Antiproliferative Activity of EO Extracted from Different Aromatic Plants on Different Cell Lines

Abstract

Essential Oils (EOs) are used in many products to be intended for human utilization. Despite their pharmacological applications in the folk and traditional medicine, studies on EO anti-proliferative properties are still limited. The aim of this study was to investigate the anti-proliferative properties of hydrol distilled EO from 16 aromatic plants grown in Yemen. The cytotoxicity of the EOs was determined against seven cell lines namely; HeLa, MCF7, MDA-MB 231, CEMs, WEHI-3B, 3T3 and CHO cell lines using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) assay. Lantana camara EO was the most cytotoxic extract against all tested cell lines with an IC50 value of ≤ 0.01 % (v/v) except the non-tumorous cell lines CHO with IC50 value of 0.025 % ± 0.005 % (v/v). While Cinnamomum zeylanicum EO showed high cytotoxic activity against Hela, MDA-MB 231, 3T3 and CHO cell lines with IC50 value of ≤ 0.010 % (v/v). EO from Ocimum basilicum and Mentha piperita were among the ones with no activity or IC50 value of > 0.010 % (v/v). The results demonstrated the potential of the EO from aromatic plants from Yemen for possible source of cancer treatment.

Keyword: Cytotoxicity; Antioxidants; EOs; Cell lines; Medicinal plants

Introduction

Essential oils (EOs) recently have got substantial importance in phytomedicine [1,2] due to their increase application in various industries of perfumery, cosmetics, pharmaceutical and foods. They have shown great biological activities, such as antimicrobial and antioxidant activities [3-5]. EOs are concentrated oily liquids extracted from different plant parts, such as flowers, buds, seeds, twigs, bark, herbs, wood, fruits or roots. They are very complex mixtures of complex compounds derived from terpenes and their oxygenated compounds, such as monoterpenes and sesquiterpenes, with the general formula (C5H8)n. These compounds could include alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides. They have shown to possess antibacterial, antifungal, antiviral, and antioxidant activities [6,7]. In addition, some EOs have been used in cancer treatment [2], aromatherapy, food preservation [8] and fragrance industries.

EOs represent good source for a vast array of bioactive biological compounds with good antioxidants, anticancer and antibacterial activities as well as food additives and preservatives. Cancer chemotherapy has life-threatening side effects manifested by the complication of the chemotherapy in addition to the anticancer drug resistance necessitate the search for natural anticancer agents that proven to have fewer side effects to cancer patients. As a result, there are great efforts to replace synthetic anticancer compounds by natural secondary metabolites, such as EOs. For identification and development of novel anticancer agents, natural products remain the potential source for anticancer drug discovery, since the majority of the anticancer drugs used are of natural origin [9,10]. Compounds isolated from natural products played an important role in anticancer therapy, since about half of the anticancer drugs used during the last decades are of natural resources [11,12]. Their contents of phytochemicals that can suppress cancer initiation and development, inhibit cellular proliferation, reduce inflammatory process and malignancy transformation. EOs considered a promising resource as anticancer [13,14]. At present time various industries, are looking into sources of alternative, more natural and environmentally friendly. In other words, pharmaceutical firms are mainly interested in the discovery of active chemical structures from which can develop and prepare synthetic analogues. These are more controllable from point of reproducibility, patentability, safety, and are more economically viable. Therefore, the present work, aimed to screen EO from sixteen herbal plants grown in Yemen for their antiproliferative activity against seven cell lines.

Materials and Methods

Plant materials

Plant materials were collected during summer 2008, from three different regions in Yemen. They were identified by Dr Abdulwali Ahmed Al-Khalidi, the botanists at the Department of Biology, Faculty of Sciences, Taiz University. The plants, which screened for their anti-proliferative activities are shown Table 1.

Extraction of EOs

Two hundred grams (200g) of plant samples were subjected to hydrodistillation for approximately three hours using a Clevenger...
Human cervical cancer cells (HeLa), human breast cancer cell lines (MCF-7), human mammary cancer cell lines—estrogen negative (MDA-MB-231), human colon carcinoma cell lines (HT-29), mouse fibroblast cell lines (3T3) and murine monomyelocytic leukemia cell lines (WEHI-3) were obtained from ATCC. While Chinese hamster ovary cell line (CHO) from ECACC and T4-lymphoplastoid cell lines (CEMss) from NIH (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: USA). Cell lines were grown in RPMI 1640 supplemented with 10% fetal calf serum, 1% penicillinstreptomycin and 1% amphotericin B. Flasks containing cell lines were incubated in a humidified incubator at 37 °C. Cultures were frequently examined under inverted microscope (Micros, Austria). Once cells reached 80% confluency, media was removed and the cells were washed 3 times with 7mL of PBS (Phosphate Buffer Saline). Two milliliters of trypsin was added to the adherent cells and were incubated for 5 minutes. The flask was tapped gently to detach cells from the wall of the flask to appear as single cells. Ten milliliters of RPMI 1640 with 10% FCS were added and incubated in CO2 incubator at 37 °C. After 24 hours incubation, content of each well was decanted and cells were treated with different concentrations of EOs (in a concentration range of 0.015 to 2.0µl/mL, 0.1% DMSO) for 72 hours. The MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetraazolium bromide) assay used has been described by Mossman (1983) [15], powder was purchased from Amresco and the DMSO (Dimethylsulphoxide) was purchased from Sigma Aldrich, Germany. Twenty microliters of 5mg/mL MTT (Micro culture Tetrazolium) solution was added into each well. Plates were covered with aluminum foil and incubated at 37 °C (5% CO2) for 4 hours in dark in order to allow the active live cells to convert water soluble yellow MTT solution into water insoluble purple formazan. After 4 hours of incubation, the media containing MTT solution was aspirated. The remaining purple formazan was dissolved by adding 100µl DMSO into each well. MTT assay reading was performed using ELISA plate reader at 450 nm (TECAN, SunriseTM, Männedorf, Switzerland). The IC50 value (Concentration at which 50% of the cells are viable and another 50% cells killed) was determined from the dose-response curve (%cell viability versus concentration of EO or cisplatin).

Cytotoxicity assay (MTT)

EOs were solubilized with DMSO and diluted in RPMI 1640 media to give final concentrations of 10µl/mL. Substock solution of 100µM cisplatin was prepared from the stock solution 1mg/mL as a control for the test system. Cells were washed 3 times with 7mL of PBS and 2.5mL of trypsin were added to the adherent cells and were incubated for 5 minutes in the CO2 incubator. Once the cells were detached from the flask, 10 mL of RPMI with 5% FCS was added. Cells density was determined using a hemocytometer. One hundred microliters of cell suspension were plated in each well of 96 well plates at concentration of 1 x 105 cells/mL. After 24 hours incubation, content of each well was decanted and cells were treated with different concentrations of EOs (in a concentration range of 0.015 to 2.0µl/mL, 0.1% DMSO and cisplatin (as negative and positive controls, respectively). The cells were incubated in CO2 incubator at 37 °C for 3 days (72 hours). The MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetraazolium bromide) assay used has been described by Mossman (1983) [15], powder was purchased from Amresco and the DMSO (Dimethylsulphoxide) was purchased from Sigma Aldrich, Germany. Twenty microliters of 5mg/mL MTT (Micro culture Tetrazolium) solution was added into each well. Plates were covered with aluminum foil and incubated at 37 °C (5% CO2) for 4 hours in dark in order to allow the active live cells to convert water soluble yellow MTT solution into water insoluble purple formazan. After 4 hours of incubation, the media containing MTT solution was aspirated. The remaining purple formazan was dissolved by adding 100µl DMSO into each well. MTT assay reading was performed using ELISA plate reader at 450 nm (TECAN, SunriseTM, Männedorf, Switzerland). The IC50 value (Concentration at which 50% of the cells are viable and another 50% cells killed) was determined from the dose-response curve (%cell viability versus concentration of EO or cisplatin).

Table 1: Aromatic plants, site of their collection, and parts used for EOs hydrodistillation.

| Botanical Name          | Site of Collection Part Used | Botanical Name          |
|-------------------------|-----------------------------|-------------------------|
| Artemisia abrotanum     | Thamar                      | Aerial Name             |
| Chenopodium ambrosioides| Imported (India)             | Bark                    |
| Cinnamomum zeylanicum   | Imported (India)             | Fruits                  |
| Clove Eugenia caryophyllata | Imported (India)         | Seeds                   |
| Conyza incana (Vah) wildd | Taiz-Hojarash              | Seeds                   |
| Coriandrum sativum      | Sana’a                      | Fresh aerial parts      |
| Eucalyptus camaldulensis | Sana’a                      | Dried leaves            |
| Lantana camara          | Sana’a                      | Fresh leaves            |
| Mentha piperita         | Amman                       | Fresh leaves            |
| Ocimum basilicum        | Sana’a                      | Fresh leaves            |
| Origanum majorana hortensis | Sana’a                  | Fresh aerial part       |
| Rosmarinus officinalis  | Sana’a                      | Fresh leaves            |
| Pulicaria jaubertii     | Sana’a                      | Fresh aerial parts      |
| Schinus molle           | Sana’a                      | Fruits                  |
| Tagetes minuta          | Sana’a                      | Fresh aerial parts      |
| Thymus laeigatus        | Sana’a-Alhimah              | Dried aerial parts      |

Cell cultures and maintenance

Human cervical cancer cells (HeLa), human breast cancer cell lines (MCF-7), human mammary cancer cell lines—estrogen negative (MDA-MB-231), human colon carcinoma cell lines (HT-29), mouse fibroblast cell lines (3T3) and murine monomyelocytic leukemia cell lines (WEHI-3) were obtained from ATCC. While Chinese hamster ovary cell line (CHO) from ECACC and T4-lymphoplastoid cell lines (CEMss) from NIH (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: USA).
Results and Discussion

Seven cell lines have been exposed to increasing concentrations of EOs, namely HeLa, MCF-7, MDA-MB-231, 3T3, CEMss, WEHI-3B, and CHO cell lines. Cell survival was determined by the MTT assay. In vitro cytotoxic activity of the EOs is shown in Table 2. The EOs revealed different cytotoxic activities towards the seven cell lines under investigation. Results present the IC50 values of the EOs (the EO concentration needed to reduce proliferation by 50% after 72 h of incubation compared to control wells). IC50 values were determined by plotting dose response curve for the EO in the range of (0.0015 to 0.100 % v/v). The IC50 value of the reference drug cisplatin ranged from 3.1 to 16.1 μg/mL against all tested cell lines. Lantana camara EO exhibited the most effective cytotoxic activity towards most of the cell lines tested with an IC50 ≤ 0.01 % v/v, except CHO cell lines in which the IC50 value was found to be (0.025±0.005 % v/v). In contrast, Lantana camara EO exhibited less pronounced cytotoxicity against CHO cell line, a non-tumorous cell line, which makes it promising as a good anticancer candidate for further investigations. The cytotoxicity of oleanolic acid extracted from Lantana camara has been described previously and found to exhibit promising anticancer activity against A375 (malignant skin melanoma) cell lines [16]. Meanwhile, the leaf extract was found to exhibit a strong antioxidant effect using the DPPH and the reducing power assays [17, 18]. The other active EO with cytotoxic activity was the essential of Cinnamomum zeylanicum in which the IC50 value towards HeLa, MDA-MB-231, 3T3 and CHO was shown to be (0.006±0.0003, 0.008±0.0002, 0.006±0.0009 and 0.006±0.0007 % v/v, respectively). In addition, EOs of Eucalyptus camaldulensis and Thymus laevigatus were found to have marked cytotoxicity against some cell lines. They were found to be active against the leukemic cell lines, CEMss (IC50 = 0.0063 ± 0.0012 and 0.007 ± 0.0005 % v/v respectively) and WEHI-3B (IC50= 0.0063 ± 0.0006 and 0.0065 ± 0.0007 % v/v respectively). In addition EO from Schinus molle was found to be active against WEHI-3B (IC50 = 0.008 ± 0.0002 % v/v), Tagetes minuta against MDA-MB-231 (IC50 = 0.003 ± 0.0006 % v/v) and Coriandrum sativum against CEMss (IC50 = 0.0065 ± 0.0007 % v/v). Meanwhile, all the EOs were shown to exhibit less or no cytotoxic activity towards the normal epithelial cell lines CHO except the Cinnamomum zeylanicum EO (Table-2). Cytotoxicity of the EOs could be attributed to their constituents of complex mixtures of monoterpens and sesquiterpenes as reported in the literature [19]. Antitumor activity of EOs has been reported by various authors, such as the EO of thyme, which contains carvacrol, has a marked in vitro cytotoxic activity against tumor cells [20]. In this study, the EOs displayed the strongest antiproliferative activity was found to show moderate antioxidant activity (unpublished work) suggesting that antioxidant effects have moderate effects on the antiproliferative activity. This observation has been supported statistically by Baharum and his coworkers [21], who reported that the anti-cancer activities of Theobroma cacao plant extracts showed a negative moderate correlation with their antioxidant activity.

Table 2: The cytotoxicity effects of different levels of EOs.

| Essential Oil                  | Cell Lines IC50 (Mean ±STD) (%v/v) |
|-------------------------------|-----------------------------------|
|                               | HeLa     | MCF-7    | MDA-MB-231 | CEMss   | WEHI-3B | 3T3     | CHO      |
| Artimisia abrotanum           | 0.042±0.0045 | 0.062±0.008 | 0.10±0.005  | 0.025±0.006 | ND      | ND      | 0.0125±0.004 |
| Chenopodium ambrosioides      | 0.055±0.0070 | 0.021±0.003  | 0.020±0.007  | 0.025±0.003  | 0.0125±0.003 | 0.023±0.007  | 0.035±0.015 |
| Cinnamomum zeylanicum         | 0.007±0.0003 | 0.012±0.005  | 0.008±0.002  | 0.028±0.005  | ND      | ND      | 0.006±0.0009  | 0.006±0.0007 |
| Conyza incana (vah) wildd     | 0.036±0.008  | 0.027±0.005  | 0.012±0.003  | 0.0125±0.004  | 0.0127±0.008  | 0.0150±0.005  | 0.018±0.007  |
| Coriandrum sativum            | no activity | no activity | no activity | 0.0065±0.007  | 0.025±0.009  | 0.050±0.008  | 0.09±0.008  |
| Clove Eugenia caryophyllata   | 0.024±0.004  | 0.025±0.005  | 0.032±0.006  | 0.015±0.0016  | ND      | 0.027±0.005  | 0.06±0.008  |
| Eucalyptus camaldulensis       | 0.13±0.031  | 0.107±0.021  | 0.055±0.003  | 0.0063±0.0012  | 0.0063±0.0006  | 0.011±0.04  | 0.023±0.004  |
| Lantana camara                | 0.008±0.0035 | 0.0063±0.0008  | 0.0063±0.0004  | 0.003±0.0002  | 0.0068±0.0006  | 0.010±0.003  | 0.025±0.005  |
| Mentha piperita               | no activity | no activity | 0.210±0.05  | 0.0125±0.003  | 0.025±0.006  | 0.120±0.020  | no activity |
| Ocimum basilicum              | no activity | no activity | 0.210±0.04  | 0.025±0.004  | 0.0185±0.0004  | 0.035±0.008  | 0.060±0.005  |
| Origanum majorana             | 0.15±0.030  | 0.110±0.020  | 0.11±0.04  | 0.036±0.005  | 0.03±0.005  | 0.025±0.006  | 0.040±0.006  |
| Pulicaria jauberti            | 0.080±0.020  | 0.060±0.007  | 0.060±0.0055  | 0.070±0.0035  | 0.0125±0.004  | 0.038±0.008  | 0.080±0.006  |
| Rosmarinus officinalis         | 0.130±0.027  | 0.112±0.018  | 0.110±0.02  | 0.025±0.007  | 0.026±0.006  | 0.060±0.009  | 0.080±0.009  |

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## Stock solution of the EO was prepared by dissolving the EO in DMSO (final conc. No more than 1%) and diluted by culture media to give a concentration of 1µl EO/mL media.

ND: NOT Determined

### Conclusion

Lantana camara EO was the most cytotoxic extract against all tested cell lines, except the non-tumorous cell lines CHO. While Cinnamomum zeylanicum EO showed high cytotoxic activity against HeLa, MDA-MB 231, 3T3 and CHO cell lines. EOs from Ocimum basilicum and Mentha piperita were among the ones with no activity or low IC50 value. The EOs from different plants were shown to exhibit less or no cytotoxic activity towards the normal epithelial cell lines CHO except the Cinnamomum zeylanicum EO. Chemical constituent analysis of those effective oils will be beneficial for further development of new chemotherapeutic agents. These results demonstrated the potential beneficial use of the EOs extracted from aromatic plants from Yemen as possible source of cancer treatment.

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### Conflicts of Interest

The authors declare no conflict of interest.

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### Table: Antiproliferative Activity of EO Extracted from Different Aromatic Plants on Different Cell Lines

| Plant Species        | Cisplatin (µg/mL)  | Schinus molle       | Tagetes minuta     | Thymus laevigatus | Cipletin (µg/mL)  |
|----------------------|--------------------|---------------------|--------------------|-------------------|------------------|
|                      | 0.06±0.016         | 0.045±0.005         | 0.04±0.006         | 0.025±0.004       | 0.008±0.0002     |
|                      | ND                 | 0.0065±0.0007       | 0.03±0.0006        | 0.03±0.0035       | 0.01±0.007       |
|                      | ND                 | 4.2±0.05            | 0.027±0.006        | 0.018±0.003       | 0.007±0.0005     |
|                      | ND                 | ND                  | ND                 | ND                | ND               |
|                      | ND                 | ND                  | ND                 | ND                | ND               |
|                      | ND                 | ND                  | ND                 | ND                | ND               |
|                      | ND                 | ND                  | ND                 | ND                | ND               |
|                      | ND                 | ND                  | ND                 | ND                | ND               |
|                      | ND                 | ND                  | ND                 | ND                | ND               |

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