Supporting Information

***Rec. Nat. Prod*. 12:5 (2018) 432-444**

Evaluation of the Anti-Gout Potential of *Calluna vulgaris* L. (Ericaceae) in Rats

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S.1. Phytochemical analysis

S.1.1. Reagents and Substances

Caffeic acid and chlorogenic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). For the mobile phase, acetonitrile of HPLC grade was purchased from VWR Chemicals (France) and formic acid from Cristal R Chim (Romania). Methanol of HPLC grade used for preparation of standard solutions was purchased from VWR Chemicals (France). Water of high purity used in all experiments was obtained using a Milli-Q Ultrapure purification system (Millipore, USA). All chemicals for spectrophotometrical determinations were of analytical grade and were purchased from Merck (Germany).

S.1.2. Total Phenolic Content

Total phenolic content of the extract from *C. vulgaris* (ECV) was determined by Folin Ciocalteu spectrophotometric method. Five mL of Folin Ciocalteu reagent were added to an aliquot of 1 mL ECV, homogenized and then mixed with 15 mL sodium carbonate 7.5%. After 2h the absorbance of the solution was recorded at 760 nm against a blank with a Shimadzu PC-1800 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). A calibration curve was constructed using standard gallic acid in a range of concentrations from 10 to 150 mg/100 mL. The results were expressed in mg gallic acid equivalents (GAE)/g of extract.

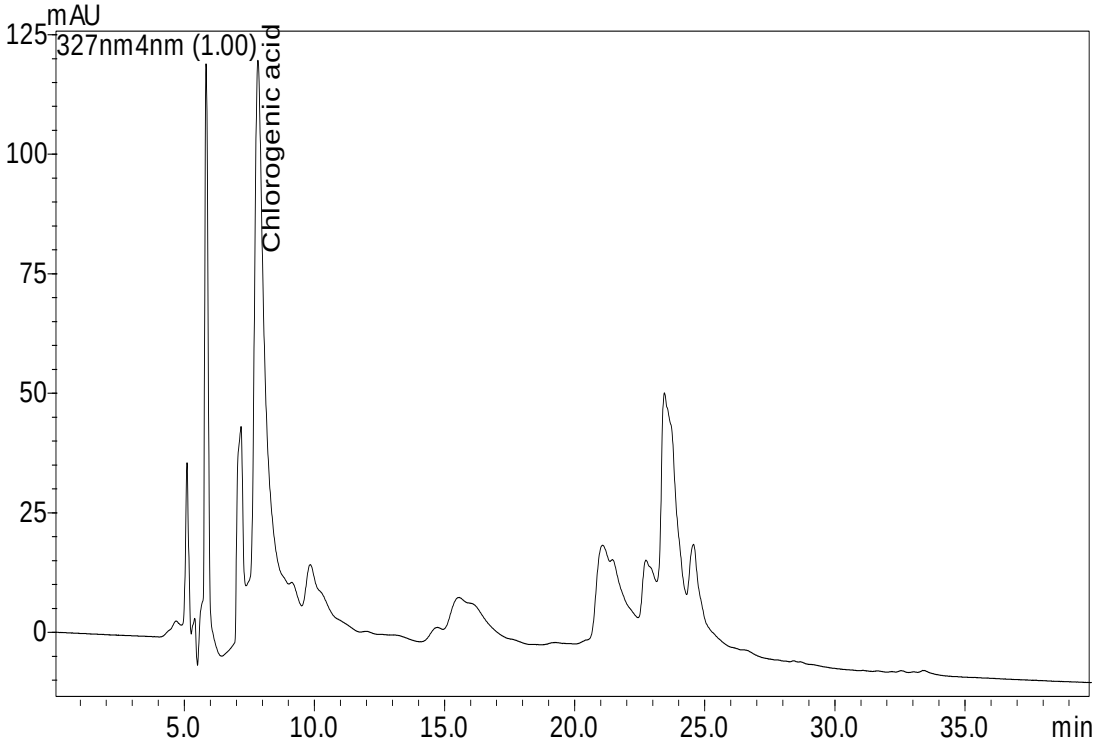
S.1.3. Total Flavonoid Content

The total flavonoid content was determined spectrophotometrically by the aluminum chloride method. Ten mL of ECV mixed with methanol and distilled water was centrifuged at 6000 rpm for 10 minutes. Later, an aliquot of 2 mL from supernatant was treated with 2 mL 10% sodium acetate and afterwards with 1 mL 0.25% aluminum chloride, the mixture being incubated at room temperature for 15 minutes. The absorbance was determined at 430 nm with a Shimadzu PC-1800 UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). Results were expressed in mg rutin/g of extract.

S.1.4. HPLC-PDA-MS Analysis of Phenolic Acid

The analysis of phenolic acid components from ECV was performed by a HPLC-PDA-MS method. A LC2010 Shimadzu system (Shimadzu, Kyoto, Japan) consisting of a LC-20AD pump, DGU-20A5 degasser, SIL-20A auto sampler and CTO-20AC column oven was used. The instrument was equipped with a SPD-M20A photodiode array (PDA) detector and LCMS-2010EV mass spectrometer (MS). The MS detector was a single quadrupole. The chromatographic separation was performed using a LiChrosorb RP-18 column (5 µm, 25 x 0.4 cm, Merck, Germany) thermostated at 40°C with gradient elution. The eluents were acetonitrile (A) and ultrapure water with 0.1% formic acid (B). The gradient elution was: 0–10.0 min, 18% A; 10.0-15.0 min, increase to 30% A; 15.0–18.0 min, increase to 100% A; 18.0-35.0 min, 100% A; in 35 min, return to 18% A and equilibration for 5 minutes (flow rate 0.43 mL/min) The parameters for MS detection were: capillary voltage 1.5 kW, 250°C dissolvation temperature, 200°C interface temperature. The electro-spray ionization (ESI) in negative ionization mode was used for mass spectra registration. The standard solutions were prepared by successive dilutions from 1 mg/mL concentration in the range of 0.003-0.1 mg/mL with a pure compound of Chloregenic acid obtained from Sigma-Aldrich. The identification of each compound was performed by comparing the retention time, UV spectra and molecular mass of the peaks from the extract with those of the reference standards. The main components of the ECV were quantified by external standard method using calibration curves obtained by plotting peak area versus concentration. The regression equation was expressed as y = 3E+07x + 710377 (where x – concentration of chlorogenic acid and y-peak area). The coefficient of correlation (R^2) was 0.9990. The limit of

detection (LOD) and quantification (LOQ) were found to be 0.002mg/mL and 0.004mg/mL, respectively. The analyses were performed in triplicate for each sample.



S2: HPLC chromatogram of ethanol extract of *Calluna vulgaris* L