

Evaluation of some phenolic derivatives contents in the vegetative growth stage of medicinal plant *Ziziphora clinopodioides* Lam. from North Khorasan province

Azadeh Taheri ¹, Monireh Cheniany ^{2*}, Ali Ganjeali ³, Afsaneh Arefi-Oskouie ⁴

¹ PhD, Department of Biology-Plant Physiology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

² Assistant professor, Department of Biology-Plant Physiology, Faculty of Science, Ferdowsi University of Mashhad, Iran

³ Professor, Department of Biology-Plant Physiology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

⁴ Professor, Department of Basic Sciences, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

E-mail of the corresponding author: *Cheniany@um.ac.ir

Received: 04/12/2022

Accepted: 26/11/2023

Introduction

Ziziphora clinopodioides Lam. is a perennial plant from the Lamiaceae family, with botanical characteristics as stemmy bushes, different stems at the wooden stem, a height of 10-50 cm, with oval, pointed and circular leaves, hairless or full of hair, and numerous flowers in the inflorescence, without peduncle or peduncle with the length of 4 mm in the form of end compact (Mozaffarian, 2012).

In addition to essential primary metabolites, organic plants are also capable of the synthesis of low molecular-weight organic compounds (less than 150 kDa) known as secondary metabolites (Oksman-Caldentey & Inze, 2004). The quantity and quality of phenolic compounds as a group of plant secondary metabolites are different, in particular at many levels, from the population, variety, and species, to genera and higher taxa (Holeski et al, 2012). The production of secondary metabolites is strongly dependent on the growing stages and can occur at any step of growth. The amount changes of these compounds also occur in different plant tissues, and parts, and during the growing season (Fenner, 1998; Shukla et al, 2009).

Based on the literature, the effect of age or growth stage on the content of phenolic derivatives of *Z. clinopodioides*, has not been conducted so far. Therefore, the present study is the first work reporting the content changes of phenolic derivatives (phenol, flavonoid, flavone and

flavonol, *ortho*-diphenol, and phenolic acid) during the vegetative growth stage of three populations of *Z. clinopodioides* in the controlled greenhouse conditions.

Material and methods

The seeds of *Z. clinopodioides* were collected from three regions of North Khorasan province ('Darkesh and Havar', 'Teymourtash', and 'Reine'). After drying in the shade, the seeds were planted in pots filled with soil and sand in a ratio of 1:1. The growing plants were harvested in two growth periods (a two-month growth stage and a five-month growth stage). The separated parts (aerial part and root) were dried in an oven at 40 °C for 48 hours. The extraction process from samples was done according to the method of Annegowda et al. (2012). The extract powders were used to prepare methanolic extract (with a concentration of 1 mg/ml in methanol 80% v/v) for the next biochemical analysis. The phenolic derivatives content of the extracts includes total phenolics (Tohidi et al, 2017), flavonoids (Khorasani Esmaeili et al, 2015), flavones & flavonols (Kosalec et al, 2004), *ortho*-diphenols (Carrasco-Pancorbo et al, 2005), and phenolic acids (Matkowski et al, 2008) were measured using the spectrophotometric method for each compound. The obtained data were analyzed using a two-way analysis of variance and Duncan's test to compare the means between populations at a significance level of 5% ($P < 0.05$) by SPSS software.

Results and discussion

The results of a two-way analysis of the variance (the effect of age, population, and their interaction) showed that there was a significant difference between two samples (aerial part and root) for all the phenolic derivatives contents. An exception was found in the content of flavone and flavonol in the root extracts, which did not show a significant difference at the $P < 0.05$ level. This result indicates the effect of physiological age, population, and their interaction on the content of analyzed secondary metabolites in *Z. clinopodioides*. According to the results and with increasing the age of plant, during the vegetative growth stage in controlled greenhouse conditions, the amount of phenolic derivatives decreased significantly in the aerial parts of three populations. The investigation of phenolic content in the stages of vegetative growth, formation of flower buds, and full flowering of *Fumaria vaillantii* showed that the value of these compounds decreased with increasing the age, and the vegetative stage was introduced as the best one to harvest the plants (Moghaddam et al, 2018). Different processes of biosynthesis or degradation of polyphenols, their transport systems, the interaction between plant organs as well as the phenological stage of each organ are involved in the distribution of various phenolic constituents (Hudaib et al, 2002; Yosr et al, 2013).

The comparison of total phenolic content among the investigated populations showed that 'Teymourtash' population has the highest amount of total phenol, with more total phenol content in the root than the aerial part. The changes of flavonoid content were also studied under the influence of the vegetative growth stage, population diversity, and the plant part; and the maximum amount of flavonoid content was obtained from the two-month-old plant aerial part of 'Darkesh and Havar' population. Ali et al. (2014) reported higher content of flavonoids in the leaves and stems of thyme (*Thymus numidicus* Poir.) than in the roots.

Flavone and flavonol contents showed a significant difference between the aerial part and root extracts. Total flavone and flavonol content in the aerial part of the two-month plants of 'Darkesh and Havar' and 'Reine' populations was approximately two times higher than those in the

roots, while there was no noticeable change in the content of these compounds between the aerial part and root of 'Teymourtash' population.

Regardless of plant organ or tissue, 'Darkesh and Havar' population is referred to as the "selected population" in terms of the maximum production of phenolic and flavonoid compounds in both stages of vegetative growth. Considering the same conditions of cultivation, the results can emphasize the effect of genetics on the synthesis and accumulation of these compounds in different populations, which, can in turn lead to the creation of a pathway for the production of secondary compounds according to the needs of a plant during the growth period.

Conclusion

The results of the present research show that the content of phenolic compounds is significantly affected by growth stages, age, plant part, and plant population. Based on this, the aerial part of *Z. clinopodioides* plants in the two-month stage, which is younger, has more phenolic derivatives than ones in the five-month stage. 'Darkesh and Havar' and then 'Teymourtash' were reported as the selected populations in terms of maximum production of phenolic derivatives in both stages of vegetative growth.

Keywords: *Flavonoid compounds, Phenolic compounds, Pot culture, Ziziphora clinopodioides.*

Acknowledgment

This research was financially supported by Ferdowsi University of Mashhad, Iran (Grant no. 3/50558).

Declaration of conflict of interest: *The authors declare that there is no conflict of interest.*

Statement of Ethics: *This article is the authors' own original work, which has not been previously published elsewhere.*