Supporting Information

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Chemical Composition, Cytotoxic, Antimicrobial and Antioxidant Activities of Essential oil from *Anthriscus caucalis* M.Bieb Grown in China

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S1: Isolation and identification of the essential oils

The essential oil was obtained by hydrodistillation for 5 h from materials (500 g), using a Clevenger-type apparatus, and dried over anhydrous sodium sulfate. It was stored at 4°C in dark vials until they were analysed.

Gas chromatography analysis:

Gas chromatography (GC) analysis was carried out using a Hewlett Packard 6890 system, equipped with HP-5MS capillary column (30 m×0.25 mm, 0.25 μm film thickness) and connected to a FID detector. Oven temperature was held at 60°C for 1 min and then programmed to 280°C at a rate of 6°C/min, hold at 280 °C for 2 min. Injector and detector (FID) temperature were 280°C. The carrier gas used was helium at a constant flow rate of 1.2 mL/min [1].

Gas chromatography- mass spectrometry

The GC-MS analysis was carried out on Hewlett Packard 6890 gas chromatograph (Agilent) attached to a HP-5MS fused silica column, interfaced with a Hewlett Packard 5975C mass selective detector operated by HP Enhanced ChemStation software. The analytical conditions for GC were the same as those mentioned for GC-FID. The injection volume was 0.2 μL of 1% solution prepared in n-hexane with split ratio 1:50. Mass spectra were acquired in EI mode at 70 eV. The mass range was from *m/z* 50 to 550.

Identification of components

Identification of the constituents was performed on the basis of their retention time, retention indices (relative to C_{10}–C_{30} n-alkanes, under the same experimental conditions), and computer matching with NIST MS Search 2.2 Mass Spectral Database for GC-MS as well as by comparisons of their mass spectra with those of authentic samples or with data already available in the literature.

S2: Cytotoxic activity test

MCF-7 (human breast adenocarcinoma cell line) and HepG2 (liver hepatocellular cells) cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM/L glutamine, and antibiotics (200 U/mL of penicillin and 50 μg/mL of streptomycin). The cells were grown at 37 °C in 5% CO_2 and humidified air atmosphere.

The Cytotoxic activity of the essential oil was estimated by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay against the aforementioned cancer cell lines [2]. The cells (5×10^3 cells per well) were seeded in 96-well cell culture plates in 200 μL of culture medium RPMI 1640 and grown for 24 hours to allow cell attachment. The essential oil was solubilized in DMSO, and afterwards diluted with culture medium for use. Doxorubicin was used as a positive control. The dilutions of the oil

were added to the wells, except the negative control wells where only culture medium was added. All samples were done in triplicate. The microplates were incubated for 24, 48, and 72 h. After incubation, 20 µL of MTT (5 mg/mL in PBS) were added to each well and incubated for 4 hours under the same culture conditions. Formazan crystals were dissolved in 100 µL DMSO. The optical density was measured at 570 nm using an enzyme linked immunosorbent assay (ELISA) reader.

Anticancer activity is expressed as the concentration of the essential oil producing 50% inhibition of cell growth (IC_{50}). The percentages of cell growth were calculated as follows:

$$\text{Cell growth (\%)} = [\text{A (sample)} / \text{A (control)}] \times 100\% \quad (1)$$

S3: Antibacterial activity test

Four bacterial strains were selected for antibacterial test, including the Gram positive bacteria, *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633) and the Gram negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853).

Agar diffusion methods: Antibacterial activities of the essential oil were assessed using the disc agar diffusion method recommended by CLSI [3]. Filter paper discs (6 mm in diameter) were impregnated with 15µL of essential oil or chloramphenicol as a positive control and incubated at 37°C for 18 h. Antibacterial activity was assessed by measuring the inhibition zone. This is the diameter of the zone visibly presenting the absence of bacterial growth, including the 6 mm disk. All the tests were repeated in triplicate.

Determination of the minimum inhibition concentration (MIC): The MIC values were performed in 96 well-microplates using the microdilution assay according to the literature previously described by Ellof (1998) with slight modifications [4]. The essential oil was diluted and transferred into each well (100 µL per well). Chloramphenicol was used as the reference antibiotic control. The inoculum was added to all wells (100 µL per well). And the 12th well was considered as growth control (it contained only the culture medium and strain). The plates were incubated at 36 °C for 18 h. 20 µL of 1% TTC (2,3,5-triphenyl tetrazolium chloride) aqueous solution was used as an indicator of microbial growth [5]. Experiments were carried out in triplicates to minimize experimental error.

S4: Antioxidant activity test

The essential oil was subjected to screening for the possible antioxidant activity by two methods namely DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging [6] and ferric reducing antioxidant power (FRAP) assays [7, 8].

Free Radical Scavenging Activity by DPPH Method

The antioxidant activity of the essential oil was compared to that of a BHT (butylated hydroxytoluene) standard and assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay [9]. Free radical scavenging activity of the essential oil was measured by spectrophotometer at 517 nm. A methanol solution of DPPH was prepared immediately before the assay. Various concentrations of each 2 mL sample were added to 2

mL of DPPH solution. The reaction mixture were shaken and allowed to stand for 30 min at shade. The absorbance of the samples was measured by a spectrophotometer at 517 nm. The experiment was repeated for three times. The DPPH radical-scavenging activity of the essential oil was calculated according the following equation:

$$\text{DPPH radical scavenging activity} = (A_0 - A_1)/A_0 \times 100\% \quad (2)$$

Where A_0 is the control absorbance, and A_1 is the sample absorbance (mixture with the essential oil). The antioxidant activity was expressed as IC_{50} value.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) of the essential oil was quantified using the method proposed by Benzie and Strain (1996) [10], with slight modifications. The FRAP reagent was prepared by mixing sodium acetate buffer (300 mmol/L, pH 3.6), a solution of TPTZ (10 mmol/L) in 40 mmol/L HCl, and 20 mmol/L $FeCl_3 \cdot 6H_2O$ using the proportion 10:1:1 (v/v/v). A 20 µL of appropriately diluted sample extract and 180 µL of FRAP reagent were mixed in a 96-well plate and incubated at 37 °C for 40 min in the dark. In the case of the blank, 20 µL methanol was added to 180 µL FRAP reagent. The absorbance of the resulting solution was measured at 593 nm using a plate reader. An analytical curve with different concentrations of Trolox (linearity: 5–100 µg/mL; $R^2 = 0.998$) was plotted to quantify the ferric reducing antioxidant power of the essential oil. The potential antioxidant activity was expressed as Trolox equivalent antioxidant capacity in µmol Trolox × g^{-1}.

S5: References

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