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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

(Sigma–Aldrich), at 36.5 °C, with 5% $CO_2$ or in a BOD-type chamber. The cells were used from the 4th passage.

*Cytotoxic activity of the essential oils*

The cytotoxic activity of the essential oils against different cell lines was screened using the Colorimetric Assay *In Vitro* Toxicology – XTT Kit (Roche Diagnostics). For the experiments, $1 \times 10^4$ cells were seeded into microplates with 100 μl of culture medium (1:1 HAM F10/DMEM or DMEM alone) supplemented with 10% fetal bovine serum containing concentrations of the essential oils that ranged from 3.12 to 400 μg/ml. Negative (no treatment), solvent (0.02% DMSO, dimethylsulfoxide, Sigma-Aldrich), and positive (25% DMSO) controls were included. The classic chemotherapeutic agents doxorubicin (DXR, Pharmacia Brasil Ltda., 98% purity), (*S*)-(+)-camptothecin (CPT, Sigma–Aldrich, ≥90% purity), and etoposide (VP16, Sigma–Aldrich, ≥98% purity) were also tested. After incubation at 36.5 °C for 24 h, the culture medium was removed. The cells were washed with 100 μl of PBS (phosphate buffered saline) to remove the treatments and exposed to 100 μl of culture medium HAM -F10 without phenol red. Then, 25 μl of XTT was added, and the cells were incubated at 36.5 °C for 17 h. The absorbance of the samples was determined using a multi-plate reader (ELISA – Tecan – SW Magellan vs 5.03 STD 2P) at a wavelength of 450 nm and reference length of 620 nm.

The experiments were performed in triplicate; 50% inhibition of cell growth ($IC_{50}$) was used as the analysis parameter calculated by Prism Graphpad (version 5.0) software. The One-Way ANOVA analysis was used to compare the mean values ($p < 0.05$).

## Results and discussion

Table 1 shows the chemical composition of the essential oils investigated in this study. Most of the compounds identified in these EO are monoterpenes, sesquiterpenes and phenylpropanoids. Diterpenes were detected only in the essential oil of *T. riparia* (TE-EO).

(*E*)-caryophyllene (10.5%), germacrene D (35.0%) and 2,6-di-*tert*-butyl-4-methylphenol (43.0%) were identified as the major constituents in the essential oil of *B. sulphurea* (BS-EO). These compounds were also the main chemical constituents of a sample of the essential oil from a specimen of *B. sulphurea* from Southeast Brazil (Aguiar et al., 2013).

Limonene (21.3%) and (*E*)-anethole (70.2%) were the major compounds in the essential oil of *F. vulgare* (FV-EO). These compounds have also been found to be the major constituents in essential oils of specimens of *F. vulgare* from different countries (Cosge et al., 2008; Garcia-Jimenez et al., 2000; Wakabayashi et al., 2015). On the other hand, methyl-chavicol and α-phellandrene, which are often detected in the essential oil of *F. vulgare* and were reported as its major compounds in some studies (Chung et al., 2011; Ozcan and Chalchat, 2006), were not detected in FV-EO.

Limonene (10.4%), dihydrotagetone (11.8%), α-terpinolene (18.1%) and (*E*)-ocimenone (13.0%) were found to be the major compounds in the essential oil of *T. erecta* (TE-EO). These monoterpenes have been reported among the main constituents of the essential oil of other *T. erecta* specimens (Baslas and Singh, 1981; Krishna et al., 2004; Ogunwande and Olawore, 2006; Sefidkon et al., 2004; Sharma et al., 1961; Singh et al., 2003). On the other hand, the oxygenated monoterpenes (*Z*)-ocimenone, (*Z*)-tagetone, linallyl acetate and linalool, which were found in the essential oil of other *T. erecta* specimens (Baslas and Singh, 1981; Sharma et al., 1961; Singh et al., 2003), were not detected in TE-EO.

Finally, the major constituents in the essential oil of *T. riparia* (TR-EO) were identified as being fenchone (6.1%), dronabinol (11.0%), aromadendrene oxide (14.7%) and (*E,E*)–farnesol (15.0%). The chemical composition of TR-EO differs from most of the previously investigated *T. riparia* essential oils in the low content of diterpenes. 13-Epimanoyl oxide (7.2%) was the only diterpene identified in TR-EO. The oxygenated diterpenes calyculone, 14-hydroxy-9-*epi*-caryophyllene, *cis*-muurolol-5-en-4-ol, which were identified as major constituents in the essential oil of other *T. riparia* specimens (Fernandez et al., 2014; Gazim et al., 2010, 2014) were not detected in TR-EO.

Table 2 shows the $IC_{50}$ values for the normal and tumor cell lines treated with TE-OE, TR-OE, BS-OE, and FV-OE. Our results showed that the $IC_{50}$ values for tumor cell lines treated ranged from $6.93 \pm 0.77$ to $161.60 \pm 1.41$ μg/ml for TE-OE; from $77.46 \pm 1.75$ to $272.37 \pm 18.45$ μg/ml for TR-OE; from $229.23 \pm 10.40$ to $334.17 \pm 15.50$ μg/ml for BS-OE, and from $112.78 \pm 13.74$ to $406.00 \pm 1.57$ μg/ml for FV-OE. Regarding the normal cell line V79, the $IC_{50}$ values were $19.50 \pm 5.96$, $76.33 \pm 3.44$, $96.50 \pm 1.19$ and $448.00 \pm 19.52$ μg/ml for TE-OE, TR-OE, BS-OE, and FV-OE, respectively.

TE-OE exerted the most pronounced antiproliferative effect against the tumor cell lines. The lowest $IC_{50}$ values were $7.47 \pm 1.08$ and $6.93 \pm 0.77$ μg/ml for the B16F10 and HT29 cell lines, respectively, which were significantly lower than the $IC_{50}$ value obtained for the normal cell line ($19.50 \pm 5.96$ μg/ml). On the other hand, recent studies using the MTT assay reported that *Tagetes erecta* extracts in EtOH and EtOAc did not significantly affect the cell viability of H460 (pleural carcinoma) and Caco-2 (colon carcinoma) cells as compared with the control groups (Vallisuta et al., 2014).

FV-EO was more cytotoxic to B16F10 tumor cells ($IC_{50} = 112.78 \pm 13.74$ μg/ml) than to normal cells ($IC_{50} = 448.00 \pm 19.52$ μg/ml); the selectivity index was 3.97 (Tables 2 and 3). However, according to American National Center Institute, only extracts with $IC_{50}$ values lower than 30 μg/ml against experimental tumor cell lines constitute promising anticancer agents for drug development (Suffness and Pezzuto, 1990). In this sense, TR-EO and BS-EO showed $IC_{50}$ values greater than 30 μg/ml for all cell lines tested, and were more cytotoxic to normal line to which the tumor cell lines.

Table 3 shows the selectivity indices of the essential oils tested against the tumor cell lines and the non-tumor cell line. For all natural products tested, the selectivity indexes were greater than those observed for the chemotherapeutic agents, especially VP16. Literature papers have considered that a value greater than or equal to 2.0 is an interesting selectivity index (Suffness and Pezzuto, 1990). This value means that the compound is more than twice more cytotoxic to the tumor cell line as compared with the normal cell line. According to Bézivin et al. (2003), the selectivity index is interesting in the case of values greater than three.

Treatments with TE-EO and FV-EO afforded the highest selectivity indices, which ranged from 0.23 to 2.81 for TE-EO and from 1.10 to 3.97 for FV-EO (Table 3). These findings demonstrated that TE-EO constitutes a promising essential oil for the development of anticancer drugs, because it provided indices greater than 2. For FV-EO, although the selectivity indices suggested promising antitumor activity, the $IC_{50}$ values were greater than that recommended by Suffness and Pezzuto (1990). In addition, it was not possible to calculate the selectivity index for the majority of the cancer cell lines treated with FV-EO, since it was more cytotoxic to the normal cell line.

The cytotoxicity of the essential oils used in present study can be attributed to the major chemical constituents in the essential oils tested. (*E*)-anethole, the major compound found in FV-EO, caused a concentration and time-dependent cell death in freshly isolated rat hepatocytes (Nakagawa and Suzuki, 2003). Limonene,

**Table 1**
Chemical composition and yields (w/w) of the essential oils of *B. sulphurea* (BS-EO), *F. vulgare* (FV-EO), *T. erecta* (TE-EO) and *T. riparia* (TR-EO).

| Compound | $RI_{exp}$ | $RI_{lit}$ | % RA | | | | Identification |
|---|---|---|---|---|---|---|---|
| | | | BS-EO | FV-EO | TE-EO | TR-EO | |
| α-pinene | 938 | 939 | | 1.5 | 1.3 | | RL, MS, Co |
| camphene | 954 | 953 | | | 0.2 | 0.6 | RL, MS |
| sabinene | 978 | 976 | | 0.8 | 0.8 | 1.0 | RL, MS |
| β-pinene | 983 | 980 | | | tr | 0.5 | RL, MS, Co |
| myrcene | 988 | 991 | | 1.7 | | | RL, MS |
| α-phellandrene | 1007 | 1005 | | | 0.5 | tr | RL, MS |
| limonene | 1033 | 1031 | | 21.3 | 10.4 | 1.2 | RL, MS, Co |
| (*Z*)-β-ocimene | 1040 | 1043 | 0.5 | | 4.2 | 0.5 | RL, MS |
| (*E*)-β-ocimene | 1043 | 1050 | | 0.9 | 0.7 | | RL, MS |
| dihydrotagetone | 1049 | 1054 | | | 11.8 | | RL, MS |
| α-terpinolene | 1084 | 1088 | | | 18.1 | | RL, MS |
| fenchone | 1095 | 1094 | | 1.2 | | 6.1 | RL, MS, Co |
| (*E*)-tagetone | 1151 | 1146 | | | 6.9 | | RL, MS |
| camphor | 1151 | 1143 | | | | 2.0 | RL, MS, Co |
| borneol | 1173 | 1165 | | | | 0.8 | RL, MS, Co |
| terpinen-4-ol | 1184 | 1177 | | | | 0.7 | RL, MS |
| α-terpineol | 1197 | 1189 | | | | 1.0 | RL, MS |
| estragol | 1198 | 1195 | | 1.8 | | | RL, MS |
| verbenone | 1218 | 1230 | | | 9.7 | | RL, MS |
| (*E*)-ocimenone | 1238 | 1239 | | | 13.0 | | RL, MS |
| piperitone | 1252 | 1252 | | | 8.8 | | RL, MS |
| (*E*)-anethole | 1287 | 1293 | | 70.2 | | | RL, MS, Co |
| piperitenone | 1335 | 1342 | | | 9.7 | | RL, MS |
| α-copaene | 1399 | 1376 | | | | 0.8 | RL, MS |
| β-elemene | 1416 | 1391 | 2.1 | | | 1.5 | RL, MS |
| α-gurjunene | 1426 | 1409 | | | | 4.5 | RL, MS |
| (*E*)-caryophyllene | 1441 | 1428 | 10.5 | | 1.2 | 1.6 | RL, MS, Co |
| α-*trans*-bergamotene | 1460 | 1436 | | | | 0.4 | RL, MS |
| precocene I | 1458 | 1467 | | | 1.4 | | RL, MS |
| α-humulene | 1467 | 1467 | 0.5 | | | 0.7 | RL, MS, Co |
| germacrene D | 1488 | 1480 | 35.0 | | 0.9 | tr | RL, MS |
| aromadendrene | 1490 | 1491 | | | | 0.5 | RL, MS |
| viridiflorene | 1500 | 1493 | | | | 1.3 | RL, MS |
| bicyclogermacrene | 1507 | 1494 | 5.8 | | | 0.6 | RL, MS |
| *E,E*-α-farnesene | 1503 | 1508 | 0.4 | | | 3.1 | RL, MS |
| 2,6-di-*tert*-butyl-4-methylphenol | 1523 | 1519 | 43.0 | | | 0.3 | RL, MS, Co |
| δ-cadinene | 1527 | 1524 | 0.4 | | | 2.7 | RL, MS |
| *cis*-nerolidol | 1531 | 1539 | | | | 1.5 | RL, MS |
| germacrene-D-4-ol | 1572 | 1574 | | | tr | 5.4 | RL, MS |
| spathulenol | 1575 | 1576 | 0.5 | | | 0.3 | RL, MS |
| caryophyllene oxide | 1577 | 1581 | 1.0 | | tr | tr | RL, MS |
| viridiflorol | 1590 | 1590 | | | | 2.4 | RL, MS |
| α-cadinol | 1650 | 1653 | | | | 3.0 | RL, MS |
| α-muurolol | 1655 | 1657 | | | | 0.5 | RL, MS |
| t-cadinol | 1664 | 1660 | | | | 5.6 | RL, MS |
| aromadendrene oxide | 1672 | 1668 | | | | 14.7 | RL, MS |
| *E,E*-farnesol | 1702 | 1706 | | | | 15.0 | RL, MS |
| 13-epimanoyl oxide | 1996 | 2002 | | | | 7.2 | RL, MS |
| dronabinol | 2190 | 2202 | | | | 11.0 | RL, MS |
| Total | | | 99.7 | 99.4 | 99.8 | 99.2 | |
| Monoterpene hydrocarbons | 0.5 | 26.2 | 18.1 | 4.1 | | | |
| Oxygenated monoterpenes | | 1.2 | 78.0 | 10.6 | | | |
| Sesquiterpene hydrocarbons | 54.7 | | 2.1 | 17.9 | | | |
| Oxygenated sesquiterpenes | 1.5 | | 0.2 | 55.6 | | | |
| Others | 43.0 | 72.0 | 1.4 | 11.0 | | | |
| Yield (w/w) | 0.24% | 1.60% | 0.53% | 1.26% | | | |

$RI_{exp}$: Retention index determined relative to *n*-alkanes ($C_8$–$C_{20}$) on the Rtx-5MS column. $RI_{lit}$: Retention index from the literature (Adams, 2005). Compound identification: RL, comparison of the RI with those of the literature (Adams, 2005); RA: relative area (peak area relative to the total peak area in the GC-FID chromatogram), average of three replicates; MS, comparison of the mass spectra with those of the Wiley 7, NIST 08, and FFNSC 1.2 spectral libraries as well as with those of literature (Adams, 2005); Co: co-elution with standard compounds available in our laboratory. tr: relative area lower than 0.1%. Yields were expressed as the average of three replicates (3 × 150 g for FV-EO, TE-EO and TR-EO and 3 × 100 g for BS-EO).

one the major compounds found on the BS-EO and TE-EO, combined with docetaxel significantly enhanced the cytotoxicity to normal prostate epithelial cells (DU-145) (Rabi and Bishayee, 2009).

Our results demonstrated that TE-EO exerted a selective and cytotoxic activity against tumor cell lines. Therefore, this essential oil should be considered a promising source to develop specific antitumor drugs.

## Author's contributions

HJD contributed in the essential oil extractions and GC-FID and GC–MS analyses. PFO, JMA, JLD and RAMO contributed to biological assays and helped in the draft of the manuscript. AEMC collected the plant, confected the herbarium samples to taxonomic identification, supervised the GC-FID and GC–MS analyses and helped in

**Table 2**
$IC_{50}$ values found for the different cell lines after treatment with the essential oils of *T. erecta* (TE-EO), *T. riparia* (TR-EO), *B. sulphurea* (BS-EO), *F. vulgare* (FV-EO) and the positive control: etoposide (VP-16), (*S*)-(−)camptothecin (CPT) and doxorrubicin (DXR).

| Treatments | $IC_{50}$ (μg/ml)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | V79 | B16F10 | HT29 | MCF-7 | HeLa | HepG2 | MO59J | U343 | U251 |
| TE | 19.50 ± 5.96 | 7.47 ± 1.08 | 6.93 ± 0.77 | 63.42 ± 2.42 | 26.02 ± 5.52 | 161.60 ± 1.41 | 38.69 ± 5.51 | 83.56 ± 10.81 | 75.56 ± 7.11 |
| TR | 76.33 ± 3.44 | 272.37 ± 18.45 | 77.46 ± 1.75 | 129.57 ± 10.68 | 155.70 ± 19.09 | 140.97 ± 11.29 | 217.97 ± 13.55 | 221.30 ± 9.80 | 109.90 ± 2.83 |
| BS | 96.50 ± 1.19 | 230.00 ± 19.50 | 268.70 ± 8.23 | 253.75 ± 15.91 | 334.17 ± 15.50 | 241.07 ± 9.49 | 229.23 ± 10.40 | 243.53 ± 0.87 | 236.30 ± 2.33 |
| FV | 448.00 ± 19.52 | 112.78 ± 13.74 | NE | NE | NE | NE | 406.00 ± 1.57 | NE | NE |
| VP-16 | 1.28 ± 0.05 | 48.91 ± 8.53 | 325.40 ± 6.79 | 82.67 ± 15.62 | 225.50 ± 31.82 | 235.37 ± 6.47 | 58.94 ± 3.10 | 2.18 ± 0.99 | 42.97 ± 0.40 |
| CPT | 3.27 ± 0.14 | 20.17 ± 1.98 | 7.34 ± 1.47 | 36.09 ± 12.46 | 19.38 ± 0.76 | 11.87 ± 1.96 | 15.55 ± 0.65 | 5.71 ± 1.05 | 11.14 ± 2.79 |
| DXR | 0.57 ± 0.27 | 3.81 ± 0.18 | 101.62 ± 24.15 | 5.39 ± 1.35 | 21.90 ± 9.09 | 62.13 ± 2.04 | 6.98 ± 2.13 | 0.70 ± 0.35 | 16.28 ± 2.51 |

[a] The values are mean ± standard deviation. $IC_{50}$ (concentration inhibiting 50% growth). V79 (normal hamster lung fibroblasts); B16F10 (murine melanoma); HT29 (human colon carcinoma); MCF-7 (human breast adenocarcinoma); HeLa (human cervical adenocarcinoma); HepG2 (human hepatocellular carcinoma); and MO59J, U343 and U251 (human glioblastoma). NE – not effective.

**Table 3**
Selectivity of the cytotoxicity of *T. erecta* (TE-EO), *T. riparia* (TR-EO), *B. sulphurea* (BS-EO) and *F. vulgare* (FV-EO) essential oils to tumor cells as compared with V79 cells.

| Treatments | Selectivity index[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | B16F10 | HT29 | MCF-7 | HeLa | HepG2 | MO59J | U343 | U251 |
| TE | 2.61 | 2.81 | 0.30 | 0.74 | 0.12 | 0.50 | 0.23 | 0.25 |
| TR | 0.28 | 0.98 | 0.58 | 0.49 | 0.54 | 0.35 | 0.34 | 0.34 |
| BS | 0.41 | 0.35 | 0.38 | 0.28 | 0.39 | 0.42 | 0.39 | 0.40 |
| FV | 3.97 | NE | NE | NE | NE | 1.10 | NE | NE |
| VP16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.60 | 0.00 |
| CPT | 0.10 | 0.40 | 0.40 | 0.00 | 0.10 | 0.20 | 0.60 | 0.30 |
| DXR | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.70 | 0.00 |

[a] The selectivity index is the ratio of the $IC_{50}$ values of the treatments on V79 cells to those in the cancer cell lines. B16F10 (murine melanoma); HT29 (human colon carcinoma); MCF-7 (human breast adenocarcinoma); HeLa (human cervical adenocarcinoma); HepG2 (human hepatocellular carcinoma); and MO59J, U343, and U251 (human glioblastoma). NE–not effective

the draft of the manuscript. DCT supervised the biological tests. All the authors have read the final manuscript and approved the late submission.

## Conflicts of interest

The authors declare no conflicts of interest.

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