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Investigation of phenolic acids and some biological activities of *Nepeta macrosiphon*

Saeed Mollaei[1*], Hadeseh Abbasi Holasu[1], Behvar Asghari[2], Mostafa Ebadi[3], Hossein Hashempour[1]

1. Tabriz, Azarbaijan Shahid Madani University, Department of chemistry (*Corresponding author:: s.mollaei@azaruniv.ac.ir)
2. Qazvin, Imam Khomeini International University, Faculty of Agriculture and Natural Resources, Department of Horticultural Sciences Engeneering
3. Tabriz, Azarbaijan Shahid Madani University, Department of Biology

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Introduction

Nepeta is one of the largest families of Lamiaceae with more than 250 different species in the world. The most species of this genus are found in Iran, Pakistan and India. In Iran, more than 67 species of this genus have been reported, and more than 60% of these species are endomic. (Mozaffarian, 1996; Jamzad et al., 2000). Some species of them in Iran have wide uses as medicinal plants, and include *N. ispahanica*, *N. binaloudensis*, *N. bracteata*, *N. ponogosperma* and *N. pungens*.

Phenolic acids are a subgroup of phenolic compounds that are found in plants in free or derived forms (Klick and Herrmann, 1998; Lam et al., 2001). Many phenolic acids such as cinnamic and benzoic acid derivatives are found in all plants. However, only a small part of phenolic acids are seen as free acid (Verpoorte et al., 2002; Shahidi and Naczk, 2004). Research results have determined that phenolic compounds have a high potential in the management of chronic diseases related to oxidative stress and inhibition of alpha-glucosidase enzymes (Shetty et al., 2004; McCue et al., 2004).

N. macrosiphon is one of the endomic plants of Iran's region, and no comprehensive study has been done on the metabolites and biological properties of this plant. Therefore, in this study, for the first time, the free and soluble esters phenolic acids of different organs of *N. macrosiphon* were investigated. Also, the antioxidant properties and inhibition of alpha-glucosidase enzyme activity of the extracts and fractions of each organ were also evaluated.

Materials & Methods

Nepeta macrosiphon was collected from East Azarbaijan Province, Zonuz region during the flowering season, and then dried in a dark place away from sunlight. 4 grams of different parts of the plant were mixed with 40 ml of n-hexane solvent and placed in an ultrasonic bath for 30 minutes, and then filtered. The obtained pulp was mixed with 100 ml of 80% ethanol solvent and ultrasonicated for 30 minutes. The dried extracts were mixed in 30 ml of methanol, and the pH was adjusted at 2, and then centrifuged for 5 minutes. The supernatant was separated, and extracted by n-hexane solvent. Then, diethyl ether and ethyl acetate solvents were added to the aqueous phase. Finally, the resulting organic phase containing free phenolic acids was dried. 80 ml NaOH, 2 M was added to the remaining aqueous phase and the solution remained for 4 hours. Next, by adjusting its pH to 3-4, the solution was placed in a centrifuge. It was extracted by separator funnel using n-hexane solvent. Then, extraction was done by adding diethyl ether and ethyl acetate solvents to the separated aqueous phase. The obtained extract containing dissolved phenolic ester acids was dried.

A high-performance liquid chromatography (HPLC) device with a Welch Ultisil XB-C18 reverse phase column was used to separate and identify the phenolic acids of the extracts. Separation was done at room temperature and wavelength of 280 nm, and the total analysis time for each extract was 55 minutes. The total phenolic and flavonoid contents were evaluated using Folin-Ciocâlteu reagent and aluminum chloride colorimetric, respectively. The antioxidant activity of the extracts was also investigated using the DPPH method. In the following, alpha-glucosidase inhibition was evaluated using spectroscopic method.

Results & discussion

According to the results, the flowers and leaves extracts of the plant had the highest yield, and the lowest yield belonged to the stems extract. Also, the highest amount of phenolic compounds is related to the flowers extract and the extract obtained from the stems also had the lowest amount. The results of total flavonoid content showed that the highest flavonoid content is related to the flowers extract. Based on the analysis of phenolic acids, the highest amount of free phenolic acids was related to flowers. Rosmarinic acid was the main phenolic acid in the flowers, followed by para-coumaric acid. In the leaves of the plant, para-coumaric acid and rosmarinic acid were the main phenolic acids which found in this organ. By examining the soluble phenolic ester acids in different organs, it was also determined that the highest amount of these compounds is present in the flowers.

The lowest amount is related to the stems, in which only caffeic acid was detected. Also, only vanillic acid was present in the roots. Caffeic acid had the highest amount among other soluble phenolic acids in all organs. Also, caffeic acid was the main compound in the leaves.

The results of antioxidant activity showed that the highest antioxidant activity with IC_{50} value of 315.2 µg/mL is related to flowers extract, followed by the extract of leaves with IC_{50} value of 616.5 µg/mL. Also, the flowers extract containing free phenolic acids had the highest antioxidant activity, followed by the leaves extract containing free phenolic acids. It should be noted that among the extracts containing soluble phenolic ester acids, flowers extract had the highest activity. According to the results of this research and previous studies, it may be possible to guess that the high antioxidant property of the flowers and leaves fraction is related to the phenolic acids present in them and the high amount of rosmarinic, para-coumaric and caffeic acids can justify the antioxidant activity of them. According to the results of inhibition of alpha-glucosidase enzyme, flowers extract inhibited alpha-glucosidase enzyme at the lowest concentration compared to other extracts. Among the extracts, the leaves extract showed the ability to inhibit alpha-glucosidase enzyme activity in a lower concentration range. Moreover, the ability to inhibit alpha-glucosidase enzyme of the free and soluble phenolic ester acids were according to flower > leaf > root > stem.

Conclusion

Based on the results, the flower of *N. macrosiphon* has the most phenolic compounds, including rosmarinic acid and para-coumaric acid, and considering that the fraction containing free phenolic acids has a high antioxidant and alpha-glucosidase inhibition potentials, so the flower of this plant can be used in pharmaceutical and food industries.

Keywords: *Nepeta macrosiphon; Antioxidant; Alpha-glucosidase enzyme; Phenolic acid*

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Declaration of conflict of interest

All authors declare no conflict of interest.