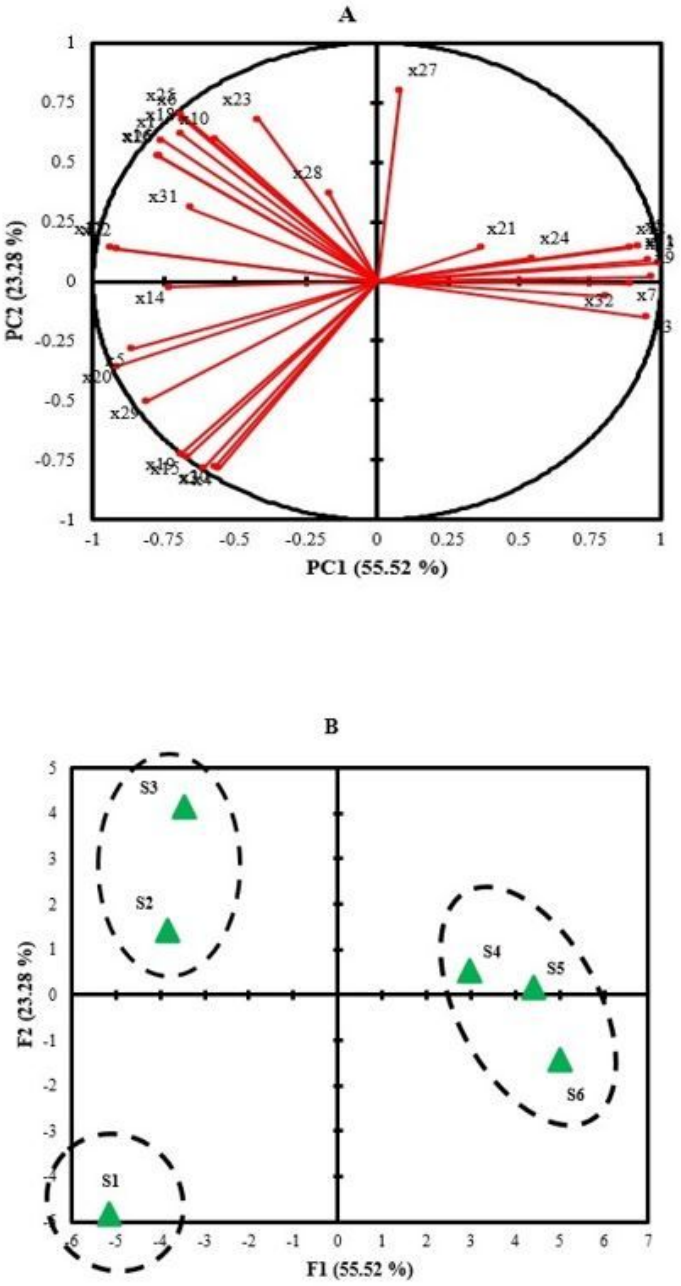

**Figure 3**

Changes in extract content (% w/w) of Heracleum persicum at different phenological stages. Essential oil content with different letters were significantly different at $p < 0.05$ (Tukey's test)




## Figure 4

Comparison of main chemical groups (%) of Heracleum persicum at different phenological stages.




## Figure 5

Biplot derived based on first and second principle components (PC) for different phenological stages of Heracleum persicum medicinal plants.

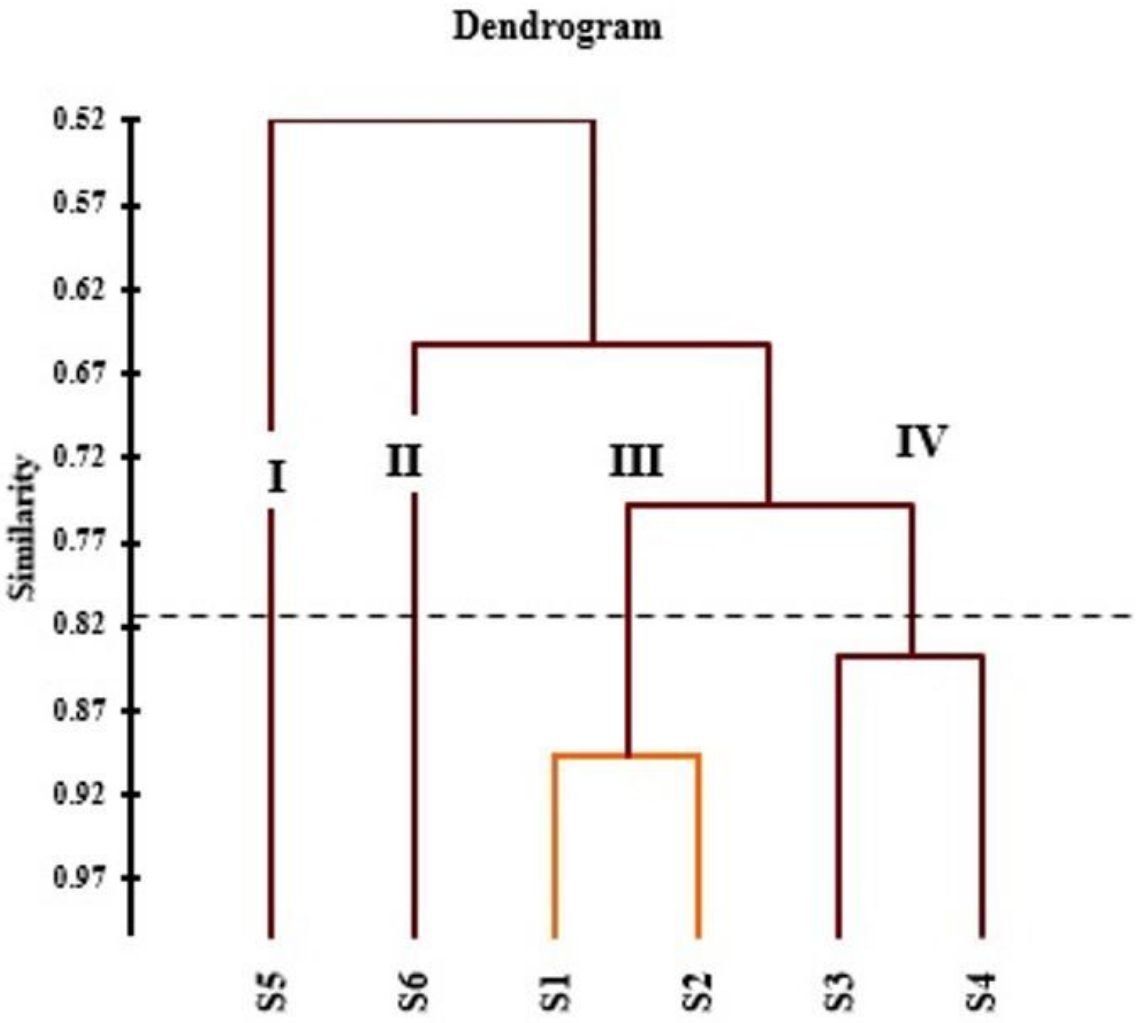



**Figure 6**

Hierarchial cluster analysis based on based on all studied traits in different phenological stages of Heracleum persicum medicinal plants.

## Supplementary Files

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