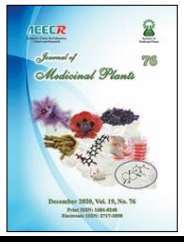
J. Med. Plants 2020; 19(76): 69-88

Institute of Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Research Article

Changes in secondary metabolite contents of *Arnica chamissonis* Less. in response to different harvest time, flower developmental stages and drying methods

Mojdeh Asadi[1], Samad Nejad Ebrahimi[2], Mehrnaz Hatami[3,*], Javad Hadian[1]

[1] *Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Tehran, Iran*
[2] *Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, G.C., Tehran, Iran*
[3] *Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak, Iran*

ARTICLE INFO

Keywords:
Arnica chamissonis
Flower development
Harvesting
Drying
Rutin

ABSTRACT

Background: The relative values of secondary metabolites may be impacted by harvesting time and post-harvest drying methods. **Objective:** The effects of various harvesting stages of flower and drying methods were investigated on the quality and quantity of secondary metabolites of *A. chamissonis* Less. grown in the field. **Methods:** The flowers were harvested in early May, June and July from four different developmental stages (stage 1: opening bud; stage 2: all radial florets or less than half of tubular florets opened; stage 3: all radial and tubular florets opened; stage 4: radial florets withering and seed formation). Also, the maximum collection of inflorescences during two years of trial was considered for drying method treatments (shade and sun drying, oven drying at 40 and 50 °C, and microwave drying at 500 and 1000 W power). **Results:** Results showed that the highest and the lowest total phenols were obtained in the second and first harvest date at flower maturity stages of 3 and 4, respectively. However, the highest luteolin and apigenin contents were obtained in the third harvest time at flower development stage 1. In the second experiment, the flower heads treated by shade contained the highest total phenols content followed by oven drying at 40°C. Moreover, oven drying was the most favorable method for preserving of other main secondary metabolites including total flavonoid, rutin, luteolin and apigenin. **Conclusion:** Different harvesting dates at various flower bud phenological stages had significant effect on total phenols, flavonoids, rutin, luteolin and apigenin contents.

Abbreviations: PCA, principal component analysis; UPGMA, un-weighted pair group method with arithmetic mean.

* Corresponding author: m-hatami@araku.ac.ir

doi: 10.29252/jmp.19.76.69
Received 3 January 2020; Received in revised form 26 October 2020; Accepted 1 November 2020

1. Introduction

The genus *Arnica* contains thirty-two species in the family of Compositae (Asteraceae) and mainly distributed to the boreal-montane area of the northern hemisphere [1]. *Arnica chamissonis* Less. a North American species, is a herbaceous perennial, medicinally interesting crop to the pharmaceutical and cosmetic industries as an valuable source of secondary metabolites such as phenolic acids, flavonoids, essential oils and terpenoids [2-5], presenting powerful antibacterial, antifungal, antioxidant and anti-swelling activities [2-7]. Furthermore, extracts of *A. chamissonis* exhibit anti-inflammatory and antiradical activity that might be beneficial in retarding the frightful progress of free radical-related disorders [2, 8].

Cultivation of *A. chamissonis* is necessary due to the more stringent rules to protect the species in countries where wild collection has been practiced and because of the increased demand from the industry [9]. Moreover, *A. chamissonis* is an herbal alternative for the critically threatened mountain *Arnica* species [4-8]. In addition, due to its low ecological demand the cultivation of *A. chamissonis* is easier than *Arnica montana* L. [8, 10], which is important in the aspect of raw material production for different industries. Several factors affect quantity and quality of bioactive compounds in industrially valuable plants such as optimization of the cultivation conditions, harvest time and post-harvest (e.g., drying) practices. The content of chemical metabolites in *Arnica* differs among plant organs, type of vegetation, temperature rate during cultivation, and agro-ecological factors [11-12]. Previous investigations showed that the yield of flower heads and the quantity and composition of the chemical compounds in field grown arnica were impacted by application of organic-mineral fertilizers and humic substances [13-14], stage of flower head maturity and establishment of plantation steps [13-17]. Considerable less research in this subject has been devoted to *A. chamissonis* [17-18]. However, various developmental stages of flowers in *A. montana* displayed strongly significant differences in sesquiterpenes and quercetin contents [5].

The relative values of chemical metabolites may also be impacted by the drying and pre-drying operations [19]. Drying is an important procedure in the post-harvest process, which may diminish the moisture content of freshly harvested plant parts to prevent the microbial growth and decrease the rate of biochemical reactions [20, 21]. Drying methods may either be carried out by traditional open sun/and or shade drying as well as microwave/oven drying methods [21, 22]. However, development of enzymatic and non-enzymatic reactions occurring during drying processes of fresh plant materials may lead to significant changes in the quantity and composition of chemical metabolites [21, 23]. Since *Arnica* is not reported in the flora of Iran, and its cultivation was done for the first time by the authors of this article through current research, there are no recorded methods for its cultivation, harvesting and processing. Therefore, the aims of this study were (1) to investigate the effect of various developmental stages of flower and (2) different drying methods of inflorescence on the quality and quantity of secondary metabolites in *A. chamissonis* Less.

2. Materials and Methods

2.1. Experimental field

This study was conducted during 2015 and 2016 at the experimental field of Medicinal Plant and Drug Research Institute, Shahid Beheshti University (latitude: 35° 41´ 46″ N, longitude:

51° 25′ 23″ E, altitude: 1776 m above the sea level), Tehran, Iran. The thermo-pluviometric information/data during the study years, 2015 and 2016 was presented in Fig. 1. The research field in Tehran is classified as BSk (a local step climate) according to Köppen and Geiger climate classification system.

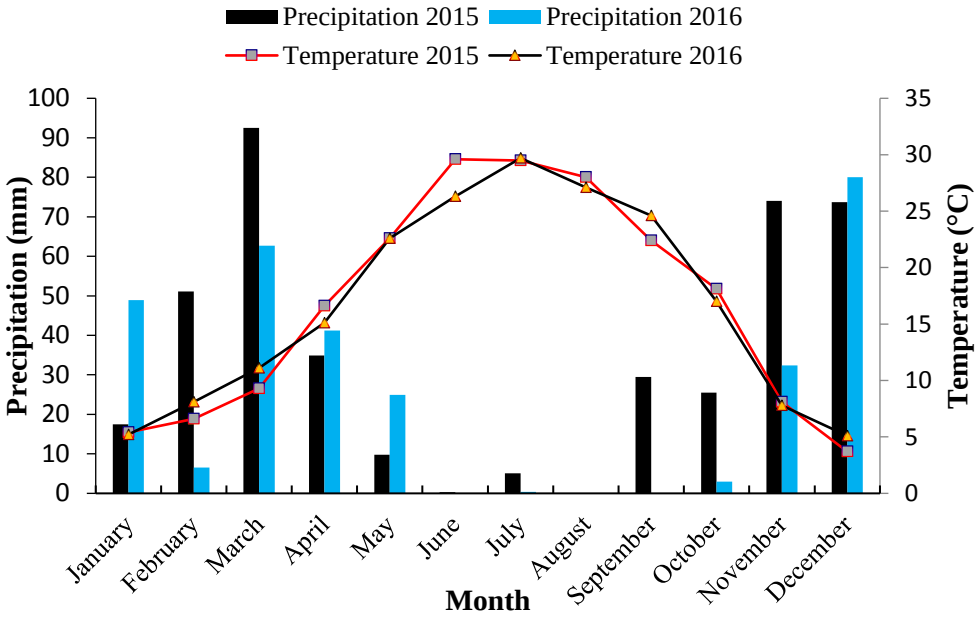


Fig. 1. The thermo-pluviometric diagram at the research field during 2015 and 2016.

2.2. Plant materials, experimental set up and treatments

The seeds (Item No. AA904) were obtained from Jelitto Perennial Seed Co., Hamburg, Germany. Seeds were planted in early February 2015 into the modules trays filled with a 1:1:2 mix of peat moss, coco peat and sand, and kept in a cold frame, which provides the right conditions of moisture (~ 65 %) and warmth (3-4 °C) that seeds need to germinate. Seed germination/emergence was commenced after 15 days of planting, and after field preparation the seedlings at four-leaf stage (8 weeks from planting) were transplanted in the end of March (when daily mean temperature was in the range of 15-20 °C) in experimental field which divided into several plots (each plot was 2.5 × 1.5 m long, and rows distance was 60 cm apart, and plant to plant distance was 40 cm in each row). The physical and chemical properties of the experimental field soil (0-30 cm in depth) before planting were shown in Table 1. After soil preparation and based on the chemical analysis of the soil, N, P, and K fertilizers was given at 40, 24, and 66.4 kg/ha basis to the soil.

The first study was performed in a factorial experiment based on randomized complete block design (RCBD) with three replications (n = 3) to observe the effect of different harvesting time and flower phenological stages on secondary metabolites of *A. chamissonis*. The plant flowers were harvested in early May, June and July from 3 plots in 4 different stages of flower development (A, stage 1: opening bud; D, stage 2: all radial florets or less than half of tubular florets opened; F, stage 3: all radial and tubular florets opened; G, stage 4: radial florets withering and seed formation) as shown in Fig. 2. The flowering pattern of *A. chamissonis* was

Table 1. The soil (0-30 cm in depth) physical and chemical characteristics of the experimental field.

Texture	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	N (%)	P (mg/kg)	K (mg/kg)	OC (%)	pH	EC (dS/m)
Sandy-loam	2.3	20.94	0.8	2.07	0.06	59.4	334.9	0.38	7.38	1.54

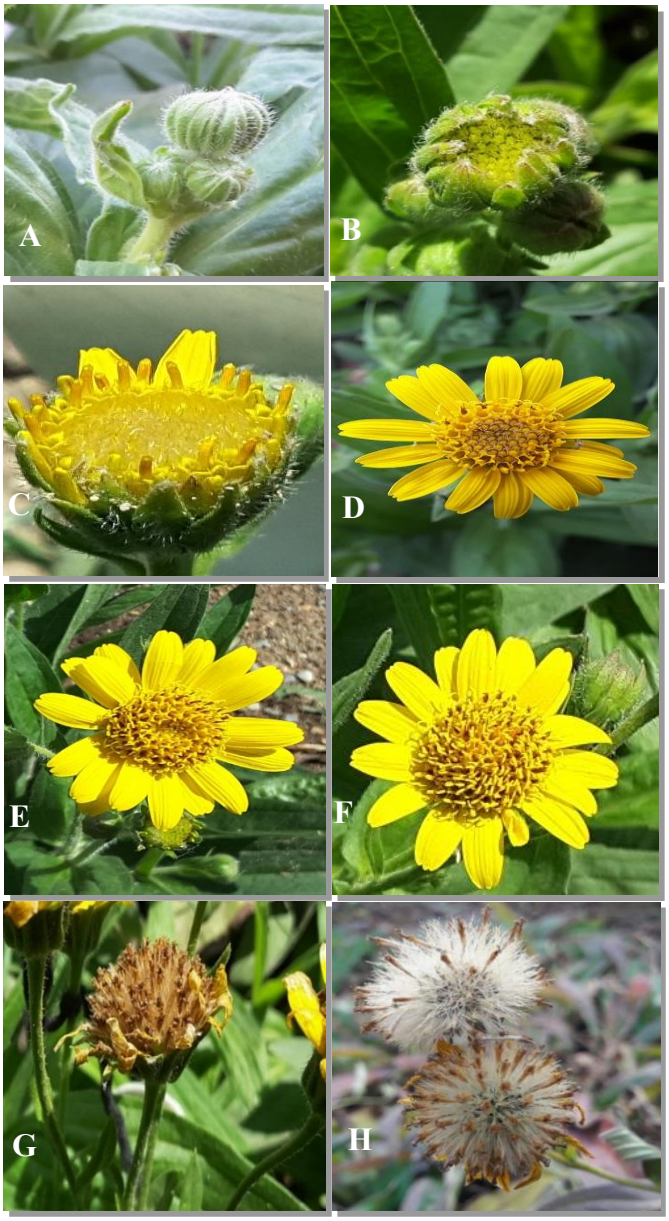


Fig. 2. Different developmental stages of flowers in *A. chamissonis*. Four stages (A, stage 1: opening bud; D, stage 2: all radial florets or less than half of tubular florets opened; F, stage 3: all radial and tubular florets opened; G, stage 4: radial florets withering and seed formation) were considered for metabolic analysis.

shown in Fig. 3A and B. As can be observed in the first year, 2015, the flowering process was completed only in one month (30 days of July), however, in the second year, 2016, the flowering period was lasted 65 days (in May, June and July). Therefore, the phenological stages of *A. chamissonis* flower of two-year old plants (in combination with harvesting time) were considered for further analysis. According to the results obtained from the first study, and also based on the pattern and length of flowering of two-year-old plants (Fig. 3B), the most abundant flowering and maximum flower head collections were observed in June harvesting compared to the other harvesting time. Therefore, the June collection of inflorescences under developmental stage of 3 was considered for drying method treatments.

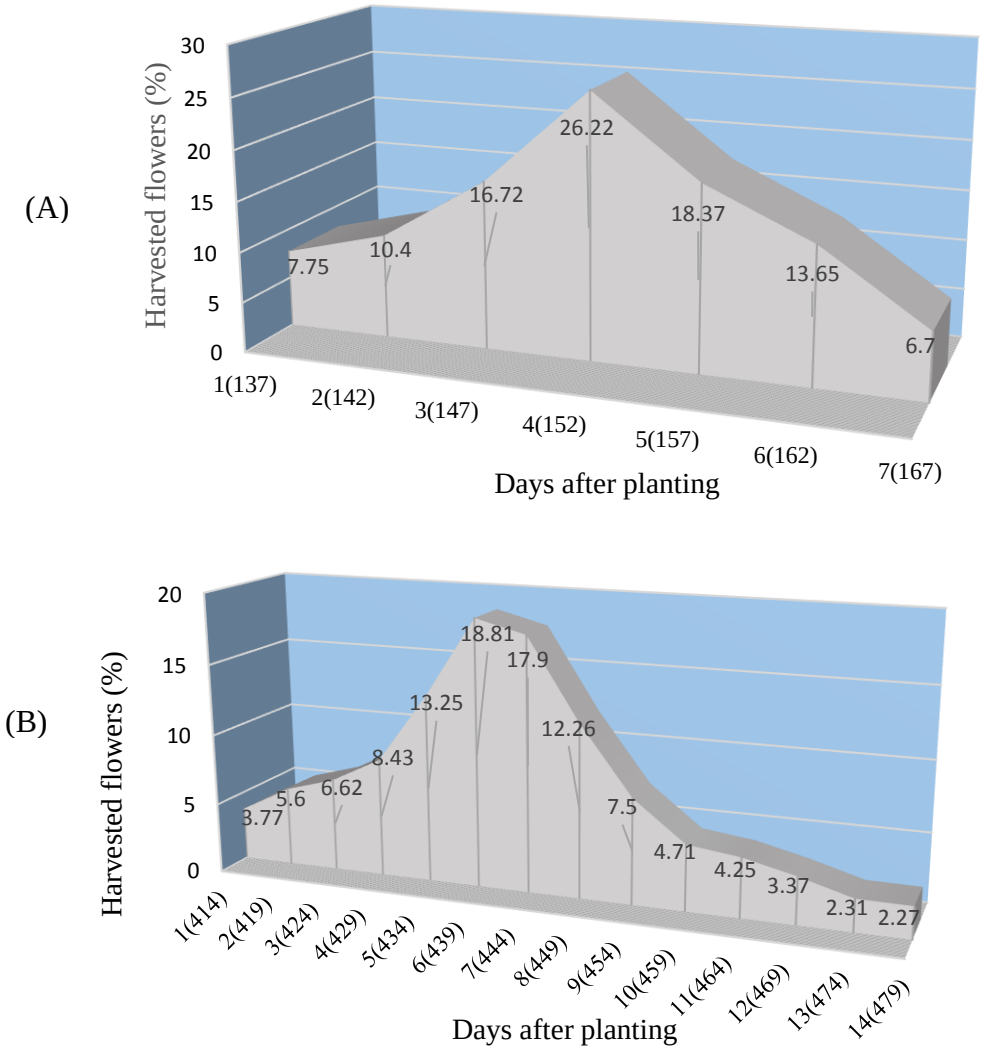


Fig. 3. The flowering pattern of *A. chamissonis* Less. in the first year (30 days of July) (A), and second year (May, June and July) (B) of growth cycle. Numbers outside and inside the brackets (parentheses) refer to the number of flower collection and days after planting, respectively.

2.3. Drying techniques

The initial moisture content of *A. chamissonis* Less. flower heads (harvested at June under stage 3) was measured by drying of samples (10 g) in a 105 °C oven for 24 h. The moisture content of plant materials was calculated based on fresh and dry weight using following equations:

Moisture content based on fresh weight = moisture weight / (moisture weight + dry weight)

Moisture content based on dry weight = moisture weight / dry weight

Then, the samples in each group were dried by the various methods/or treatments (1: shade drying inside the room at 25 °C without direct sunlight, 2: drying in sunlight and in open area, 3: oven drying at 40 and 50 °C using Memmart UF1060 laboratory oven, 4: microwave drying at 500 and 1000 W power output using a microwave oven (Panasonic Dimension 4 Microwave Oven/Combination Oven) which equipped to rotating tray and digital control of power and time was used to conduct the microwave treatment, in a completely randomized design with three replications. The flower samples (10 g) were distributed evenly on the tray in order to better absorb microwave radiations and a 10-s program was chosen to dry the samples. A digital scale was used to determine weight decrease ratio while drying and each sample was weighted in less than 15 s. The drying practices have been continued as long as the moisture content of the samples reached to 10 % based on fresh weight. Microwave samples were weighted each 10 s, while shade and sun dried samples were weighted every hour. Oven-dried samples were weighted each 30 min. The dried flowers through the aforesaid methods were extracted for further chemical analysis.

2.4. Phytochemical analyses

2.4.1. Extraction

Extraction was carried out from dried samples with a maximum moisture content of 10 %. Briefly, 10 ml of methanol was added to 200 mg of powdered flower samples and kept in an ultrasonic bath (PowerSonic 405, Hwashin Technology, South Korea) at room temperature for 30 min. After sonication, methanol extracts were centrifuged (Eppendorf R5702, 4000 rpm, 10 minutes) and filtered.

2.4.2. Determination of total phenols

The methanol extracts were used to measure the total phenols content of the samples by Folin-Ciocalteu reagent [23]. A calibration curve was generated using gallic acid at 50-1000 mg/L, and the results expressed as mg gallic acid equivalents per g of extract (mg GAE/g extract).

2.4.3. Quantification of total flavonoids

Total flavonoids content of methanol extracts was determined with the $AlCl_3$ colorimetric assay [24-25]. The calibration curve was plotted using standard rutin. Results were expressed as mg of rutin equivalents per g of extract (mg RUT/g extract).

2.4.4. Determination of flavonoid compounds by HPLC

The segregation and designation of rutin, apigenin and luteolin in methanol extract was carried out using HPLC. A Waters 2695 separation module equipped with a C_{18} column (150 mm × 4.6 mm, 3.5 μm, Waters) and an UV detector (Waters 2487) was used for this study. HPLC grade methanol along with 0.02 percent TFA (Mobile phase A) and distilled water along with 0.02 percent TFA (Mobile phase B) was used for peak segregation. Detection was carried out at 275 nm wavelength with a flow rate of

1.0 ml/min while the volume of each injection was 20 µl. Standards for apigenin and luteolin were obtained from Sigma-Aldrich Company. 0.2 mg of rutin, apigenin and luteolin standards were weighted separately and diluted in 1 ml of HPLC-grade methanol to obtain 200 ppm solutions. 3 concentrations (6-100 ppm) for plotting the standard curve were prepared from each sample and injected with 3 replications to the HPLC separation module.

2.5. Statistical analysis

Data were analyzed using SAS (version 9.1) software and MSTAT-C (Version 1.4). The means analysis was carried out using Duncan's Multiple Range Test (DMRT) and the statistical significant differences were determined at $P < 0.05$ or $P < 0.01$ levels. The principal component analysis (PCA) was used to assess possible relationships among the treatments using SPSS software (Version 10). Cluster analysis (UPGMA) using Euclidean distance and scatter plot diagram was created using PAST statistical software. Data were expressed as mean ± standard deviation.

3. Results

3.1. Harvest time

Analysis of variance (ANOVA) indicated that the harvest time and flower bud phenological stages and their interactions at 1 % probability level had a significant ($P < 0.01$) effect on accumulation of secondary metabolites such as total phenol, total flavonoid, rutin, luteolin and apigenin (Table 2).

Mean comparison of studied traits in *A. chamissonis* plants under different harvest time are given in Table 3. As shown, harvesting of *A. chamissonis* in the first date (May) caused lower total phenol (by 20.9 and 3.4 %) and flavonoid (by 20.7 and 16.1 %) contents compared to the other two harvesting dates, June and July, respectively. Results also showed that the effect of different harvesting date on changing the contents of rutin, luteolin and apigenin had a significant difference (Table 3).

Table 2. Analysis of variance (ANOVA) for the studied parameters in *A. chamissonis* Less. plants under different harvest time and flower bud phenological stages.

Source of variation (S.O.V)	df	Total phenol	Total flavonoid	Rutin	Luteolin	Apigenin
Block	2	0.76^{ns}	1.39^{ns}	0.23^{*}	0.0002^{ns}	0.001^{ns}
Harvest time (HT)	2	120.3^{**}	50.02^{**}	0.9^{**}	0.1^{**}	0.35^{**}
Phenological stage (PS)	3	437.52^{**}	69.08^{**}	55.93^{**}	0.008^{**}	3.64^{**}
HT×PS	6	63.97^{**}	32.04^{**}	5.95^{**}	0.23^{**}	0.21^{**}
Error	22	8.25	1.11	0.01	0.0005	0.001
CV (%)		11.84	6.09	5.4	5.2	3.47

*, **: Significantly different at the 5 and 1% probability level, respectively, ns: not significant.

Table 3. Mean comparison of studied traits in *A. chamissonis* Less. plants under different harvest time. Different letters in rows indicate significant differences based on DMRT ($n = 12$). The values are mean ± standard deviation (SD).

Secondar y metabolite (mg/g)	Harvest time		
	May	June	July
Total phenol	22.06 ± 1.14^{b}	27.89 ± 1.28^{a}	22.85 ± 1.08^{b}
Total flavonoid	15.03 ± 0.4^{c}	18.97 ± 0.5^{a}	17.93 ± 0.3^{b}
Rutin	5.68 ± 0.19^{a}	5.26 ± 0.12^{b}	5.16 ± 0.15^{b}
Luteolin	0.41 ± 0.05^{b}	0.41 ± 0.03^{b}	0.57 ± 0.06^{a}
Apigenin	1.0 ± 0.08^{a}	0.72 ± 0.04^{b}	1.03 ± 0.06^{a}

The maximum rutin content (5.68 mg/g) was recorded during the first harvest time, however, no statistically significant difference was found between second (June) and third (July) time of harvesting. Both luteolin and apigenin were significantly ($P < 0.01$, by 28 and 30 %) increased in third harvest date compared to the second date of harvesting (Table 3).

3.2. Interaction between harvest time and phenological stages of flower

The interaction results (Fig. 4) show that the highest (38.7 mg/g) and the lowest (11.4 mg/g) total phenols were obtained in the second and first harvest date in 2016 at flower maturity stages of 3 and 4, respectively. Generally, there was higher level of total phenols (26.7 mg/g) in the second harvest date at stage 2 than the other two dates at different phenological stages (Fig. 4). The different times of harvest at various flower bud phenological stages had significant effect on rutin, luteolin and apigenin contents (Fig. 4). The maximum and minimum rutin accumulation, 8.93 and 1.26 mg/g, was recorded for plants harvested in the first harvest date (*i.e.*, May) at flower maturity stages of 3 and 4, respectively. However, the highest luteolin and apigenin contents, 0.95 and 2.02 mg/g, were obtained in the third harvest time at stage 1.

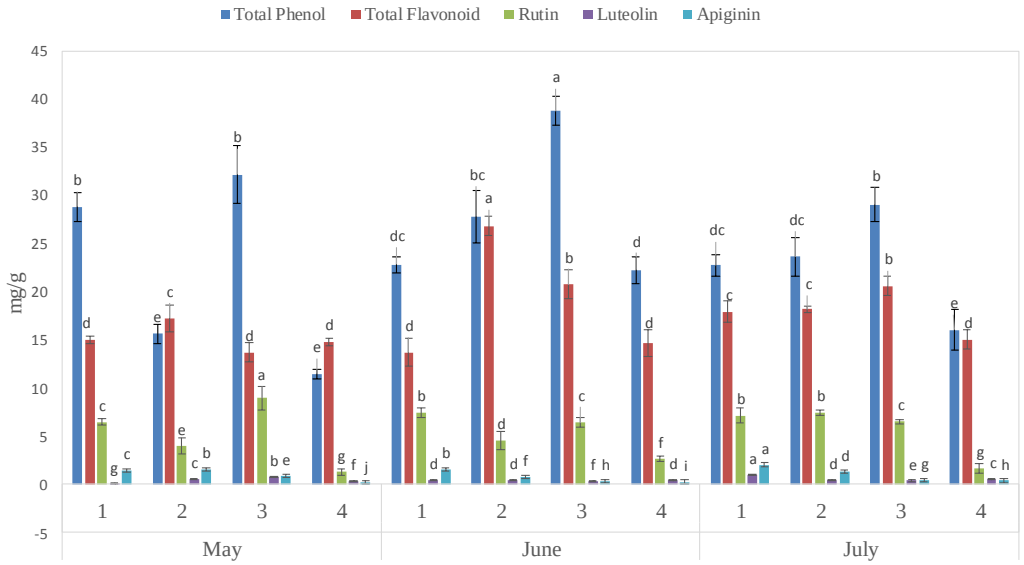


Fig. 4. Interaction of three different harvest time and four flower bud phenological stages (stage 1: opening bud; stage 2: all radial florets or less than half of tubular florets opened; stage 3: all radial and tubular florets opened; stage 4: radial florets withering and seed formation) on flower active compounds in *A. chamissonis* Less. The values are mean ± standard deviation (SD).

3.3. Drying methods

Analysis of variance (ANOVA) for the metabolic parameters in flower of *A. chamissonis* plants under different drying methods are given in Table 4. As shown, the secondary metabolite contents were significantly ($P < 0.01$) changed following drying procedures. In six drying methods, the flower heads treated by shade contained the highest (38.42 mg/g) total phenols content followed by oven drying at 40 °C

(Fig. 5A). Moreover, oven drying at temperature of 40 °C was the most favorable method for preserving of other main secondary metabolites including total flavonoid, rutin, luteolin and apigenin (Fig. 5B). Furthermore, results indicate that metabolites accumulation significantly decreased with the increase of the microwave power and oven temperature.

Table 4. Analysis of variance (ANOVA) for the studied parameters in *A. chamissonis* Less. plants under different drying methods.

Source of variation (S.O.V)	df	Total phenol	Total flavonoid	Rutin	Luteolin	Apigenin
Drying methods	5	330.8**	88.64**	55.31**	0.137**	0.6**
Error	12	5.27	9.39	2.56	0.001	0.006
CV (%)		9.2	13.63	11.33	5.28	7.49

** Significantly different at the 1% probability level.

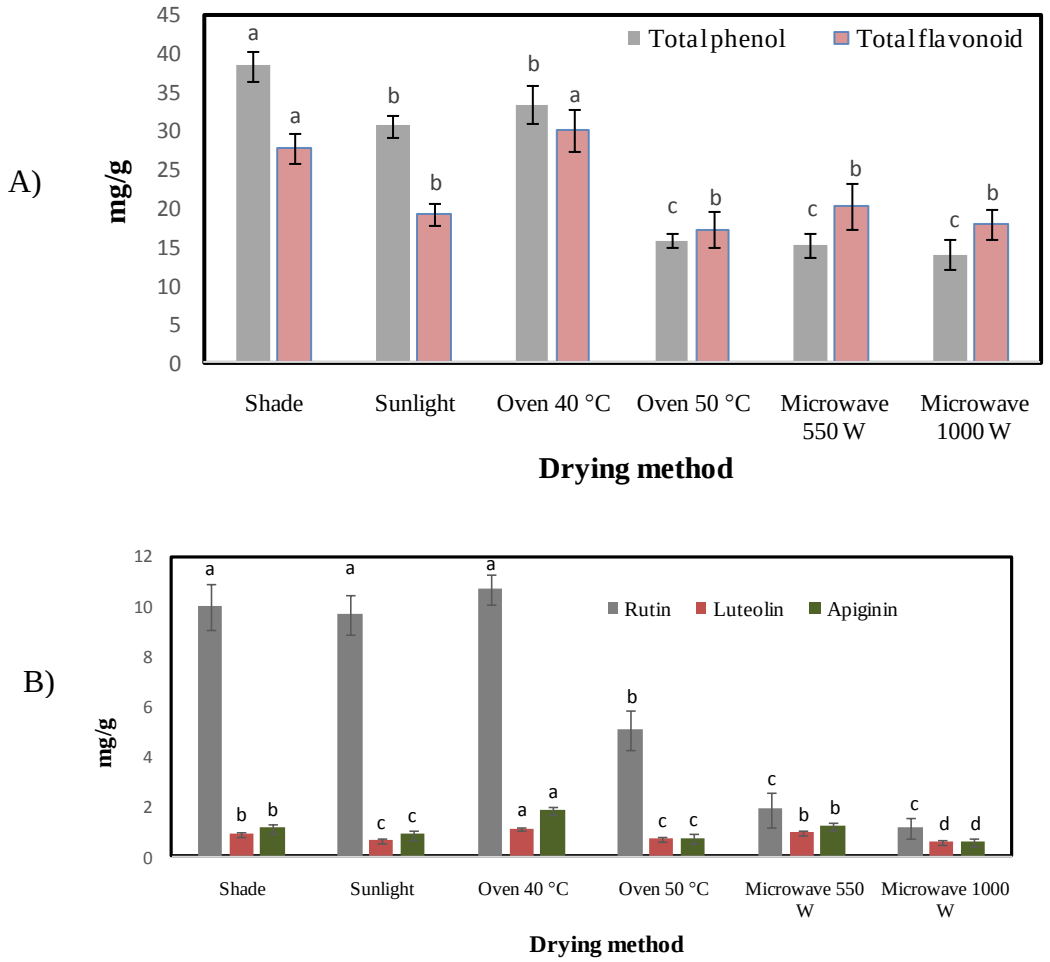


Fig. 5. Effect of different drying methods on total phenol and flavonoid (A), rutin, luteolin and apigenin (B) contents in *A. chamissonis* Less. flower. The values are mean ± standard deviation (SD).

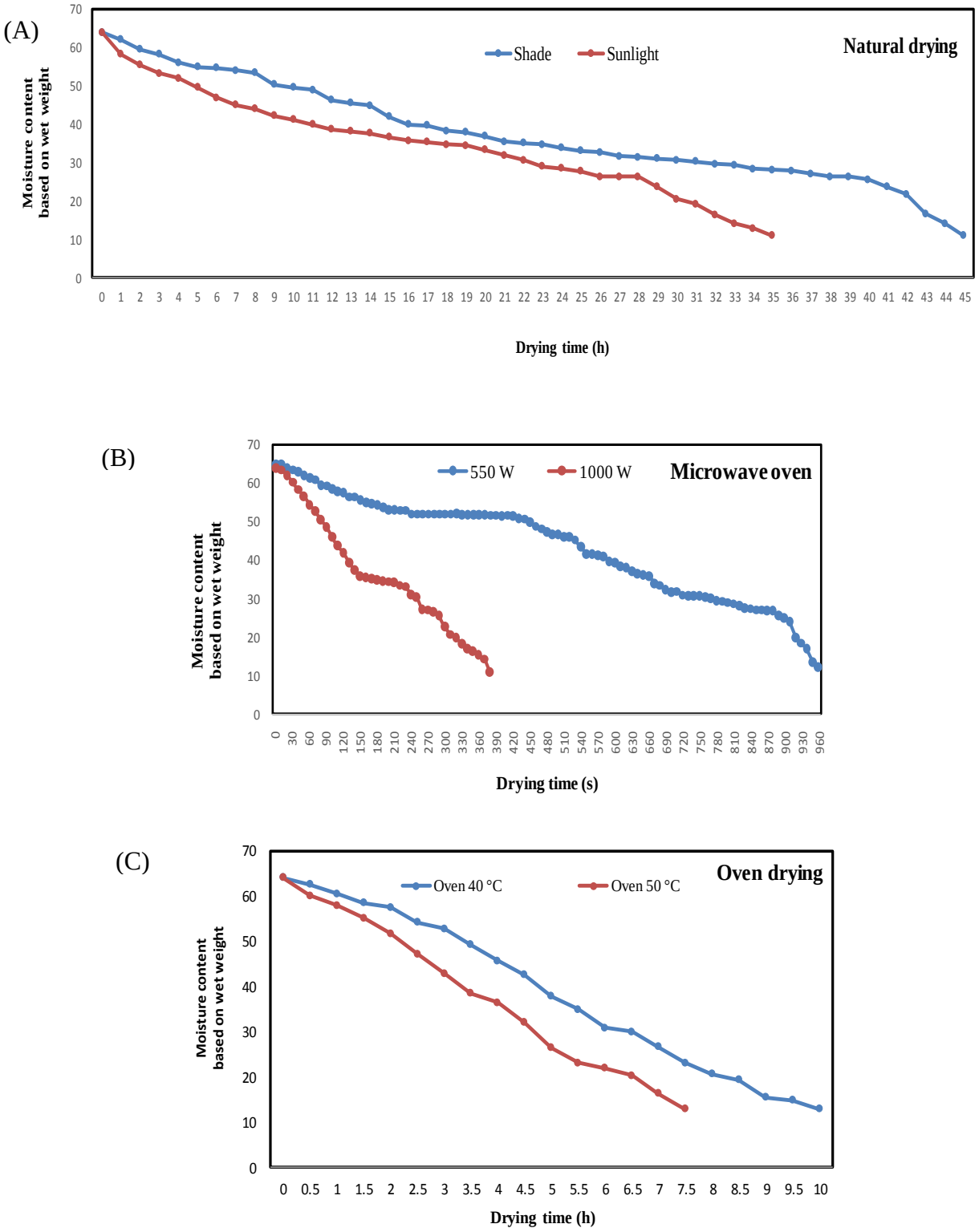


Fig. 6. The trend of time taken to reach moisture content from 64.1% to 10% based on fresh weight under employed drying methods.

The time taken to reach the moisture content from 64.1 % to 10 % based on fresh weight for the different drying methods was shown in Fig. 6. The time was longer for the natural drying treatments due to the fluctuating temperature during the drying period. The drying curves were obtained according to the variation of the sample moisture content as a function of time. Also, graphs of the moisture content ratio versus time curves for natural drying, microwave and oven drying are presented in Fig. 6A-C.

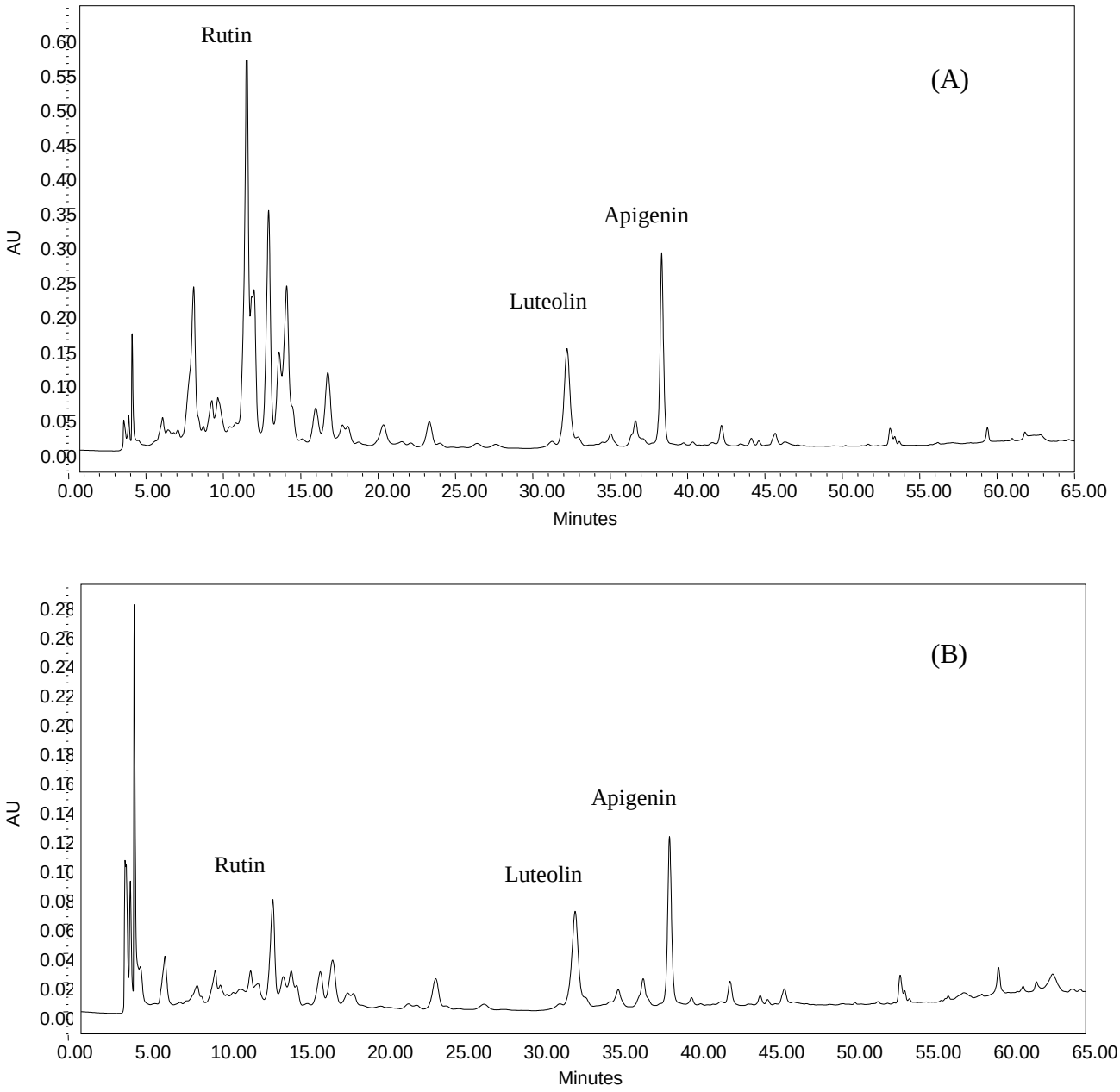


Fig. 7. HPLC chromatogram of oven-dried (40 °C) (A) and microwave-dried (1000 Watts) (B) in *A. chamissonis* Less.

In our study, the drying process with microwave reduced the moisture content of *Arnica* flowers up to 10% dry weight over a period of 6 to 16 min, which significantly reduced the phenol and flavonoids.

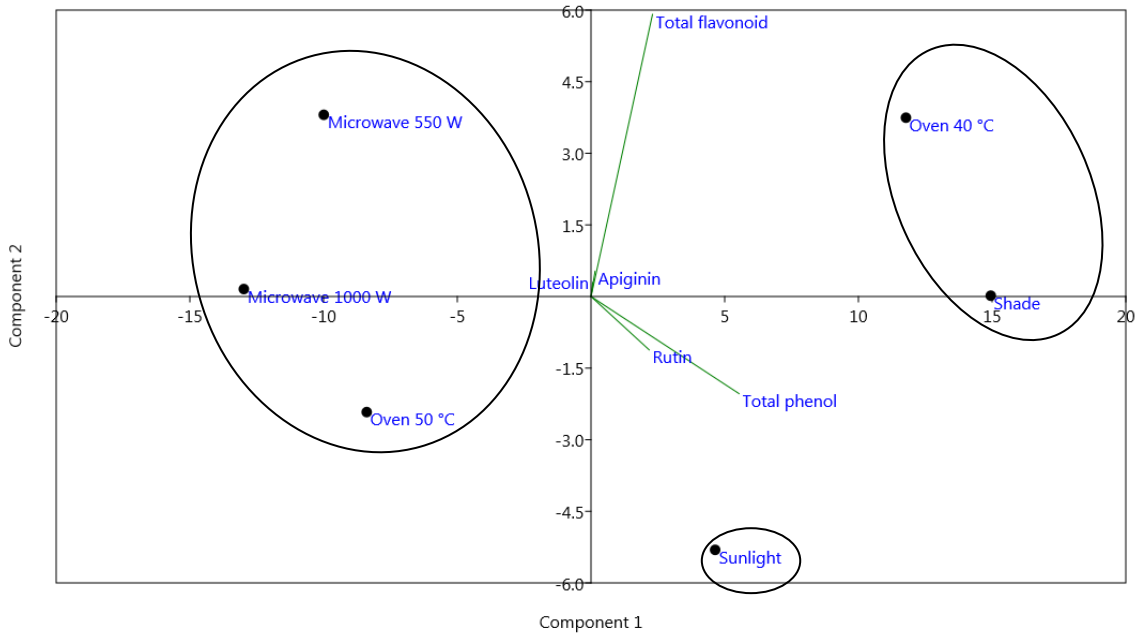
The HPLC chromatograms of two sample extract from experimental treatments, oven-dried (at 40 °C) (A) and microwave-dried (at 1000 W) (B), are shown in Fig. 7. As presented significant qualitative profiles of the phenol compounds from *A. chamissonis* was found using the different drying procedures. For example, rutin under oven drying at 40 °C showed higher peak area than microwave (at 1000 W) dried samples (*i.e.*, the oven drying of samples at 40 °C revealed significantly higher rutin content than that of microwave drying).

Multivariate statistical analysis was performed to understand treatment-variable relationships in *A. chamissonis* secondary metabolites content, presenting a clear separation among employed drying methods in terms of secondary metabolites content (Fig. 8). The differences between flavonoids metabolic profiles of the *A. chamissonis* Less. flower heads upon different drying methods were illustrated using Principal Component Analysis (PCA) and UPGMA techniques (Fig. 8A, B). Scatter plot analysis was carried out using PC1 and PC2, which explained 56.31% of the total variance (Fig. 8A). The treatments that were in close proximity to each other were more similar based on impressive characters in PC1 and PC2 and were classified in the same group.

The PCA plot demonstrated three district groups/or clusters based on the metabolites, and showed that drying treatments manipulated the distribution of metabolites in *A. chamissonis* flower heads. Cluster 1 included the oven drying at 50 °C and microwave drying at 550 and 1000 W, and cluster 2 contained drying at sunlight, however, cluster 3 comprised of all the other drying methods such as oven drying at 50 °C and shade treatments, mainly due to higher content of measured metabolites. UPGMA cluster analysis was also prepared using the Euclidean distance coefficient and the average linkage method based on all the studied traits to estimate the relationship between the employed treatments. The UPGMA analysis showed three main clusters (Fig. 8B). The second cluster included sunlight treatment only; however, the first and the third main clusters were divided into three and two sub-clusters, respectively. The results of biplot analysis (Fig. 8A) supported the findings of UPGMA cluster analysis (Fig. 8B).

4. Discussion

Changes in active compounds during different dates of the year and even different hours in a day emphasize the importance of harvesting time. Harvesting time of plant raw materials is selected as a key factor for obtaining the maximum level of active compounds in plants, however, it should be considered that the quality and quantity of these compounds are not constant and may be altered during the plant growth cycle [26]. Harvesting at an improper time not only decreases yield, but also causes decline in quality as well, since the yield of each plant part and also the amount of secondary metabolites significantly changes under different phenological stages [26, 27]. Therefore, optimization of harvesting time and the right time of harvesting may be of great importance from an agronomic and commercial point of view. In 2015 cropping year, the flowering of *A. chamissonis* lasted for 30 days (in July) and in this period, seven collections of inflorescences were made while in 2016 (*i.e.*, two-year-old plant) the flowering continued for 65 days (from mid-May to mid-July), and the flower head of *A. chamissonis* were collected



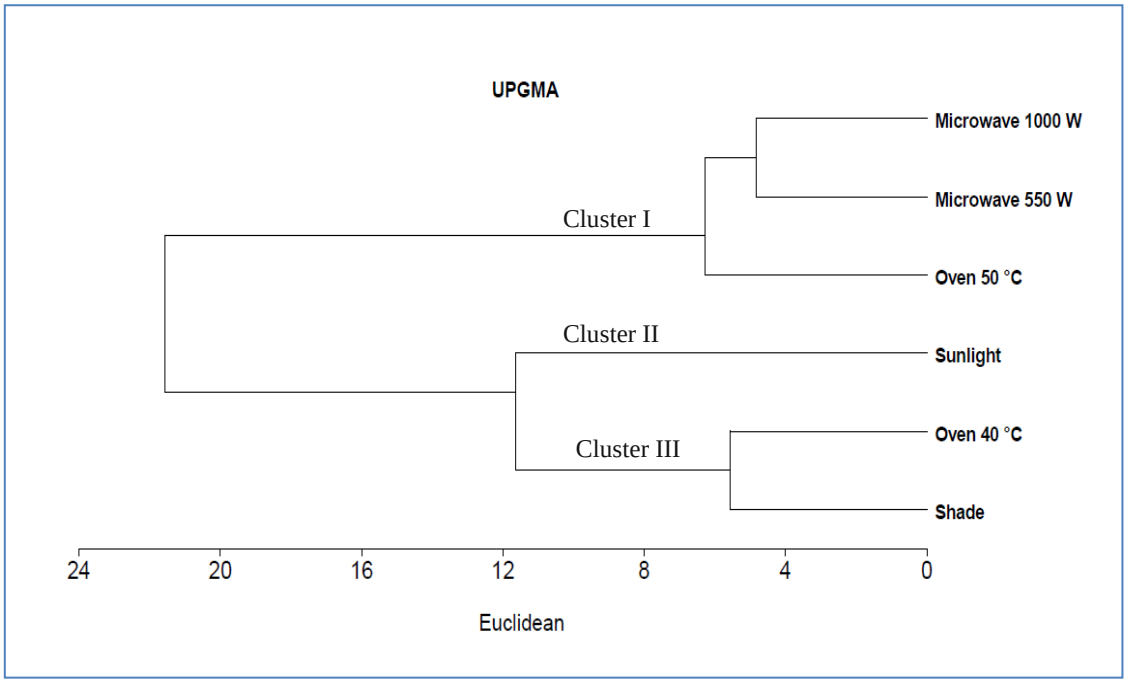


Fig. 8. (A) Biplot of the first two principal components (PC1 and PC2), and (B) dendrogram of cluster analysis for the different drying methods (shade and sun drying, oven drying at 40 and 50 °C, and microwave drying at 500 and 1000 W power) based on the measured secondary metabolites (total phenols, flavonoids, rutin, luteolin and apigenin contents).

fourteen times and the maximum abundant flowering was observed in the middle period of May-July (*i.e*., June). It may be due to the fluctuations of weather parameters such as temperature, rainfall, day length, and humidity during different years. In this case, the harvesting time should be determined based on the combination of quality and quantity of product, from an economic point of view. In a study on various stages of flower heads development in *Achillea millefolium* different coloration of isolated essential oils was observed regarding the developmental stages [28]. The obtained essential oils were blue, blue-green, brown-yellow and light-yellowish-brown in closed flower heads, young opened flower heads, fully opened flower heads, and in air-dried flower heads, respectively. Changes in oil coloration reflected the reduction in the content of chamazulene with the flower maturity [28]. The influence of various phenological stages of flower development on the quantitative and qualitative profile of volatile oils of two *Arnica* species, *A. montana* L. and *A. chamissonis* Less. were previously investigated by Kowalski *et al.* (2015) in Eastern Poland [17]. They reported that the maximum content of volatile oils in the inflorescence of the two studied species was observed in the full flowering stage, when ligulate florets were completely opened, and up to half of the disc florets (tubular) were opened. Changes in the content of volatile oils were recorded between two studied species only in the phase of yellow buds, whereas in the other developmental stages of flower head the volatile oil contents were similar.

Fluctuation of temperature and light seem to be the most important factor in the observed variations of metabolite contents. According to the results of this research, the best time to harvest of *A. chamissonis* flowers is proposed in the late June and early July at the stage of opening flower tubers to full maturity phase. The highest content of phenol in *Boerhavia diffusa* and *Sida cordifolia* was observed in a study at the full flowering stage, which was consistent with the results of our study. Increasing the content of phenol and flavonoid at the full flowering stage can be related to the role of both biotic and abiotic components of specific environment, increased protection against pathogens and the attraction of pollinators [29].

In this study, the minimum drying time was observed in 1000 W (6 ± 0.68 min) microwave treatment and the maximum drying time was observed in shade treatment (45 ± 0.64 h). This is could be due to the larger value of driving force for heat and moisture transfer [30]. The moisture content of the sample was very high during the beginning of the drying phase which leads to the high drying rates because of the higher moisture diffusion characteristic [31]. Although, the microwave drying reveals distinct benefits such as energy absorption equivalent to the residual moisture content and less drying time, it is the lack of temperature control [32-33].

According to the results of this study, the microwave and oven methods significantly reduced the drying time and increased the drying rate compared to the natural method. On the other hand, the drying time in microwave was much shorter than the oven method. Therefore, the power and temperature on the drying rate had a definite effect. Similar results have been reported by other researchers [34-36]. In a study on different drying methods of chamomile flowers the minimum (7-104 min according to the desired power) and the maximum (120 h) drying time was obtained in microwave and shadow procedures, respectively [37]. In another study on drying of parsley leaves, the time to reach 10% moisture content (based on dry weight) was

observed in microwave (900 W power) compared to 30, 40, 50 and 65 °C of oven drying, which reduced the drying time as much as 111, 92, 37, and 31 times, respectively [38].

However, our results are in contrast with a previous study performed by Gulati *et al*. [39] on optimization of drying methods in green tea (*Camellia sinensis*), who reported that medium and high power (500 to 800 Watts) microwave treatment had the best possible preservation of phenolic compounds and flavonoids. Drying with different temperatures (30, 40, and 50 °C) in *Plantago major* had a significant effect on phenol content, and the increase in drying temperature reduced the concentration of biological compounds [40]. Also, comparison of different drying methods such as air drying, freeze drying vacuum drying and oven drying in six species of the Lamiaceae family showed that the air-dried samples had significantly higher phenol compounds, rosmarinic acid and antioxidant capacity than the other two ways [41]. Similar results have also been reported in dry and shade method in *Dracocephalum moldavica* in relation to antioxidant activity relative to different oven temperatures [42]. The difference in the amount of polyphenols in various drying treatments may be due to samples exposure time to the temperature [43]. High temperatures result in the release of phenol compounds and the loss of phenol compounds attributed to the destruction of phenolic chains and cell walls [43].

The current study showed that the secondary metabolite contents in *A. chamissonis* decreased by increasing oven temperature. This result was in accordance with other study on different medicinal and aromatic plants such as peppermint and dill [44] tarragon [45] and sage [46]. It has been acknowledged that higher drying temperature may damage to the glandular trichomes in medicinal and aromatic plants [47-48]. Furthermore, increased drying temperature causes more degradation of the cell wall and plasma membrane structure [49]. Among the different drying techniques used in this work, it seems that most of the losses in secondary metabolites occurred under oven (at 50 °C) and microwave (at 500 and 1000 W) treatments. On the other hand, the content of total phenol and total flavonoid, rutin, luteolin and apigenin under microwave drying (especially at 1000 W) showed substantial losses in most of compounds which may be due to expansion of the structure of the epidermis of plants [50-51]. Rapid diffusion of microwave radiation in plant materials causes an increase in absorption of microwave energy through water molecules. Therefore, water rapidly evaporated and average drying rates accelerated. Also, it has been acknowledged that the microwave radiation besides energy conservation leads to a decreased drying times without rising surface temperature of the materials [52]. Thus, the microwave radiation treatment does not destroy the outer surface of plant materials and therefore enhance the surface feature of herbs [53]. According to a study, the employed procedures of thermal drying (*i.e.*, oven, sun-drying and microwave) caused sharp decline in the value of total phenolic compounds in dried leaves of shell ginger (*Alpinia zerumbet*), torch ginger (*Etlingera elatior*), turmeric (*Curcuma longa*), and kencur *(Kaempferia galanga*) [54]. It has been reported that the drying processes cause high/and or low levels of total phenol compounds based on the type of phenol compounds exist in the herbal material and their location in the plant cell [55].

In case of microwave drying method, literature data exhibited inconstant findings ranging from the decline to the increase of total phenol compounds based on plant material types.

However, a sharp decline in the total phenol compounds of *Enicostemma littorale* Blume was observed following microwave drying procedure [21]. Also, a higher decrease in the total phenol compounds of *Phyllanthus amarus* was observed under microwave drying treatment than that of hot-air drying [56]. However, it has been reported that drying with microwaves (450 W) remarkably enhanced the extraction of phenolic compounds of Citrus peel [57].

It has been acknowledged that different drying led to decrease or loss of some chemical compositions, because of differences in characteristics, mechanism and conditions of drying [58]. In experiments conducted with different methods of drying, and checking the amount of rosmarinic acid in *Melissa officinalis* rosmarinic degradation was observed at high temperatures of oven, and a temperature range of 40 °C was considered to maintain rosmarinic acid for drying this plant [59]. It has been acknowledged that the changes of chlorogenic acid in chrysanthemum (mums or chrysanths) flower heads significantly decreased with the increase of oven temperature (55-75 °C) [60].

5. Conclusion

In the first experiment, it has been observed that the different harvesting time at various flower bud phenological stages had significant effect on total phenols, flavonoids, rutin, luteolin and apigenin contents. The maximum and minimum rutin accumulation was recorded for plants collected in the first harvest date at flower maturity stages of 3 and 4, respectively. However, the highest luteolin and apigenin contents were obtained in the third harvest time at stage 1. In the second experiment, drying procedures had also significant effect on chemical metabolites of *Arnica* flowers. Drying methods cause a decrease/and or an increase in chemical components. In general, drying by oven 40 °C possessed the higher total flavonoid, rutin, luteolin and apigenin content, while microwave drying (at 1000 W) showed the lowest chemical composition value. Since the oven drying method reduces the amount of time required to dry *Arnica* flowers compared to the natural way and increases the drying rate, it is a suitable method for drying *Arnica* flowers. The shade drying method is recommended for maintaining the total phenol content as the best method. However, according to the results of this study, it can be concluded that oven drying at 40°C is very desirable to dry *Arnica* flowers because it will preserve phenol and flavonoids, as well as rutin, luteolin and a significant amount of apigenin.

Author contributions

M. Asadi performed the experiment and contributed in data collection. J. Hadian supervised the research. M. Hatami wrote the manuscript and edited the text. S.N. Ebrahimi advised the research. All authors read and approved the final version of manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

The authors thank the Research Council of Shahid Beheshti University, Tehran, Iran for their financial support. We also wish to thank Mr. Hamid Ahadi for their kind help in extraction and HPLC analysis. Collaboration of Tochal Pharma Co. is kindly appreciated.

References

1. Maguire B. A monograph of the genus Arnica. *Brittonia.* 1943; 4: 386-510.

2. Gawlik-Dziki U, Swieca M, Sugier D and Cichocka J. Seeds of *Arnica montana* and *Arnica chamissonis* as a potential source of natural antioxidants. *Herba. Pol.* 2009; 55: 60-71.

3. Ganzera M, Egger C, Zidorn C and Stuppner H. Quantitative analysis of flavonoids and phenolic acids in *Arnica montana* L. by micellar electrokinetic capillary chromatography. *Anal. Chim. Acta.* 2008; 614: 196-200.

4. Gawlik-Dziki U, Swieca M, Sugier D and Cichocka J. Comparison of *in vitro* lipoxygenase, xanthine oxidase inhibitory and antioxidant activity of *Arnica montana* and *Arnica chamissonis* tinctures. *Acta Sci. Pol-Hortoru*. 2011; 10: 15-27.

5. Sugier D. Essential Oil from *Arnica montana* L. Achenes: Chemical Characteristics and Anticancer Activity. *Molecules* 2019; 24: 41-58.

6. Sugier D and Gawlik-Dziki U. The influence of foliar fertilization on yielding and quality of mountain Arnica (*Arnica montana* L.) and chamisso Arnica (*Arnica chamissonis* var. foliosa). *Ann Univ Mariae Curie Sklodowska. Sectio E, Agricultura* 2009; 64: 129-139.

7. Gaspar A, Craciunescu O, Trif M, Moisei M and Moldovan L. Antioxidant and anti-inflammatory properties of active compounds from *Arnica Montana* L. *Rom. Biotechnol. Lett.* 2014; 19: 9353-9365.

8. Cassell AC, Walsh C, Belin M, Cambornac M, Rohit JR and Lubrano C. Establishment of plantation from micropropagated *Arnica chamissonsis* a phar-maceutical substitute for the endangered *A. montana*. *Plant Cell, Tissue Organ Cult.* 1999; 156: 139-144.

9. Nichterlein K. *Arnica montana* (Mountain Arnica): in vitro culture and the production of sesquiterpene lactones and other secondary metabolites. In Medicinal and Aromatic Plants VIII. 1995; pp. 47-61. Springer, Berlin, Heidelberg.

10. Nowack T. Lowland arnica-culivation in gostyńskoleszczyński region. *Wydawnictwa Polski Komitet Zielarski* 2002; 1: 18-19.

11. Albert A, Sareedenchai V, Heller W, Seidlitz HK and Zidorn C. Temperature is the key to altitudinal variation of phenolics in *Arnica montana* L. cv. ARBO. *Oecologia* 2009; 160: 1-8.

12. Cornu C, Joseph P, Gaillard S, Bauer C, Vedrinne C, Bissery A, Melot G, ossard N, Belon P and Lehot J. No effect of a homoeopathic combination of *Arnica montana* and *Bryonia alba* on bleeding, inflammation, and ischaemia after aortic valve surgery. *Br. J. Clin. Pharmacol.* 2010; 69: 136-142.

13. Pljevljakušić D, Janković T, Jelačić S, Novaković M, Menković N, Beatović D and Dajić-Stevanović Z. Morphological and chemical characterization of *Arnica montana* L. under different cultivation models. *Ind. Crops. Prod.* 2014; 52: 233-244.

14. Sugier D, Sugier P and Gawlik-Dziki U. Propagation and introduction of *Arnica montana* L. into cultivation: a step to reduce the pressure on endangered and high-valued medicinal plant species. *Sci. World J.* Article ID 414363, 2013; 1-11.

15. Pljevljakušić D, Rančić D, Ristić M, Vujisić L, Radanović D and Dajić-Stevanović Z.

Rhizome and root yield of the cultivated *Arnica montana* L., chemical composition and histochemical localization of essential oil. *Ind. Crops. Prod.* 2012; 39: 177-189.

16. Sugier D, Kołodziej B and Bielińska E. The effect of leonardite application on *Arnica montana* L. yielding and chosen chemical properties and enzymatic activity of the soil. *J. Geochem. Explor*. 2013; 129: 76-81.

17. Kowalski R, Sugier D, Sugier P and Kołodziej B. Evaluation of the chemical composition of essential oils with respect to the maturity of flower heads of *Arnica montana* L. and *Arnica chamissonis* Less. cultivated for industry. *Ind. Crops. Prod.* 2015; 76: 857-865.

18. Kumar TS, Shanmugam S, Palvannan T and Kumar VM. Evaluation of antioxidant properties of *Canthium parviflorum* Lam. leaves. *Nat. Prod. Rad.* 2008; 7: 122-126.

19. Douglas JA, Smallfield BM, Burgess EJ, Perry NB, Anderson RE, Douglas MH and Anne Glennie V. Sesquiterpene lactones in *Arnica montana*: a rapid analytical method and the effects of flower maturity and simulated mechanical harvesting on quality and yield. *Planta Med.* 2004; 70: 166-170.

20. Yuan G, Hong L, Li X, Xu L, Tang W and Wang Z. Experimental investigation of a solar dryer system for drying carpet. *Energy Procedia*. 2015; 70: 626-633.

21. Sathishkumar R, Lakshmi PTV and Annamalai A. Effect of drying treatment on the content of antioxidants in *Enicostemma littorale* Blume. *Res. J. Medicinal Plant.* 2009; 3: 93-101.

22. Capecka E, Mareczek A and Leja M. Antioxidant activity of fresh and dry herbs of some Lamiaceae species. *Food chem.* 2005; 93: 223-226.

23. Zhou K and Yu L. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. *LWT-Food Sci. Technol.* 2006; 39:1155-1162.

24. Pallab K, Tapan BK, Tapas PK and Ramen K. Estimation of total flavonoids content (tfc) and antioxidant activities of methanol whole plant extract of *Biophytum sensitivum* Linn. *J. Drug Deliv. Ther.* 2013; 3: 33-37.

25. Patel R, Patel A, Desai S and Nagee A. Study of secondary metabolites and antioxidant properties of leaves, stem and root among *Hibiscus rosa-sinensis* cultivars. *Asian J. Exp. Biol. Sci.* 2012; 3: 719-725.

26. Figueiredo AC, Barroso JG. Pedro LG and Scheffer JJ. Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour. Frag. J.* 2008; 23: 213-226.

27. Omidbeigi R. Production and manufacturing the herbs. Beh-nashr Publication, Mashhad, 2005, 1: 347.

28. Figueiredo AC, Barroso JG, Pais MSS and Scheffer JJ. Composition of the essential oils from leaves and flowers of *Achillea millefolium* L. ssp. millefolium. *Flavour. Frag. J.* 1992; 7: 219-222.

29. Langenheim JH. Higher plant terpenoids: a phytocentric overview of their ecological roles. *J. Chem. Ecol.* 1994; 20: 1223-1280.

30. Methakhup S, Chiewchan N and Devahastin S. Effects of drying methods and conditions on drying kinetics and quality of Indian gooseberry flake. *LWT-Food Sci. Technol.* 2005; 38: 579-587.

31. Sharma GP, Verma RC and Pathare PB. Thin-layer infrared radiation drying of onion slices. *J. Food Eng.* 2005; 67: 361-366.

32. Feng H. Analysis of microwave assisted fluidized-bed drying of particulate product with a simplified heat and mass transfer model. *Int. Commun. Heat Mass Transf.* 2002; 29: 1021-1028.

33. Walde SG, Velu V, Jyothirmayi T and Math RG. Effects of pretreatments and drying methods on dehydration of mushroom. *J. Food Eng.* 2006; 74: 108-115.

34. Azizi M, Rahmati MM, Ebadi TM and Hassanzadeh-Khayyat M. The effects of different drying methods on weight loss rate, essential oil and chamazulene contents of chamomile (*Matricaria recutita*) flowers. *Iran. J. Med. Aromat. Plants* 2009; 25: 182-192.

35. Funebo T, Ohlsson, T. Microwave-assisted air dehydration of apple and mushroom. *J. Food Engineering* 1998; 38: 353-367.

36. Soysal Y. Microwave drying characteristics of parsley. *Biosyst. Eng.* 2004; 89: 167-173.

37. Rahmati M, Azizi M and Hasanzadeh Khayat M. Study on the effects of different drying methods on weight loss rate, essential oil and chamazolene contents of chamomile (*Matricaria recutita* CV. Germania (Diploid)) flowers. *J. Hort. Sci.* 2010; 24: 129-137.

38. Parker JC. Developing aherb and spice industry in Callide Valley, Qeensland. A Report for the Rural Industries Research and Development Corporation (RIRDC), RIRDC 1999; Publication No: 99/45, RIRDC Project No: DAQ-194A.

39. Gulati A, Rawat R, Singh B, Ravindranath SD. Application of microwave energy in the manufacture of enhanced-quality green tea. *J. Agric. Food Chem.* 2003; 51: 4764-4768.

40. Zubair M, Nybom H, Lindholm C and Rumpunen K. Major polyphenols in aerial organs of greater plantain (*Plantago major* L.), and effects of drying temperature on polyphenol contents in the leaves. *Sci. Hortic.* 2011; 128: 523-529.

41. Hossain MB, Barry-Ryan C, Martin-Diana AB and Brunton NP. Effect of drying method on the antioxidant capacity of six Lamiaceae herbs. *Food Chem.* 2010; 123: 85-91.

42. Mohtashemi S, Babalar M, Mirjalili MH, Ebrahimzadeh Moosavi M and Adib J. Effects of different drying methods on drying rate, essential oil content and antioxidant activity of *Dracocephalum moldavica* L. 2010; In: Proceedings of National Young Researchers Congress of Biology, 13th-17th February, Tehran, Iran. (In Persian).

43. Heras-Ramírez ME, Quintero-Ramos A, Camacho-Dávila AA, Barnard J, Talamás-Abbud R, Torres-Muñoz JV and Salas-Muñoz E. Effect of blanching and drying temperature on polyphenolic compound stability and antioxidant capacity of apple pomace. *Food Bioproc. Tech.* 2012; 5: 2201-2210.

44. Ayyobi H, Peyvast GA, and Olfati JA. Effect of drying methods on essential oil yield, total phenol content and antioxidant capacity of peppermint and dill. *Ratarstvo i Povrtarstvo* 2014; 51: 18-22.

45. Arabhosseini A, Padhye S, van Beek TA, van Boxtel AJ, Huisman W, Posthumus MA, and Müller J. Loss of essential oil of tarragon (*Artemisia dracunculus* L.) due to drying. *J. Sci. Food Agric.* 2006; 86: 2543-2550.

46. Hamrouni-Sellami I, Rahali FZ, Rebey IB, Bourgou S, Limam F and Marzouk B. Total phenolics, flavonoids, and antioxidant activity of sage (*Salvia officinalis* L.) plants as affected by

different drying methods. *Food Bioproc. Tech.* 2013; 6: 806-817.

47. Díaz-Maroto MC, Pérez-Coello MS, Gonzalez Vinas MA and Cabezudo MD. Influence of drying on the flavor quality of spearmint (*Mentha spicata* L.). *J. Agric. Food Chem.* 2003; 51: 1265-1269.

48. Yousif AN, Durance TD, Scaman CH and Girard B. Headspace volatiles and physical characteristics of vacuum-microwave, air, and freeze-dried oregano (*Lippiaber landieri* Schauer). *J. Food Sci.* 2000; 65: 926-930.

49. Lewicki PP, Pawlak G. Effect of drying on microstructure of plant tissue. *Dry. Technol.* 2003; 21: 657-683.

50. Yousif AN, Scaman CH, Durance TD, Girard B. Flavor volatiles and physical properties of vacuum-microwave-and air-dried sweet basil (*Ocimum basilicum* L.). *J. Agric. Food Chem.* 1999; 47: 4777-4781.

51. Lin TM, Durance TD and Scaman CH. Characterization of vacuum microwave, air and freeze dried carrot slices. *Food Res. Int.* 1998; 31: 111-117.

52. McLoughlin CM, McMinn WAM and Magee, TRA. Microwave-vacuum drying of pharmaceutical powders. *Dry. Technol.* 2003; 21: 1719-1733.

53. Szumny A, Figiel A, Gutiérrez-Ortíz A and Carbonell-Barrachina AA. Composition of rosemary essential oil (*Rosmarinus officinalis*) as affected by drying method. *J. Food Eng.* 2010; 97: 253-260.

54. Chan EWC, Lim YY, Wong SK, Lim KK, Tan SP, Lianto FS and Yong MY. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chem.* 2009; 113: 166-172.

55. Spitaler R, Winkler A, Lins I, Yanar S, Stuppner H and Zidorn C. Altitudinal variation of phenolic contents in flowering heads of *Arnica montana* cv. ARBO: a 3-year comparison. *J. Chem. Ecol.* 2008; 34: 369-375.

56. Lim YY and Murtijaya J. Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *LWT-Food Sci. Technol.* 2007; 40: 1664-1669.

57. Kammoun Bejar A, Kechaou N, Boudhrioua and Mihoubi N. Effect of microwave treatment on physical and functional properties of orange (*Citrus sinensis*) peel and leaves. *Int. J. Food Process. Technol.* 2011; 2: 109-116.

58. Guo YR, An YM, Jia YX and Xu JG. Effect of Drying Methods on Chemical Composition and Biological Activity of Essential Oil from Cumin (*Cuminum cyminum* L.). *J. Essent. Oil-Bear. Plants.* 2018; 21: 1295-1302.

59. Argyropoulos D, Heindl A. and Müller J. Assessment of convection, hot-air combined with microwave-vacuum and freeze-drying methods for mushrooms with regard to product quality. *Int. J. Food Sci. Technol.* 2011; 46: 333-342.

60. Shi XF, Chu JZ, Zhang YF, Liu CQ and Yao XQ. Nutritional and active ingredients of medicinal chrysanthemum flower heads affected by different drying methods. *Ind. Crops. Prod.* 2017; 104: 45-51.

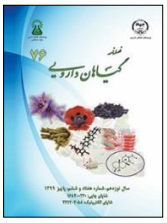
How to cite this article: Asadi M, Nejad Ebrahimi S, Hatami M, Hadian J. Changes in Secondary Metabolite Contents of *Arnica chamissonis* Less. in response to different harvest time, flower developmental stages and drying methods. ***Journal of Medicinal Plants*** 2020; 19(76): 69-88.
doi: 10.29252/jmp.19.76.69

فصلنامه گیاهان دارویی ۱۳۹۹؛ ۱۹(۷۶): ۶۹-۸۸

فصلنامه گیاهان دارویی

Journal homepage: www.jmp.ir

پژوهشکده گیاهان دارویی
جهاد دانشگاهی



مقاله تحقیقاتی

تغییر محتوای متابولیت‌های ثانویه گیاه آرنیکا (*Arnica chamissonis* Less.) در اثر زمان‌های مختلف کاشت، مراحل نموی گل و روش‌های خشک کردن

مژده اسدی[۱]، صمد نژادابراهیمی[۲]، مهرناز حاتمی[۳،*]، جواد هادیان[۱]

[۱] گروه کشاورزی، پژوهشکده گیاهان و مواد اولیه دارویی، دانشگاه شهید بهشتی، ایران

[۲] گروه فیتوشیمی، پژوهشکده گیاهان و مواد اولیه دارویی، دانشگاه شهید بهشتی، ایران

[۳] گروه گیاهان دارویی، دانشکده کشاورزی و منابع طبیعی، دانشگاه اراک، ایران

اطلاعات مقاله

گل‌واژگان:
آرنیکا
نمو گل
برداشت
خشک کردن
روتین

چکیده

مقدمه: مقادیر نسبی متابولیت‌های ثانویه گیاهان تحت تأثیر زمان برداشت و روش‌های خشک کردن پس از برداشت قرار می‌گیرند. **هدف:** بررسی اثرات مراحل برداشت گل و روش‌های خشک کردن روی کمیت و کیفیت متابولیت‌های ثانویه گیاه آرنیکا. **روش بررسی:** گل‌ها در اوایل خرداد، تیر و مرداد در چهار مرحله (مرحله ۱: زمان بازشدن جوانه گل، مرحله ۲: زمانی که همه گلچه‌های شعاعی یا کمتر از نیمی از گلچه های لوله‌ای باز شده‌اند، مرحله ۳: وقتی که همه گلچه‌های شعاعی و لوله‌ای باز شده‌اند، مرحله ۴: زمان پژمرده شدن گل‌های شعاعی و تشکیل بذر) برداشت شدند. همچنین، حداکثر میزان برداشت گل آذین در طی دو سال آزمایش برای تیمارهای روش خشک کردن (خشک کردن در سایه و آفتاب، خشک کردن در آون ۴۰ و ۵۰ درجه سانتی‌گراد، خشک کردن با مایکروویو در ۵۰۰ و ۱۰۰۰ وات) در نظر گرفته شد. **نتایج:** نتایج نشان داد که بیشترین و کمترین فنل کل به ترتیب در تاریخ برداشت دوم و اول در مراحل بلوغ گل ۳ و ۴ بدست آمد. درحالیکه، بیشترین میزان لوتئولین و آپی‌ژنین در مرحله برداشت سوم در مرحله ۱ رشد گل در زمان برداشت سوم بدست آمد. در آزمایش دوم، گل‌های سایه خشک شده حاوی بیشترین مقدار فنل کل بودند و پس از آن خشک کردن با آون در دمای ۴۰ درجه سانتی‌گراد. علاوه بر این، خشک کردن با آون مطلوب‌ترین روش برای حفظ سایر متابولیت‌های ثانویه اصلی از جمله فلاونوئید کل، روتین، لوتئولین و آپی‌ژنین است. **نتیجه‌گیری:** تاریخ‌های مختلف برداشت در مراحل مختلف فنولوژی جوانه گل تأثیر معنی‌داری بر محتوای فنل کل، فلاونوئیدها، روتین، لوتئولین و آپی‌ژنین داشت.

مخففها: PCA، تحلیل مولفه‌های اصلی؛ UPGMA، روش جفت گروه بدون وزن با میانگین حسابی

* نویسنده مسؤول: m-hatami@araku.ac.ir

تاریخ دریافت: ۱۳ دی ۱۳۹۸؛ **تاریخ دریافت اصلاحات:** ۵ آبان ۱۳۹۹؛ **تاریخ پذیرش:** ۱۱ آبان ۱۳۹۹

doi: 10.29252/jmp.19.76.69