Chemical composition, antioxidant and anti-tyrosinase potentials of *Acacia cyclops* trunk bark using *in vitro* and *in silico* approaches

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Experimental

1. Reagents and standards

Ascorbic acid (≥ 99%), chlorogenic acid (≥ 95%), fumaric acid (≥ 99%), caffeic acid (≥ 98%), vanilic acid (≥ 97%), p-coumaric acid (≥ 98%), rosmarinic acid (≥ 96%), quercetin (≥ 95%), salicylic acid (≥ 98%), naringenin (≥ 95%), luteolin (95%) and chrysin (≥ 96%) were all purchased from sigma-aldrich. (-)-Epigallocatechin (> 97%), (-)-epigallocatechingallate (> 97%), (+)-*trans* taxifolin (> 97%), luteolin 7-glucoside (> 97%), ellagic acid (> 97%), ellagic acid (> 97%), genistein (> 97%), hispidulin (> 97%) and acacetin (> 97%) were all obtained from TRC Canada. Other chemicals; luteolin-7-rutinoside (> 97%) from Carbosynth Holdings Limited (Bratislava, Slovakia), dihydrokæmpferol (> 97%), from Phytolab (Nantes, France), apigenin 7-glucoside (>97%) from EDQM CS and nepetin (98%) was purchased from Supelco (Pennsylvania, USA). HPLC gradient grade methanol (MeOH) was obtained from Merck (Darmstadt, Germany). All of the other chemicals used were of analytical reagent grade. The water used throughout the study was purified in a Milli-Q plus system (EMD Millipore, Billerica, MA).

2. Plant material and extraction process

A. cyclops trunk bark was collected from Beja, Tunisia in Februray 2020. Botanical identification of the plant material was carried out by Professor Fethia Harzallah-Skhiri (Higher Institute of Biotechnology of Monastir, University of Monastir, Tunisia). A voucher specimen (Acy-B/20) was deposited at the Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity, Faculty of Science of Monastir, Tunisia.

A. cyclops trunk bark was air-dried for 10 days at room temperature and then finely ground. The powdered sample (50 g) was subjected to a successive extraction by maceration at room temperature using ethyl acetate (EtOAc) and methanol (MeOH). The extracts were then filtered using a Buckner funnel and Whatman filter paper. After filtration and evaporation of the solvents at 40°C (Buchi R-100 Rotavapor), we obtained the EtOAc extract (1 g, 2% w/w) and the MeOH one (1.5 g, 3% w/w). The two extracts were kept then at 4°C until analysis.

3. Estimation of total phenolic and total flavonoids contents

The total phenolic content of the MeOH extract was measured by method described by Mohti et al. 2020, with slight modifications. Using the spectrophotometric method adapted by Jlizi et al. 2023, the amount of total flavonoid content was calculated. All experiments were conducted in triplicate.

4. LC-MS/MS determination of bioactive compounds

Identification and quantification of phenolic compounds in the *A. cyclops* extract was achieved using Q-Exactive LC–MS/MS – Orbitrap (Thermo Scientific, Hemel Hempstead, UK). Chromatographic separation of compounds was gained on a Troyasil HS C18 column (3mm x 150 mm, 5 µm particle size, Troyasil Column Technologies) with a 350 µL/min gradient flow. The mobile phase was composed of A: water and B: methanol with 0.1% formic acid in both the phases. The gradient program used was: 0-1.00 min 50% B, 1-3 min 100% B, 3.00-6.00 min 100% B, 6-7 min 50% B and finally 7.00-15.00 min 50% B. The injection volume was 5 µL. Q Exactive hybrid quadrupole-Orbitrap mass spectrometer equipped with an ESI source working in both negative and positive ionization mode was applied for accurate mass measurements. The operation parameters set were: ion spray voltage, 3.8 kV; capillary temperature, 320^{o}C; sheath gas and auxiliary gas rates 45 and 10 (arbitrary units), respectively. Mass spectra were recorded covering the *m/z* range of 100–900 da. Default values were used for most other acquisition parameters (Automatic gain control (AGC) target 3×10^{6} ions). The data processing was achieved using XCalibur 2.2 software (Thermo Fisher Scientific, Waltham, MA, USA). An external calibration for mass accuracy

was carried out before the analysis. Identification of the phenolic compounds was performed by comparing retention times and mass spectra with those of authentic standards. Accurate mass data achieved for pseudo-molecular $[M-H]^-$ or $[M+H]^+$ ions were also used for further confirmation of identification. The accurate mass measurements results fit well with the elemental composition of the compounds.

5. Antioxidant activity

DPPH and ABTS radical scavenging capacity of the methanol extract from the trunk barksof *A. cyclops* was determined according to the method of Zardi-Bergaoui et al. 2018. All the experiences were carried out in triplicate and results were expressed as IC_{50} values.

6. Anti-tyrosinase activity

The anti-tyrosinase activity of *A. cyclops* extract was conducted according to the method described by Zayene et al. 2022. IC_{50} values are means ± SD. Kojic acid was used as a positive control and all measurements were conducted in triplicate.

7. Molecular Docking Procedure

The chemical compound structures of tropolone, (-)-epigallocatechin, (-)-epicatechin, caffeic acid, vanillic acid, dihydrokæmpferol and naringenin were generated and optimized using ACD (3D viewer) software, where their energies were minimized. The crystal structure of Tyrosinase protein (PDB: 2Y9X) was downloaded from the RSCB data bank. The protein was prepared by removing the complexed inhibitor ligand and water molecules. Polar hydrogens were then added, followed by appending Kollman charges. Therefore, the grid box with dimensions of 40 x 40 x 40 points, spacing of 0.375 Å and centered with coordinates x: −10.004, y: −28.28, and z: −43.443, was generated based on tropolone binding position in the target protein binding site. The molecular docking analyzes of tropolone, (-)-epigallocatechin, (-)-epicatechin, caffeic acid, vanillic acid, dihydrokæmpferol and naringenin were carried outusing AutoDock Vina software (Trott and Olson 2010). Molecule-enzyme interactions were drawn and interpreted by employing the Biovia Discovery Studio Visualizer (BIOVIA, D. S. (2017)).

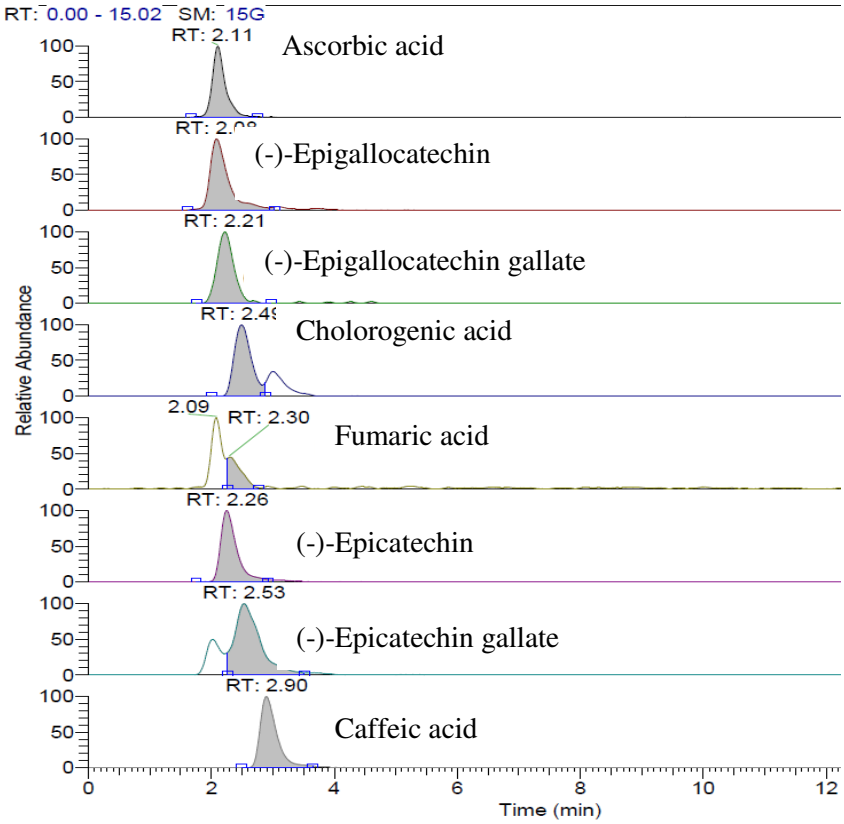
8. ADMET Analysis

The ADMET (absorption, distribution, metabolism, excretion, toxicity) profiling of the selected compounds was checked using SwissADME online server and pkCSM online tools (Ghannay et al. 2020; Kadri and Aouadi 2020; Othman et al. 2020b).

9. Statistical analysis

Statistical analysis was performed using Graph Pad Prism 7.0 (Graph Pad Software Inc., CA, and USA). All the experimental data are given as the mean ± standard error of the mean (SEM). The difference between two groups was evaluated using Student's t-test.

Significant difference among three or more groups was assessed by one-way ANOVA with a post hoc analysis.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RT: 2.21
(-)-Epigallocatechin gallate
Cholorogenic acid
2.09
RT: 2.30
Fumaric acid
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(-)-Epicatechin
RT: 2.53
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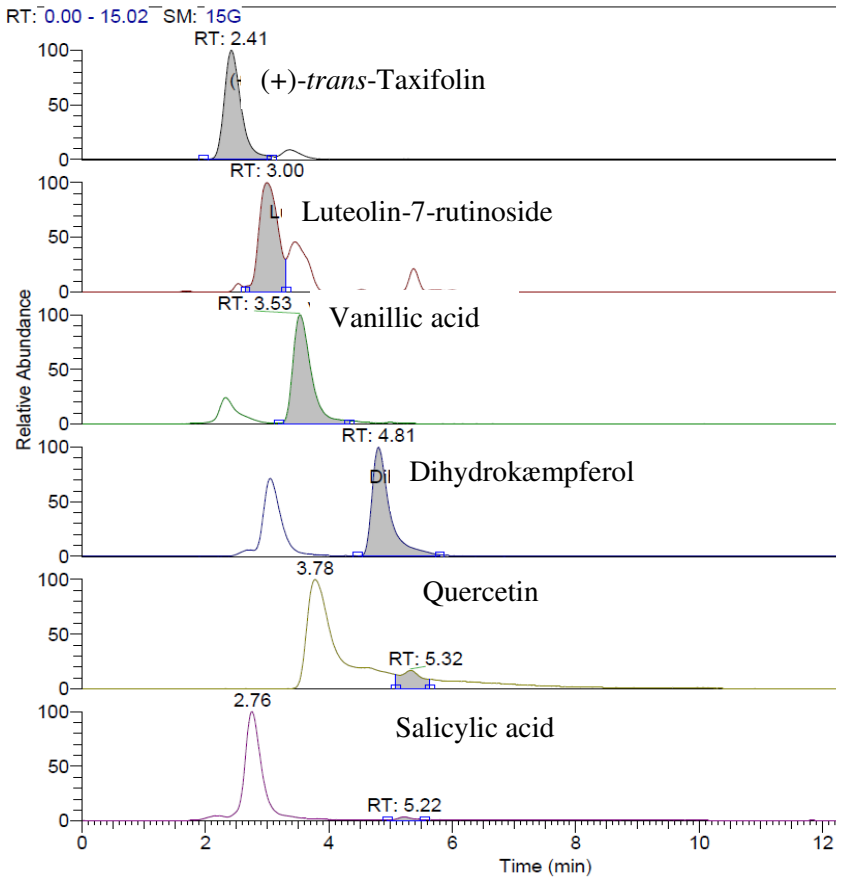
RT: 2.90
Caffeic acid
Relative Abundance
Time (min)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Luteolin-7-rutinoside
RT: 3.53
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Quercetin
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Salicylic acid
RT: 5.22
Relative Abundance
Time (min)

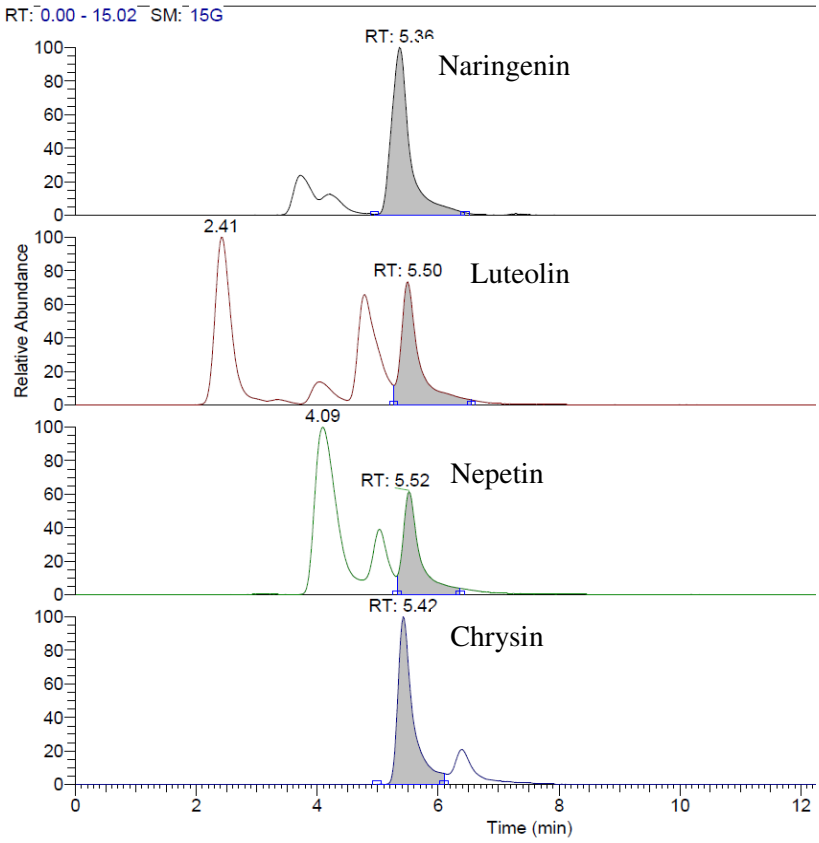

Figure S1. LC chromatogram of MeOH extract from *A. cyclops* trunk bark.