Supporting Information

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Assessment of Endemic *Cota fulvida* (Asteraceae) for Phytochemical Composition and Inhibitory Activities against Oxidation, α-Amylase, Lipoxygenase, Xanthine Oxidase and Tyrosinase Enzymes

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S1: Gas – chromatographic analysis conditions

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). HP-Innowax FSC column (60 m×0.25 mm, 0.25 μm film thickness, Agilent, Walt & Jennings Scientific, Wilmington, DE, USA) was used with a helium carrier gas at 0.8 mL/min. GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, kept constant for 10 min at 220°C, and then programmed to increase at a rate of 1°C/min to 240°C. The oil was analyzed with a split ratio of 40:1. The injector temperature was 250°C. Mass spectra were taken at 70 eV and the mass range was from *m/z* 35 to 450.

The GC-FID analysis was carried out with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). FID temperature was set at 300°C in order to obtain the same elution order with GC/MS. Simultaneous injection was performed using the same column and appropriate operational conditions.

S2: Identification of volatile constituents

Identification of the volatile constituents was based on the following: (i) comparison of GC/MS Relative Retention Indices (RRI) of the compounds on polar column determined relative to the retention times of a series of *n*-alkanes (C_8-C_{40}), with those of authentic compounds or literature data; (ii) computer matching with commercial mass spectral libraries: MassFinder software 4.0, Adams Library, Wiley GC/MS Library (Wiley, New York, NY, USA) and Nist Library, and comparison of the recorded spectra with literature data. Confirmation was also achieved using the in-house "Başer Library of Essential Oil Constituents" database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions.

S3: LC-MS/MS analysis conditions

Experiments were performed with a Shimadzu 20A HPLC system coupled to an Applied Biosystems 3200 Q-Trap LC- MS/MS instrument equipped with an ESI source operating in negative ion mode. For the chromatographic separation, a GL Science Intersil ODS 250 × 4.6 mm, i.d., 5 μm particle size, octadecyl silica gel analytical column operating at 40º C has been used. The solvent flow rate was maintained at 0.5 mL/min. Detection was carried out with PDA detector. The elution gradient consisted of mobile phases (A) acetonitrile : water : formic acid (10:89:1, v/v/v) and (B) acetonitrile : water : formic acid (89:10:1, v/v/v). The composition of B was increased from 10% to 100% in 40 min. LC-ESI-MS/MS data were collected and processed by Analyst 1.6 software.

S4: Free radical scavenging activity (DPPH assay)

The DPPH solution (0.08 mg/mL, in methanol) was freshly prepared daily, kept in the dark at 4°C between the measurements. The solutions of the essential oil (30 mg/mL), the extract (10 mg/mL) and gallic acid (0.1 mg/mL) were prepared in methanol. In the experiment, 100 μL of the sample (EO/ extract/ standard) solution and 100 μL DPPH solution were pipetted by multichannel automatic pipette (Eppendorf Research® plus, Germany) into 96-flat bottom well plate cells and incubated in the dark (30 min). The control well contained 100 μL methanol (instead of the sample) mixed with 100 μL of DPPH solution. The decrease in the absorbance due to scavenging of DPPH free radicals was recorded at 517 nm. Gallic acid was used as positive control.

S5: Trolox equivalent antioxidant capacity (TEAC assay)

In the experiment, 7 mM ABTS and 2.5 mM $K_2S_2O_8$ dissolved in 10 mL ultrapure water were allowed to stand in dark for 16 h at room temperature to create $ABTS^{\bullet+}$ free radical cations. Prior to the assay, $ABTS^{\bullet+}$ solution was diluted with absolute ethanol to an absorbance between 0.7-0.8 at 734 nm. The solutions of the oil and extract (5 mg/mL), and Trolox (3.0; 2.0; 1.0; 0.5; 0.25; 0.125 mM) were prepared in MeOH. In the experiment, 10 μL of the sample solution was mixed with 990 μL $ABTS^{\bullet+}$ solution. Methanol (10 μL) instead of the sample mixed with $ABTS^{\bullet+}$ solution was used as a control.

Decrease in the absorbance after 30 minutes of incubation was recorded at 734 nm to get linear Trolox equation.

S6: β-Carotene / Linoleic Acid Peroxidation Inhibition Assay

In the experiment, 5 mL β-carotene solution (1 mg/mL) in chloroform was added to flask containing linoleic acid (120 mg) and Tween-20 (1200 mg). The content of flask was vigorously shaken after that chloroform was evaporated under the vacuum. After evaporation, pure water (300 mL) was added and shaken vigorously. BHT (1 mg/mL) was used as standard. In the cell of the deep well plate, 10 µL of the sample (oil/ extract/ standard) and 2 mL of β-carotene emulsion were mixed. After that, 300 µL of the mixture was placed by multichannel automatic pipette (Eppendorf Research® plus, Germany) into a 96-well microplate and incubated at 50°C for 2 h. Control was prepared without sample or standards according the same procedure.

S7: Microtiter Assay for Determination of Xanthine Oxidase Inhibition

In the experiment, 100 µL sample solution (oil/ extract/ standard), 1.75 mL buffer and 40 µL xanthine oxidase (0.33 U/mL in sodium phosphate buffer pH=7.5) were pipetted in quartz cuvette and pre-incubated for 10 min at 25ºC. The reaction was initiated with addition of the substrate solution (100 µL 0.5 mM xanthine in buffer). Then, the mixture was subjected to a second incubation for 12 min at 25ºC. The sample blanks contained all reaction reagents and 40 µL buffer instead of enzyme. The control cuvette contained all the reagents without the sample (the solvents of the samples instead were added). The standard inhibitor of xanthine oxidase, allopurinol (1 mM in DMSO) was used as the positive control.

S8: Microtiter Assay for Determination of α-Amylase Inhibition

As a positive control experiment, acarbose (0.01 - 0.1 mg/mL in buffer) was used. In the experiment, 20 mM sodium phosphate buffer (pH 6.9) was pipetted in the 96-well flat bottom plates with multichannel automatic pipette (Eppendorf Research® plus, Germany), then 25 µL sample solution and 50 µL α-amylase (0.8 U/mL in buffer) were added and incubated for 10 min at 37ºC. After incubation, 50 µL substrate solution was added to the mixture. The mixture was subjected to a second incubation for 10 min at 37ºC. The reaction was stopped by addition of 25 µL HCl (1 M). Finally, 100 µL IKI reagent was added to the wells. The sample blanks contained all reaction reagents and 50 µL buffer instead of enzyme. The control wells contained all reaction reagents and 25 µL solvents of the samples (instead of the sample solution).

S9: Microtiter Assay for Determination of Lipoxygenase Inhibition

The solutions of the oil (5 mg/mL), extract (1 mg/mL) and NDGA (0.1 mg/mL) were prepared in methanol. The substrate solution (4 mM) was prepared as follow: 50 mg linoleic acid, 50 mg Tween-20, 500 µL NaOH (1 M) were mixed and the total volume (40 mL) was adjusted with the buffer (pH=8). The enzyme solution was prepared in buffer (273000 U/mL). In the experiment, 50 µL of the sample solution (oil/ extract/ standard), 2.5 mL buffer and 50 µL of lipoxygenase were mixed in quartz cuvette and incubated for 10 min at 25ºC. Then, the reaction was initiated by adding 25 µL of linoleic acid.

S10: Microtiter Assay for Determination of Tyrosinase Inhibition

In the experiment, four test mixtures were prepared: A (control), B (control blank), C (sample), and D (sample blank), which contained the following reaction mixtures: A, 120 µL of phosphate buffer (0.1 M, pH 6.8) and 40 µL of tyrosinase (33.3 U/mL) in the buffer; B, 160 µL of the buffer; C, 80 µL of the buffer, 40 µL of tyrosinase (33.3 U/mL) in the buffer, 40 µL of the sample-buffer solution containing

DMSO; D, 120 μL of the buffer and 40 μL of the sample solution containing DMSO. Pipetting was performed with multichannel automatic pipette (Eppendorf Research® plus, Germany). As a positive control experiment, kojic acid (0.01-0.1 mg/mL in buffer) was used. The contents of each well were mixed and then preincubated at 23°C for 10 min, before 40 μl L-DOPA (2.5 mM) in the same buffer was added. After incubation at 23°C for 15 min, the absorbance values at 475 nm of the mixtures was measured using an ELISA microplate reader (Biotek Powerwave XS).

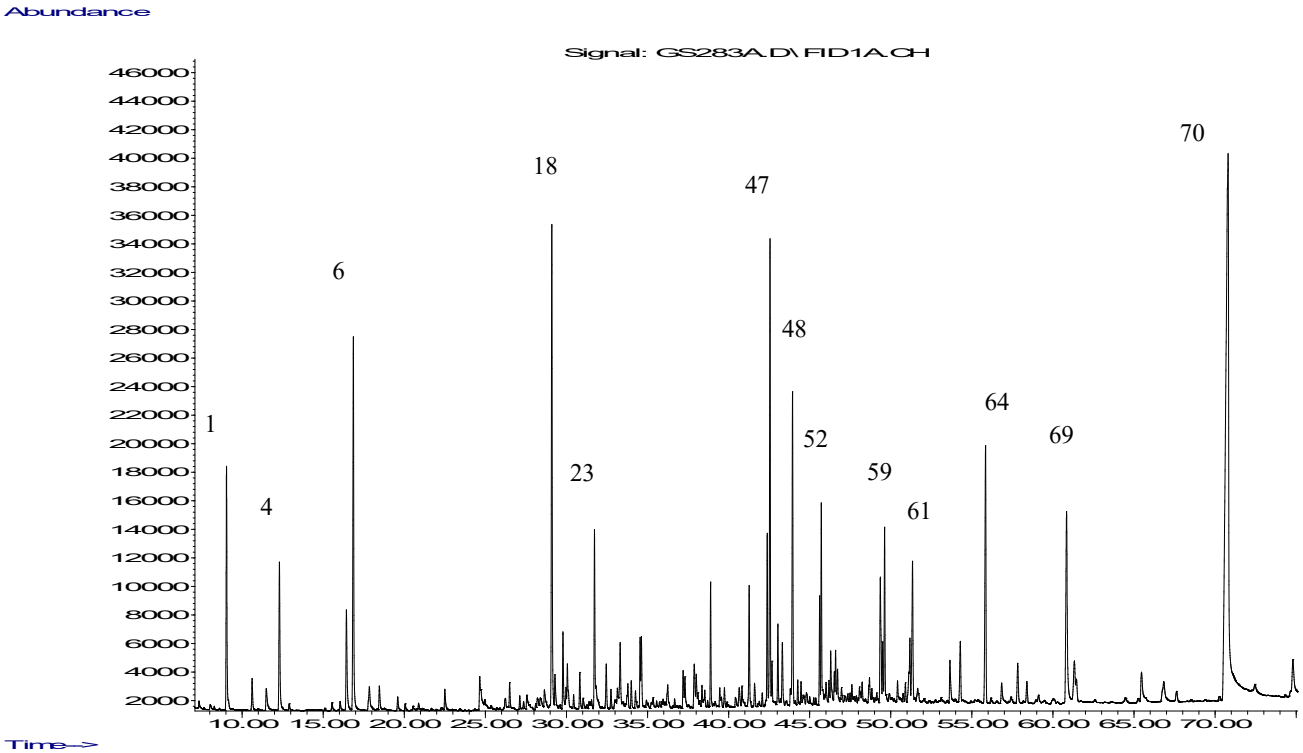

Figure S11: Gas-chromatographic profile of the essential oil of *Cota fulvida*.
Numeration of the peaks is depicted according to list of the detected compounds in Table 1.

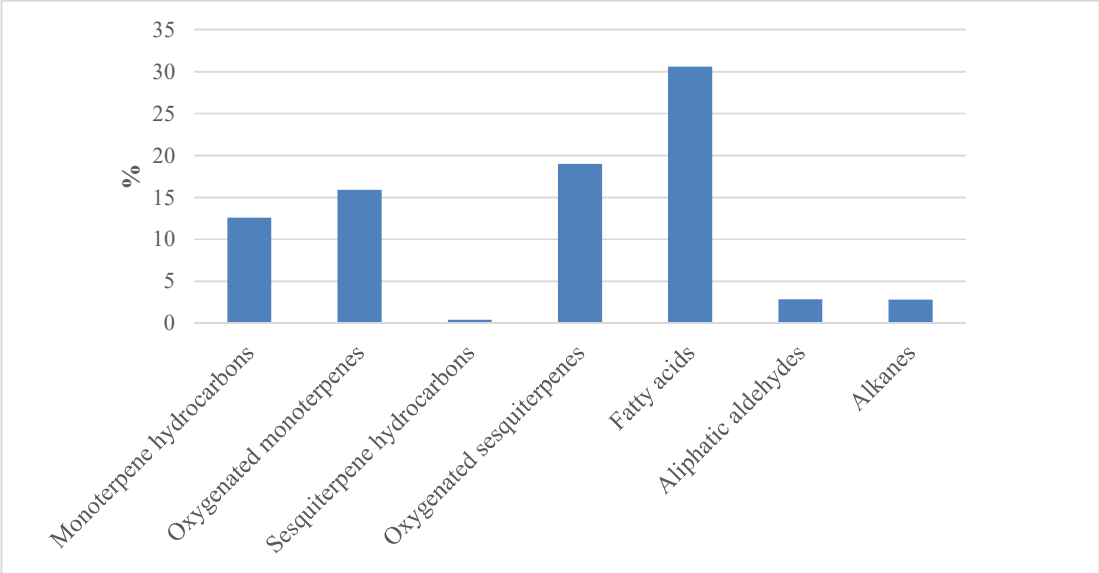

Figure S12. Distribution of the main compound groups detected in the essential oil of *Cota fulvida*

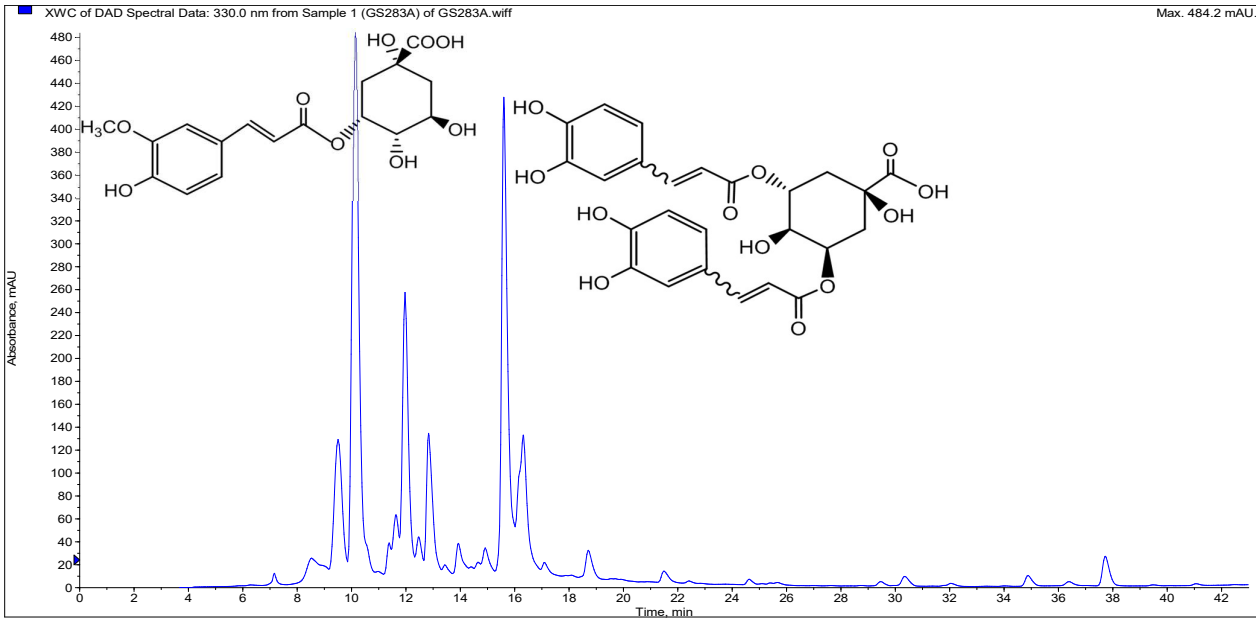

Figure S13: Chromatographic profile of *Cota fulvida* methanol extract obtained with liquid chromatography