Journal of Saudi Chemical Society (2015) **19**, 97–100

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Antioxidant and cytotoxic activities of essential oil of *Ocimum canum* Sims. from India

M. Tamil Selvi, R. Thirugnanasampandan *, S. Sundarammal

PG and Research Department of Biotechnology, Kongunadu Arts and Science College, GN Mills, Coimbatore 641 029, Tamil Nadu, India

Received 22 October 2011; accepted 31 December 2011
Available online 9 January 2012

KEYWORDS

Lamiaceae;
Free radical scavenging;
MTT assay;
DNA fragmentation

Abstract Chemical composition analysis of essential oil isolated from the leaves of *Ocimum canum* Sims. reported the presence of thirty six compounds. The major compound was camphor (39.77%). *In vitro* antioxidant activity of the isolated oil showed dose dependent free radical scavenging activity against DPPH (IC_{50} 523.55 ± 0.001 μg/mL) and hydroxyl radicals (491.12 ± 0.002 μg/mL), metal chelating (781.38 ± 0.001 μg/mL) and prevention of deoxyribose degradation (168.50 ± 0.003 μg/mL) activities were also calculated. Antioxidant activity of oil was less observed when compared with positive controls such as ascorbic acid and BHT (12.49 ± 0.002 and 30.14 ± 0.005, respectively). Significant cytotoxicity (IC_{50} value of 60 μg/mL) and DNA fragmentation was observed in breast cancer cells (MCF-7).

1. Introduction

The genus *Ocimum* belongs to the Lamiaceae. It is collectively called as Basil, is a diverse and rich source of aromatic essential oil. These essential oils are being used as pharmaceutical agents because of their antimicrobial, antiemetic, antidiabetic, antifertility, antiasthmatic, antistress and anticancer activity (Makker et al., 2007). *Ocimum canum* Sims. is a traditional medicinal plant distributes throughout Tamil Nadu and it is commonly known as Nai Tulasi in Tamil. The plant branches out from its base, with angle stems and open foliage. The plant shows a pungent, aromatic flavor and is commonly cultivated for culinary purposes. *O. canum* is used specially for treating various types of diseases and lowering blood glucose and also treats cold, fever, parasitic infestations on the body and inflammation of joints and headaches (Ngassoum et al., 2004). Essential oil from the leaves of *O. canum* posses antibacterial and insecticidal properties (Bassole et al., 2003).

2. Experimental

2.1. Plant material

The plant material *Ocimum canum* Sims. was collected from local areas of Coimbatore. To avoid causalities some of the plants were maintained under green house condition at Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu.

* Corresponding author. Tel.: +91 9894793074.
E-mail address: rtsampandan@yahoo.com (R. Thirugnanasampandan).
Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org10.1016/j.jscs.2011.12.026

2.2. Essential oil extraction

Freshly collected leaves of *O. canum* were extracted by hydrodistillation for 3 h using a Clevenger-type apparatus. The extracted essential oil was dried over anhydrous sodium sulphate and the purified oil was filled in small vials, tightly sealed and stored in a refrigerator (4 °C) until further analysis.

2.3. Chemical composition analysis

2.3.1. Gas chromatography (GC) analysis

Gas chromatography (GC) analysis was carried out using Varian 3800 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fit with DB 5 MS capillary column (30 m × 0.25 mm). The injector temperature was set at 280 °C, and the oven temperature was initially maintained at 45 °C then set to 300 °C at the rate of 10 °C/min and finally held at 200 °C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. The percentage of composition of the essential oil was calculated by the GC peak areas.

2.3.2. Gas chromatography/ mass spectrometry (GC/MS) analysis

Gas chromatography coupled with mass spectroscopy was performed using Varian 3800 gas chromatography equipped with Varian 1200L single quadrupole mass spectrometer. The GC condition was the same as described earlier. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperatures was maintained at 250 °C. The compounds were identified based on the comparison of retention indices (RI), retention time (RT) and mass spectra. Library search was carried out using the NIST and Wiley GC/MS spectral database and by co comparing with the mass spectral data and retention indices in the literature (Adams, 1995).

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging activity

Radical scavenging activity was determined according to Blois (1958). Different concentrations of the essential oil was dissolved in DMSO and mixed individually with 0.1 mM DPPH and 50 mM Tris–HCl buffer (pH 7.4). Reaction mixture was incubated at 37 °C for 30 min and then absorbance was measured at 517 nm. The percentage of DPPH scavenging activity was calculated using the following equation; %Inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_B, absorption of blank sample, A_A, absorption of test sample.

2.4.2. Hydroxyl radical scavenging activity

Reaction mixture includes 7.5 mM $FeSO_4$, 7.5 mM 1, 10-phenanthroline, 0.2 M phosphate buffer (pH 7.8), 30 mM H_2O_2 and different concentrations of the essential oil. The reaction was started by adding H_2O_2. After incubation at room temperature for 5 min, the absorbance of the mixture was read at 536 nm was measured (Zhao et al., 2006).

2.4.3. Metal chelating activity

Briefly, 2 mM $FeCl_2$ was added to different concentrations of the essential oil. Further, reaction was initiated by the addition of 5 mM ferrozine. The mixture was vigorously shaken and left to stand at room temperature for 10 min. Absorbance was read at 562 nm (Dinis et al., 1994).

2.4.4. Prevention of deoxyribose degradation

The procedure of Halliwell et al. (1987) using Fenton reaction-induced decomposition of deoxyribose has been adapted for this assay. The different concentration of the test sample was mixed with 20 mM deoxyribose, 0.1 M $NaPO_4$, 20 mM H_2O_2 and 50 mM $FeSO_4$. The reaction mixture was incubated for 60 min at 37 °C. Then 2 mL of 10% ice cold trichloroacetic acid was added and 1 mL aliquot of the samples was added with 1 mL of 1% TBA. The TBA/sample mixture was heated in a water bath at 95 °C for another 60 min. The absorbance was read at 532 nm.

2.5. Cytotoxicity

2.5.1. Cell lines and culture

The breast (MCF-7) cell lines were maintained in a humidified incubator at 37 °C and in a 5% CO_2 atmosphere. DMEM containing 10% FBS supplemented with antibiotics penicillin and streptomycin (100 μg/mL).

2.5.2. MTT assay (Lau et al., 2004)

Briefly, after being harvested from culture flasks, the cells were counted using a hemocytometer and cell viability was determined by trypan blue exclusion. 1×10^4 cells were incubated in 96 well plates containing 100 μL of the growth medium per well. Cells permitted to adhere for 24 h and then treated with various concentration of the test sample (essential oil) dissolved in medium for 48 h; 20 μl of 5 mg/mL MTT in phosphate buffer saline was added to each well and the plate was incubated at 37 °C for 4 h. The medium was removed and 100 μL of DMSO added to each well. After incubation at 37 °C for 10 min, absorbance at 570 nm of the dissolved solutions was measured by a microplate ELISA reader. The absorbance of control cells was considered as 100%.

2.5.3. DNA fragmentation (Tong et al., 2004)

Briefly, MCF-7 cells after treatment with different concentrations of test sample (essential oil) for 24 h were centrifuged at 1500 rpm for 10 min and washed with PBS. The resultant pellet was suspended in 250 μL of lysis buffer (10 mM EDTA, 50 mM Tris–HCl, 0.5% SDS) for 15 min at 55 °C. Lysed cells were then digested with proteinase-K (500 μg/mL) at 55 °C for 1 h and followed by incubation with 200 μg/mL DNase-free RNase at 55 °C for 90 min. The DNA was extracted twice with 250 μL of phenol:chloroform:isoamyl alcohol (25:24:1) for 1 min and centrifuged at 12,000 rpm for 5 min. The aqueous phase was further extracted with chloroform:isoamyl alcohol (24:1) and centrifuged. DNA was precipitated from aqueous phase with 0.1 volume of 2 M NaCl and 2.5 volumes of chilled ethanol and kept at −20 °C overnight. The precipitated DNA was centrifuged at 12,000 rpm for 10 min and dissolved in Tris–EDTA buffer (pH 8.0) and electrophoresed in 1.5% aga-