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Chemical composition, antibacterial activity, and cytotoxicity of essential oil from *Nepeta ucrainica* L. spp. *kopetdaghensis*

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ABSTRACT

Chemical composition, antibacterial activity, and cytotoxicity of essential oil (EO) obtained from the aerial parts of *Nepeta ucrainica* L. spp. *kopetdaghensis* was investigated in the present study. Chemical composition of the EO was analyzed using GC–MS method. Sixty compounds representing 98.7% of the total oil were characterized. The oil was predominated by sesquiterpene hydrocarbons, and the main components were germacrene D (53.0%), bicyclogermacrene (6.4%), β-bourbonene (4.3%), β-elemene (3.3%), spathulenol (3.2%), cubenol (2.8%), *trans*-caryophyllene (2.8%), germacrene A (2.1%), and δ-cadinene (2.0%). The antibacterial activity of the EO was investigated against 6 bacterial strains using serial dilution method. The essential oil showed a good activity against Gram-positive bacteria particularly *Staphylococcus aureus* (minimum inhibitory concentration [MIC] = 14 μg/mL, minimum bactericidal concentration [MBC] = 14 μg/mL), but Gram-negative bacteria were resistant to the oil. Also, the cytotoxic effects of the EO on human ovarian carcinoma A2780 cell line and human breast adenocarcinoma MCF-7 cell line were examined using 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide [MTT] assay. The EO was found to be cytotoxic against the tested cell lines, with IC_{50} values less than 50 μg/mL for both A2780 and MCF-7 cells.

1. Introduction

Essential oils are prosperous and indispensable sources of biologically active natural products (Bakkali et al., 2008; Burt, 2004). Essential oils are reputed for their ethno-medical, culinary, and cosmeceutical applications. Biological activities of EOs include, but are not limited to, antibacterial, antifungal, antiviral, insecticidal, antioxidant, and anti-cancer properties (Burt, 2004; Kordali et al., 2005). The composition of EOs is usually predominated by the presence of terpenoid compounds. The same as other important natural products such as phenolics, flavonoids, and coumarins; terpenoids are secondary metabolites with various biological activities (Zwenger and Basu, 2008). The hydrophobic skeleton and hydrophilic functional groups of terpenoids (in oxygenated terpenoids) enables easy transport of these phytochemicals across biological membranes of microbial and mammalian cells, thereby allowing them to reach cytosol and interact with several enzymes, receptors, and transcription factors (Derouiche et al., 2013).

Nepeta is a large genus belonging to the Lamiaceae family. This genus contains about 280 species, the majority of which are aromatic plants (Jamila et al., 2011; Naghibi et al., 2010). Several species of this genus have been reported to possess medicinal properties such as diuretic, diaphoretic, anti-tussive, antispasmodic, anti-asthmatic, febrifuge, emmenagogue, and sedative effects (Formisano et al., 2011). *Nepeta* spp. has been extensively investigated as a source of natural products with potential anti-tumor,

Abbreviations: A2780, human ovarian carcinoma; DMSO, dimethyl sulfoxide; EO, essential oil; FBS, fetal bovine serum; LPS, lipopolysaccharides; MBC, minimum bactericidal concentration; MCF-7, human breast adenocarcinoma; MHA, Mueller Hinton agar; MIC, minimum inhibitory concentration; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide; *N. ucrainica* L. spp. *kopetdaghensis*, *Nepeta ucrainica* L. spp. *kopetdaghensis*; RI, retention indices; SCDB, soybean casein digest broth; TTC, 2,3,5-triphenyltetrazolium chloride.

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anti-microbial, and anti-fungal properties (Bisht et al., 2010; Salehi et al., 2007; Zomorodian et al., 2012). Phytochemical analyses of *Nepeta* spp. have revealed the presence of several bioactive phytochemicals such as phenolics, flavonoids, and terpenoids (Formisano et al., 2011). *Nepeta ucrainica* L. spp. *kopetdaghensis*, one of the species in the *Nepeta* genus, is a perennial plant that has only been found in the Kopet Dagh mountain range (Northeast of Iran) between Iran and Turkmenistan. It grows to a height of 17–50 cm, with woody, tough and branching root. The pale-green leaves are 2–4.5 cm long and 1–2.5 cm wide. The plant produces blue flowers from May to June (Komarov, 1963).

To our knowledge, the cytotoxic and antibacterial activities of the EO obtained from *N. ucrainica* L. spp. *kopetdaghensis* have not been investigated. Therefore, the present study aimed to investigate the chemical composition, antibacterial (against both Gram-positive and Gram-negative strains), and cytotoxic (against MCF7 and A2780 cell lines) properties of the EO obtained from the aerial parts of *N. ucrainica* L. spp. *kopetdaghensis*.

2. Materials and methods

2.1. Chemicals

Fetal bovine serum (FBS), trypsin–EDTA, L-glutamine and penicillin/streptomycin were purchased from GIBCO. Trypan blue, MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide), and RPMI1640 medium were purchased from Sigma. Mueller Hinton broth (agar) and soybean casein digest broth (SCDB) obtained from Himedia (Mumbai, India). Tween 80 and 2,3,5-triphenyltetrazolium chloride (TTC) were ordered from Merck (Darmstadt, Germany). All solvents were of analytical grade.

2.2. Plant material

The aerial parts of *N. ucrainica* L. spp. *kopetdaghensis* were collected during the flowering stage of plant, from Quchan, Khorasan-Razavi province, Iran, in May 2013. The plant was identified by Mrs. Souzani and a voucher specimen (no. 12914) was deposited at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.

2.3. Isolation of EO

Essential oil was isolated using hydro-distillation of air-dried material for 3 h, with a Clevenger-type apparatus. The oil was obtained as a greenish yellow liquid with a yield of 0.13% (v/w). The oil was dried over anhydrous sodium sulphate and stored at 4 °C in dark until use. The EO was dissolved in *n*-hexane for gas chromatography and mass spectrometry analysis.

2.4. GC–MS analysis

The GC–MS analyses were performed using an Agilent 5975 apparatus with a HP-5 MS column (30 m × 0.25 mm i.d., 0.25 μm film thicknesses) interfaced with a quadruple mass detector and a computer equipped with Wiley 7n.Library. Other analytical settings were: oven temperature: 50 °C (5 min), 50–250 °C (3 °C/min), 250 °C (10 min); injector temperature 250 °C; injection volume: 0.1 μL; split ratio: 1:50; carrier gas: helium at 1.1 mL/min; ionization potential: 70 eV; ionization current: 150 μA; and mass range: 35–465 mui. Identification of individual compounds was made by comparison of their mass spectra and retention indices (RI) with those of authentic samples and those given in the literature (Adams, 2007). Quantification of the relative amount of each individual component was performed according to the area percentage method without consideration of calibration factor.

2.5. Determination of antibacterial activity

2.5.1. Bacterial strains and culture media

The obtained EO from *N. ucrainica* L. spp. *kopetdaghensis* was tested against 3 Gram-positive (*Staphylococcus aureus* PTCC 1337, *Micrococcus luteus* PTCC 1110 and *Bacillus cereus* PTCC 1241) and 3 Gram-negative (*Pseudomonas aeruginosa* PTCC 1074, *Salmonella typhi* PTCC 1609 and *Escherichia coli* PTCC 1338) bacteria. Bacterial strains were cultured overnight at 37 °C on Mueller Hinton agar (MHA).

2.5.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

In order to determine MIC and MBC values, serial two-fold dilutions of EO emulsion were prepared as follows: For the first concentration (15%, v/v equivalent to 135 μg/mL), 1.20 mL of the EO was added to a mixture of polysorbate 80 (400 μL) and SCDB (6.40 mL). The second concentration (10%, v/v equivalent to 90 μg/mL) was similarly prepared. Then, two-fold dilutions were prepared by mixing 4 mL of the previous concentration with the same volume of culture broth, until reaching the final concentration of 14 μg/mL. All samples were homogenized by vortexing before each dilution. Then, 200 μL of each EO concentration in a sterile 96-well microtitre plate was inoculated with 20 μL of a 10^6 CFU/mL overnight bacterial culture. Cell density was determined using a Neubauer hemocytometer under light microscope after staining with trypan blue. All measurements were performed in triplicate. Moreover, negative and positive controls were used for each tested strain. After an overnight incubation at 37 °C, 20 μL of 2,3,5-triphenyltetrazolium chloride (TTC) (5 mg/mL) was added to each well as a colorimetric indicator of bacterial growth and incubated for 30 min at 37 °C. The MIC was determined as the lowest concentration of the EO that showed no color change.

MBC was determined according to the method of Rios et al. (1988). Briefly, 100 μL from each well (without any red dye production) was subcultured on the Mueller Hinton agar plates and incubated at 37 °C for 24 h. The lowest concentration without any bacterial growth represented MBC.

2.6. Cell culture

A2780 (human ovarian carcinoma) and MCF-7 (human breast adenocarcinoma) cells were provided by the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. Cells were maintained in RPMI 1640 supplemented with 10% fetal calf serum 1% (w/v), glutamine, penicillin (100 U/mL) and streptomycin (100 μg/mL). Cultures were incubated at 37 °C under 5% CO_2 in a humidified atmosphere.

2.7. Cell viability assay

Evaluation of cytotoxicity using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay is based on the reduction of tetrazolium dye by mitochondrial enzyme of viable cells (Mosmann, 1983). Briefly, A2780 and MCF7 cells were plated at a density of 10^5 cells/well and 10^4 cells (100 μL per well) into 96-well plates in a humidified atmosphere at 37 °C in 5% CO_2, respectively. Control wells contained no EO, and blank wells contained only growth medium for background correction. After an overnight incubation at 37 °C to allow cell attachment, EOs were solubilized in DMSO, and then diluted in culture medium for use. The maximum concentration of dimethyl sulfoxide (DMSO) in each well was kept below 0.5%. The EO dilutions (10–200 μg/mL) were added to seeded wells in triplicate and incubated for a further 24,