SUPPLEMENTARY MATERIAL

Phenolic compounds and antioxidant activity of *Nepeta fissa* - first report from Iran

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ABSTRACT

In recent years, scientific interest in natural products has burgeoned. The genus *Nepeta* is one of the most important medicinal plants belongs to family Lamiaceae. In this study, the total phenolic and flavonoid contents, antioxidant potential and distribution of individual phenolic compounds by HPLC-MS/MS were determined in native *Nepeta fissa* Benth species. The total phenolic and flavonoid contents and antioxidant capacity (by DPPH and FRAP assays) values were measured in *N. fissa* as 43.07 mg GAE/g dw, 3.77 mg q/g dw, 197.85 µg/mL (IC_{50} value) and 1.15 µmol Fe^{++}/g dw, respectively. The most abundant flavonoid, phenolic acid, and anthraquinone in the analyzed *N. fissa* extracts were rutin, ferulic acid, and chrysophanol, respectively. As a conclusion, results of present study showed that *N. fissa* was rich in some phenolic compounds and exhibited antioxidant activity. The obtained results can provide new safe resources to the development of new products for the pharmaceutical industries.

Keywords: Anthraquinone; Flavonoid; HPLC-MS/MS; Phenolic acid

Experimental

1. Plant sampling

Aerial parts of wild growing *Nepeta fissa* (July 2017) at flowering stage was collected from Qotur region (Khoy- West Azerbaijan province), Iran with 37° 39' N, 44° 46' E and 1387 m elevation. The voucher herbarium sample (HUPS-499) of the collected *N. fissa* species have been deposited at herbarium of Urmia University of Medical Sciences. The climate condition of this region is semi-humid Mediterranean with an annual rainfall of 400-500 mm. The soil in this area is loam. Aerial parts of *N. fissa* dried in the shade at room temperature (about 30 °C) for 5 days.

2. Preparation of plant extracts

Dried aerial parts (1 g) of *N. fissa* species were powdered by a laboratory mill and their methanolic (MeOH) extracts (80 %, v/v) were obtained using ultrasound (Elmasonic, Germany) process at 25 ºC for 30 min. Then, the extracts were centrifuged (3000 *x g*, 5 min, model ROTANTA 380/380R, Hettich, Germany). The recovered extracts after centrifugation kept at 4 °C in dark glass, until the time of HPLC analysis and phytochemical tests (Alirezalu et al. 2018).

3. Phenolic compound analysis by HPLC-MS/MS

In the present study most important phenolic compounds of *N. fissa* species including rutin, catechin, quercetin, kaempferol, and myricetin (flavonoids), ferulic acid, coumaric acid, gallic acid, caffeic acid, and chlorogenic acid (phenolic acids), and emodin, chrysophanol, aloe emodin, and physcion (anthraquinones) were measured with HPLC Alliance waters e2695 (Milford, MA, USA), with a Quattro Micro API (Atmospheric Pressure Ionisation) triple quadrupole LC-MS/MS (Waters, Micromass, Manchester, UK). The separations and identification of phenolic compounds were conducted by an Agilent ZORBAX SB-C18 (4.6×150 mm, 5micron). The injection volume was 50 µL and flow rate of the mobile phase was maintained at 0.5 mL/min. Solvent A was HPLC water containing 1% formic acid (v/v), and solvent B was acetonitrile. The gradient conditions were as follows: 0 min, 20% B; 18 min, 100% B increase linearity and held for 2 min, the initial conditions were held for 3 min as a re-equilibration step. The detection wavelengths of detector were set at selected positions including: 254, 272, 280 and 310 nm. Acquisition of obtained data was performed with Mass Lynx (version 4.1) software.

The MS/MS system fitted with an electrospray ionization source (ESI) in negative and positive modes. Mass spectrometric detection conditions were: capillary voltage, 3.5 kV; cone, 40 V; extractor, 2 V; RF lens, 0.2 V; source temperature, 120 °C; desolvation

temperature, 380 °C; desolvation gas and cone gas (nitrogen 99.99% purity) flow rates, 600 and 60 L/h, respectively. The analyzer settings were: resolution, 15.0 and 12.0 (unit resolution) for LM1 and LM2 resolution respectively; 14.0 and 12.0 for HM1 and HM2 resolution respectively; ion energy 0.5 and 1 respectively; entrance and exit energies, -2 and 2 (V); multiplier, 600 (V); collision as (argon, 99.995%) pressure 8.80×10^{-3} mbar.

4. Measurement of total phenol (TPC) and flavonoid (TFC) contents

The TPC and TFC of the *N. fissa* species extracts were measured by Folin-Ciocalteu (Slinkard and Singleton, 1977) and $AlCl_3$ (Shin et al., 2007) assays, respectively. The contents were expressed as gallic acid (mg GAE/g dw) and quercetin equivalents (mg q/g dw), respectively.

5. Measurement of antioxidant activity by DPPH and FRAP assays

The antioxidant activity of the *N. fissa* species extracts were measured using DPPH (Nakajima et al., 2004) and FRAP (Benzie and Strain, 1996) assays, respectively. The contents were expressed as DPPH IC_{50} (µg/mL) and µmol Fe^{++}/g dw, respectively.

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