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Short communication

Cytotoxicity screening of essential oils in cancer cell lines

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ABSTRACT

This study evaluated the cytotoxicity activity of the essential oils of *Tagetes erecta* L., Asteraceae (TE-OE), *Tetradenia riparia* (Hochst.) Codd, Lamiaceae (TR-OE), *Bidens sulphurea* (Cav.) Sch. Bip., Asteraceae (BS-OE), and *Foeniculum vulgare* Mill., Apiaceae (FV-OE), traditionally used in folk medicine, against the tumor cell lines murine melanoma (B16F10), human colon carcinoma (HT29), human breast adenocarcinoma (MCF-7), human cervical adenocarcinoma (HeLa), human hepatocellular liver carcinoma (HepG2), and human glioblastoma (MO59J, U343, and U251). Normal hamster lung fibroblasts (V79 cells) were included as control. The cells were treated with essential oil concentrations ranging from 3.12 to 400 μg/ml for 24 h. The cytotoxic activity was evaluated using the XTT assay; results were expressed as IC_{50}, and the selectivity index was calculated. The results were compared with those achieved for classic chemotherapeutic agents. TE-OE was the most promising among the evaluated oils: it afforded the lowest IC_{50} values for B16F10 cells (7.47 ± 1.08 μg/ml) and HT29 cells (6.93 ± 0.77 μg/ml), as well as selectivity indices of 2.61 and 2.81, respectively. The major BS-EO, FV-EO and TE-EO chemical constituents were identified by gas chromatography mass spectrometry as being (*E*)-caryophyllene (10.5%), germacrene D (35.0%) and 2,6-di-*tert*-butyl-4-methylphenol (43.0%) (BS-EO); limonene (21.3%) and (*E*)-anethole (70.2%) (FV-EO); limonene (10.4%), dihydrotagetone (11.8%), α-terpinolene (18.1%) and (*E*)-ocimenone (13.0%) (TE-EO); and fenchone (6.1%), dronabinol (11.0%), aromadendrene oxide (14.7%) and (*E*,*E*)–farnesol (15.0%) (TR-EO). 2,6-di-*tert*-butyl-4-methylphenol (43.0%), (*E*)-anethole (70.2%) and α-terpinolene (18.1%), respectively. These results suggest that TE-OE may be used to treat cancer without affecting normal cells.

Introduction

The search for new drugs that display activity against several types of cancer has become one of the most interesting subjects in the field of natural products research. In this area, plants have played a dominant role in the development of sophisticated traditional medicine systems, especially those with a long history in the treatment of cancer (De Mesquita et al., 2007). Reports on the use of herbs are as old as humanity and have demonstrated that plant-derived essential oils exert better therapeutic activity than their isolated major compounds. In addition, the essential oils are the products of extraction of a plant species, so they are more concentrated and may exhibit higher toxicity than the original plant (Bisset, 1994).

Tagetes erecta L., Asteraceae, an ornamental plant known as marigold, is commonly used to treat bronchitis, rheumatic pain, cold, and respiratory diseases, and which can also be employed as stimulant and muscle relaxant (Neher, 1968). The essential oil from *T. erecta* leaves displays schistosomicidal properties and is utilized as antihelminthic in the Amazonia region (Stasi and Hiruma-Lima, 2002; Tonuci et al., 2012). The monoterpenes α-terpinolene, (*E*)-ocimenone, limonene, (*Z*)-β-ocimene, linalool, dihydrotagetone, piperitone, piperitenone and (*E*)-tagetone are the main chemical constituents of this essential oil (Baslas and Singh, 1981; Krishna et al., 2004; Ogunwande and Olawore, 2006; Sefidkon et al., 2004; Sharma et al., 1961; Singh et al., 2003; Tonuci et al., 2012).

Tetradenia riparia (Hochst.) Codd., Lamiaceae, possesses a variety of medicinal properties in cases of cough, dropsy, diarrhea,

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fever, headaches, malaria, and toothache (Campbell et al., 1997). The essential oil from *T. riparia* leaves displays repellent (Omolo et al., 2004), insecticidal (Dunkel et al., 1990), ascaricidal (Peter and Deogracious, 2006), antimalarial (Campbell et al., 1997), antimicrobial and antinociceptive actions (Gazim et al., 2010). Its oil presents a complex mixture of monoterpenes, sesquiterpenes and diterpenes. The oxygenated diterpenes calyculone, 9β,13β-epoxy-7-abietene and 6,7-dehydroroyleanone; the oxygenated sesquiterpenes 14-hydroxy-9-*epi*-caryophyllene, *cis*-muurolol-5-en-4-α-ol, α-cadinol and ledol and the oxygenated monoterpene fenchone, perillyl alcohol, α-terpineol and β-fenchyl alcohol have been reported as the main chemical constituents of the essential oil of *T. riparia* (Fernandez et al., 2014; Gazim et al., 2010, 2014; Omolo et al., 2004).

Bidens sulphurea (Cav.) Sch. Bip., Asteraceae, many times referred to *Cosmos sulphureus* Cav., a synonymous of *B. sulphurea* in the literature, has anti-icteric and hepatoprotective effects and is traditionally used to treat malaria in Brazil (Botsaris, 2007). The essential oil extracted from the flowers of *B. sulphurea* displays schistosomicidal properties and exhibited significant antibacterial activity that support folkloric use in the treatment of some diseases as broad spectrum antibacterial agents (Aguiar et al., 2013; Ram et al., 2013). 2,6-di-*tert*-butyl-4-methylphenol and the sesquiterpenes β-caryophyllene and germacrene D are reported as the major constituents of the essential oil from *B. sulphurea* flowers (Aguiar et al., 2013).

Foeniculum vulgare Mill., Apiaceae, commonly known as "fennel", is a medicinal and aromatic plant used as carminative, digestive, lactogogue, and diuretic agent, and which can also help to treat respiratory and gastrointestinal disorders (Agarwal et al., 2008). The essential oil of fennel is used as additive in the food, pharmaceutical, cosmetic, and perfume industries (Tinoco et al., 2007), besides having important medicinal properties, such as diuretic, anti-inflammatory, analgesic, antioxidant (Gross et al., 2002), antiseptic, sedative, carminative, stimulant, and vermifugal activities (He and Huang, 2011; Tinoco et al., 2007). In the literature, (*E*)-anethole and the monoterpenes limonene and fenchone are often reported as the main constituents of this essential oil of fennel (Akgul and Bayrak, 1988; Anwar et al., 2009; Cosge et al., 2008; Garcia-Jimenez et al., 2000).

In the present study, we screened the cytotoxicity of essential oils extracted from *T. erecta*, *T. riparia*, *B. sulphurea* and *F. vulgare* against different cell lines. Despite the reports on the biological activities of these essential oils, data on their cytotoxicity are still scarce in the literature (Fabio et al., 2007; Gazim et al., 2014; Villarini et al., 2014).

Materials and methods

Plant material and essential oil extraction

Specimens of *Tagetes erecta* L., Asteraceae, *Tetradenia riparia* (Hochst.) Codd., Lamiaceae, *Bidens sulphurea* (Cav.) Sch. Bip., Asteraceae, and *Foeniculum vulgare* Mill., Apiaceae, were collected at "Sítio 13 de maio" (20°26′ S 47°27′ W 977 m) near Franca, State of São Paulo, Brazil on May 10, 2012 and identified by Prof. Dr Milton Groppo (Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo). Voucher specimens (SPFR 10014, 12421, 12020 and 12024, respectively) were deposited at the Herbarium of this institution (Herbarium SPFR).

Fresh leaves (450 g) of *F. vulgare* (FV-EO), *T. erecta* (TE-EO) and *T. riparia* (TR-EO) and fresh flowers (300 g) of *B. sulphurea* (BS-EO) were submitted to hydrodistillation in a Clevenger-type apparatus for 3 h. After manual collection of the essential oils (EO), anhydrous sodium sulfate was used to remove traces of water, which was followed by filtration. The EO were stored in an amber bottle and kept in the refrigerator at 4 °C until further analysis. The essential oil yields were calculated from the weight of fresh leaves and expressed as the average of triplicate analysis.

GC-FID and GC–MS analyses

BS-EO, FV-EO, TE-EO and TR-EO were analyzed by gas chromatography (GC) on a Hewlett-Packard G1530A 6890 gas chromatograph fitted with FID and data-handling processor. An HP-5 (Hewlett-Packard, Palo Alto, CA, USA) fused-silica capillary column (30 m × 0.25 mm i.d.; 0.33 μm film thickness) was employed. The operation conditions were as follows: the column temperature was programmed to rise from 60 to 240 °C at 3 °C/min and then held at 240 °C for 5 min; carrier gas = H_2, at a flow rate of 1.0 ml/min; injection mode; injection volume = 0.1 μl (split ratio of 1:10); injector and detector temperatures = 240 and 280 °C, respectively. The components relative concentrations were obtained by peak area normalization (%). The relative areas were the average of triplicate GC-FID analyses.

GC–MS analyses were carried out on a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with an AOC-20i autosampler. The column consisted of Rtx-5MS (Restek Co., Bellefonte, PA, USA) fused silica capillary (30-m length × 0.25-mm i.d. × 0.25-μm film thickness). The electron ionization mode was used at 70 eV. Helium (99.999%) was employed as the carrier gas at a constant flow of 1.0 ml/min. The injection volume was 0.1 μl (split ratio of 1:10). The injector and the ion-source temperatures were set at 240 and 280 °C, respectively. The oven temperature program was the same as the one used for GC. Mass spectra were taken with a scan interval of 0.5 s, in the mass range from 40 to 600 Da. BS-EO, FV-EO, TE-EO and TR-EO components identification was based on their retention indices on an Rtx-5MS capillary column under the same operating conditions as in the case of GC relative to a homologous series of *n*-alkanes (C_8–C_{24}); structures were computer-matched with the Wiley 7, NIST 08, and FFNSC 1.2 spectra libraries, and their fragmentation patterns were compared with literature data (Adams, 2005). Standard compounds available in our laboratory were also co-eluted with the essential oils to confirm the identity of some of their components.

Cell lines

Eight different tumor cell lines were used during the experiments: murine melanoma (B16F10), courtesy by Departamento de Bioquímica da Faculdade de Medicina da Universidade de São Paulo, Campus de Ribeirão Preto, São Paulo; colon adenocarcinoma (HT29), human glioblastoma (MO59J, U343, and U251) and human cervical adenocarcinoma (HeLa), obtained from the Cell Bank of Universidade Federal do Rio de Janeiro; human breast adenocarcinoma (MCF-7) and human hepatocellular carcinoma (HepG2), courtesy of Laboratório de Mutagênese do Departamento de Ciências Biológicas da Universidade Estadual Paulista, Campus de Araraquara, São Paulo. In order to compare the cytotoxic effects and the selectivity obtained on tumor cells after the treatment with the essential oils, we also included treatments in a normal cell line (Chinese hamster lung fibroblasts; V79), courtesy of Laboratório de Mutagênese da Universidade Estadual de Londrina, Paraná. The different cell lines were maintained as monolayers in plastic culture flasks (25 cm^2) in culture medium (HAM-F10 + DMEM, 1:1, Sigma–Aldrich or only DMEM) supplemented with 10% fetal bovine serum (Nutricell), antibiotics (0.01 mg/ml streptomycin and 0.005 mg/ml penicillin; Sigma–Aldrich), and 2.38 mg/ml Hepes