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Anticancer activity of *Salvia officinalis* essential oil and its principal constituents against hormone-dependent tumour cells

G. Privitera[1]✉, T. Luca[1], S. Castorina[1,2], R. Passanisi[1], G. Ruberto[3], E. Napoli[3]

[1]*Fondazione Mediterranea "G.B. Morgagni", Catania 95125, Italy*

[2]*Department of Medical and Surgical Sciences and advanced technologies, University of Catania, Catania 95123, Italy*

[3]*Institute of Biomolecular Chemistry, Italian National Research Council ICB-CNR, Catania 95126, Italy*

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ABSTRACT

Objective: To investigate the *in vitro* antiproliferative action of essential oil from *Salvia officinalis* L. (*S. officinalis*) grown in Sicily (Italy), and its main components on hormone-dependent cancer cell lines. **Methods:** *S. officinalis* essential oil was prepared by hydrodistillation. The actions of the *S. officinalis* essential oil and its three principal components (α -thujone, 1,8-cineole and camphor) were evaluated in LNCaP cells (prostate carcinoma), MCF7 cells (breast carcinoma) and HeLa cells (cervical carcinoma) at various dosages and diverse time points. Cell viability and proliferation were estimated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. **Results:** *S. officinalis* essential oil at doses of 100 μg/mL and 200 μg/mL induced a significant reduction of cell viability in MCF7, LNCaP and HeLa cell lines after a 48-hour incubation. The same cell lines also showed decreased cell viability when they were treated with a mixture of three major components of the essential oil, at doses of 100 μg/mL and 200 μg/mL, after a 48-hour incubation. **Conclusions:** These preliminary results could shed light on the formulation of new therapeutic agents with antiproliferative activity. Thus supplementary investigations are fundamental to examine the molecular mechanisms of the anticancer effects of this species of *Salvia* in cancer cells and to achieve confirmation of its *in vivo* anticancer activity.

1. Introduction

Cancer is one of the most malignant illness in the present decennium, and every year a lot of people die because of diverse cancers. Hormone-dependent tumors of the breast, uterine cervix and prostate are common in the Western world[1]. Current treatments include surgery, anti-hormone therapy, radiotherapy and chemotherapy; however, drug resistance, adverse effects and costs are forcing scientists to examine other sources to discover novel anticancer molecules.

Nature contains more than 1 000 species of plants with significant anticancer properties. The investigation of natural anticancer substances from plants and herbs initiated in 1960s, when vincristine, vinblastine, camptothecin and taxol were discovered[2]. These compounds are currently used in cancer therapies; for example, taxol is an Food and Drug Administration-approved cancer agent that has been used as a second-line therapy of refractory ovarian and breast cancers, refractory or anthracycline-

✉First and corresponding author: Dr. Giovanna Privitera, Fondazione Mediterranea "G.B. Morgagni", Via del Bosco, 105 – 95125 Catania, Italy.
Tel: 0039 095 238529
E-mail: giovannaprivitera@tiscali.it

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resistant breast cancer, Kaposi's sarcoma and non-small cell lung cancer[3]. The synthetic transformation of podophyllotoxin led to the formulation of etoposide, which is used to treat small cell tumors of the lungs and testes[4].

It used to be routine practice to examine medicinal plants to find the single active compound responsible for the curative effect[5], neglecting that the biological action may be the product of the association of activities of various substances. This was mainly due to the deficiency of high-quality studies on phytocomplexes, which turned into a general absence of recognition of natural medicine by the scientific society despite the promising results of these alternative traditional approaches.

Among the well-known phytocomplexes, essential oils (EOs) from aromatic plants have shown anticancer properties. EOs are plant volatile oils that have been extensively used in conventional medicine, and those from aromatic plants (and their constituents) have shown anticancer properties. The literature contains over 100 investigations of EOs from different plants used for *in vitro* and *in vivo* experiments of different kinds of cancer (*i.e*. mouth, breast, lung, prostate, liver, colon, brain and leukaemia)[6–12].

Salvia officinalis L. (*S. officinalis*) is an odorous perpetual plant that is endemic to southern Europe and Asia and is widely used for both dietary and medicinal compositions. *Salvia* is the largest genus of the Lamiaceae family, with around 900 species described worldwide[13]. It has different biological activities that are manifested by its different components[14–16], and this 'panacea' reputation is often just linked to its EO[17], even if its chemical composition is different due to genetic aspects and environmental factors[18].

Considering the interest in the possible anticancer properties of EOs from plants and herbs, the main goal of this investigation was to evaluate the actions of *S. officinalis* EO and its main components (α -thujone, 1,8-cineole and camphor) on three human hormone-dependent cancer cells: LNCaP (prostate carcinoma), MCF7 (breast carcinoma) and HeLa (cervical carcinoma). In light of the results and data reported in the scientific literature, these findings are very interesting and encourage further investigations on the mechanism of action of *S. officinalis* EO.

2. Materials and methods

2.1. Chemicals

α -Thujone, 1,8-cineole, camphor, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Italy).

2.2. Plant materials

Specimens of *S. officinalis* at bloom stage were collected in Avola, Siracusa province, Sicily, Italy, and the plants were dried naturally at the collection point (GPS: N 36°56'20", E 14°58'51"). The plant samples were collected in the herbarium of the Agronomic Institute, University of Catania, after botanical characterization.

2.3. EO isolation and analysis

The EO of *S. officinalis* was prepared from the dried aerial part of this plant (100 g) by 3 hours of hydrodistillation as previously reported[19], and 2.15 mL of essential oil was recovered. Analytic method used and EO composition were already published in our previous work[16].

2.4. Cell cultures

Human hormone-dependent tumour cell lines, LNCaP (prostate cancer), MCF7 (breast cancer) and HeLa (cervical cancer), were kindly donated by the Department of Biomedical and Biotechnological Sciences, University of Catania, Italy. All cell culture media and supplements were purchased from Life Technologies (Carlsbad, CA 92008, USA) unless stated differently.

HeLa cells were grown in Dulbecco's Modified Eagle's Medium supplemented with 1% pyruvate, MCF7 cells were grown in Dulbecco's Modified Eagle's Medium with Glutamax-1 and LNCaP cells were grown in Roswell Park Memorial Institute media. Ten percent foetal bovine serum, 100 IU/mL penicillin and 100 μg/mL streptomycin were added in all media. Cells were cultured at 37 ℃ and 5% CO_2 in a humid environment. After 4-5 d, cells were washed with a buffer solution and trypsinized for 3-5 min.

2.5. Treatment of tumor cell lines with S. officinalis EO and a mixture of its main components

LNCaP (1.8×10^3 cells/well), MCF7 (2×10^3 cells/well) and HeLa cells (1×10^3 cells/well) cells were seeded in sterile 96-well plates and incubated overnight. After 24 h, the medium was replaced with the medium containing only 1% foetal bovine serum, and incubation continued at 37 ℃ in a humidified chamber. After serum starvation, cells were treated with increasing doses (50, 100 and 200 μg/mL) of *S. officinalis* EO or with three main components of *S. officinalis* EO (α -thujone, 1,8-cineole and camphor) and incubated for 24, 48 or 72 h. All cells treated with EO or a mixture of three main compounds were compared to the corresponding controls (cells treated with medium and DMSO only). Six wells were assigned to each treatment. Cell morphology was examined using a phase contrast microscope (Axiovert 40 CFL, Carl Zeiss Light Microscopy).

2.6. Determination of cell viability

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed to quantify cell viability as previously described[16–20]. The results were also expressed in terms of viability using: % of cell viability = [OD (550–690 nm) test product/OD (550–690 nm) negative control] ×100. A reduction of cell viability by more than 30% was considered a cytotoxic effect[21].

2.7. Statistical analysis

All analyses were repeated 10 times, and six wells were assigned to each treatment. Data were presented as mean ± standard deviation

(SD) and were evaluated with one-way ANOVA test, with $P < 0.05$ considered statistically significant.

3. Results

Cell growth was determined using the MTT assay. No activity was observed in all cell lines when they were treated with *S. officinalis* EO and a mixture of its three major components at a dose of 50 μg/mL for 24, 48 and 72 h (data not shown). Moreover, the treatment with 100 μg/mL and 200 μg/mL dose of *S. officinalis* EO caused a reduction in cell viability in all cell lines after only 48 hours of treatment. HeLa (Figure 1), MCF7 (Figure 2) and LNCaP cells (Figure 3) also showed decreased cell viability when they were treated with the EO main components at the doses of 100 μg/mL and 200 μg/mL each after only 48 hours of incubation. Moreover, a dose of 100 μg/mL produced a cytostatic effect in all cell lines after 48 h, and a dose of 200 μg/mL of *S. officinalis* EO appeared to be cytostatic in LNCaP cells and cytotoxic in MCF7 and HeLa cells. At the same treatment time, a dose of 100 μg/mL of treatment with the combination of three main components had a cytotoxic effect in MCF7 and HeLa cells and a cytostatic effect in LNCaP cells. A 200 μg/mL dose of treatment with the combination of three main components was cytotoxic in all tumour cell lines. In addition, the inhibition of cell viability was coupled with morphological changes in all treated cells, which were examined using an inverted light microscope. Cells treated with only DMSO remained normal in size and shape (data not shown).

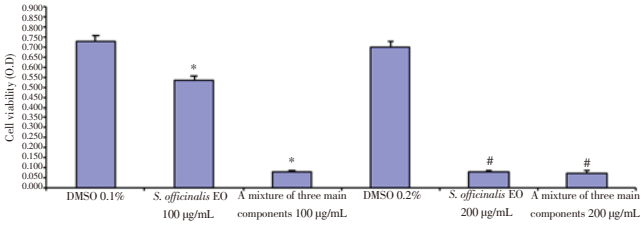

Figure 1. Effect of *S. officinalis* EO and a mixture of its three main components on HeLa cell proliferation measured by MTT test after 48 hours of incubation. (*) denotes significant differences ($P<0.05$) between cells treated with a dose of 100 μg/mL and untreated cells (DMSO 0.1% only). (#) denotes significant differences ($P<0.05$) between cells treated with a dose of 200 μg/mL and untreated cells (DMSO 0.2% only).

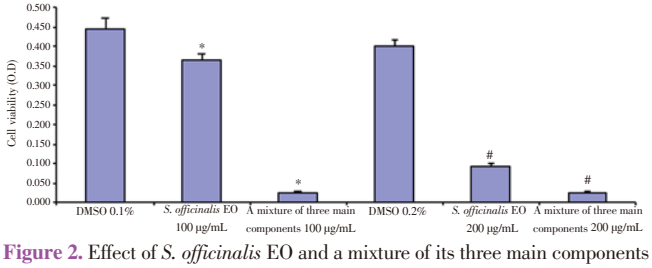

Figure 2. Effect of *S. officinalis* EO and a mixture of its three main components on MCF7 cell proliferation measured by MTT test after 48 hours of incubation. (*) denotes significant differences ($P<0.05$) between cells treated with a dose of 100 μg/mL and untreated cells (DMSO 0.1% only). (#) denotes significant differences ($P<0.05$) between cells treated with a dose of 200 μg/mL and untreated cells (DMSO 0.2% only).

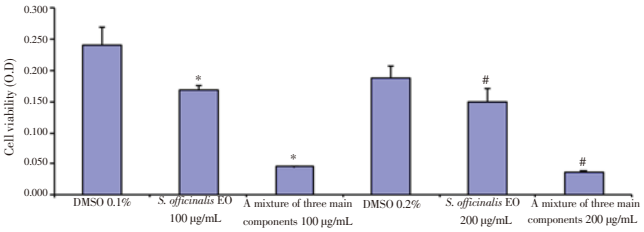

Figure 3. Effect of *S. officinalis* EO and a mixture of its three main components on LNCaP cell proliferation measured by MTT test after 48 hours of incubation. (*) denotes significant differences ($P<0.05$) between cells treated with a dose of 100 μg/mL and untreated cells (DMSO 0.1% only). (#) denotes significant differences ($P<0.05$) between cells treated with a dose of 200 μg/mL and untreated cells (DMSO 0.2% only).

4. Discussion

Breast cancer and cervical cancer are the most prevalent malignancies in women[22], and prostate carcinoma is the second most common cancer in men[23]. Conventional cancer treatments consist of anti-hormone therapy in the early stages of the disease, surgery, radiotherapy and chemotherapy; however, they often affect the patient's quality of life due to their serious side effects. Therefore alternative and less toxic therapies are urgently needed.

EOs represent an attractive source of new anticancer molecules, and the recent literature demonstrates the growing interest in this field. EOs are concentrated hydrophobic liquids that are produced by aromatic plants. Their constituents are mainly mono- and diterpenes that belong to several chemical classes (*i.e.* hydrocarbons, phenols, alcohols, ethers and esters) and have been classified on the basis of their chemical profiles. EOs are considered more effective than their components due to their more selective and synergistic action[24].

Salvia species have been widely studied for their chemical composition and pharmacological profile. Some diterpenoid quinones isolated from the roots of *S. officinalis* showed cytotoxic and DNA-damaging action in human colon carcinoma (Caco-2) cells and human hepatoma (HepG2) cells[8]. Until today the anticancer properties of the Sicilian *S. officinalis* EO and its components had not been studied in human hormone-dependent tumour cell lines. Therefore this investigation aimed to test the effects of the whole oil and a mixture of the three main components (α -thujone, 1,8-cineole and camphor) on breast, uterine and prostate cancer cells. The composition of *S. officinalis* EO was established using gas chromatography and mass spectrometry as reported in our previously published work[16].

Researchers demonstrated that α -thujone had a relevant antiproliferative activity against melanoma[25] and that low concentrations could improve CD3AK cell proliferation and function[26]. Other studies showed that α -thujone improved the cytotoxicity of colon cancer cells and inhibited lung metastasis of B16F-10 cells by inhibiting tumor cell proliferation, adhesion and invasion[27]. 1,8-Cineole showed antiproliferative effects against Molt 4B (T lymphoblast; acute lymphoblastic leukaemia) and HL-

60 cells (human promyelocytic leukaemia cells)[7] and increased the penetration of the anticancer drug, 5-fluorouracil, through human skin[20]. The activation of the PI3K/AKT and MAPK/ERK pathways and antioxidant activity may be involved in this action[12]. Camphor, 1,8-cineole and α-thujone showed cytotoxic activity[28]. EOs and their components act by various pathways and mechanisms, including apoptosis, cell cycle arrest, antimetastatic and antiangiogenic activities, DNA repair modulation, increased levels of reactive oxygen species and reactive nitrogen species[29]. Oxidative stress has been considered to be linked to the etiology of many diseases, including diabetes, atherosclerosis, chronic inflammation, viral infection, and neurodegenerative diseases[30,31].

The results of our research demonstrate, for the first time, that *S. officinalis* EO exhibits antiproliferative activity against hormone-dependent cancer cells after only 48 hours of treatment. A treatment dose of 50 μg/mL produced no significant antiproliferative effect, while an EO dose of 100 μg/mL produced a cytostatic effect in all cell lines. A treatment dose of 200 μg/mL appeared to be cytostatic in LNCaP cells and toxic in MCF7 and HeLa cells. Interestingly, the combination of the three major components of the EO produced a cytotoxic effect in MCF7 and HeLa cells and a cytostatic effect in LNCaP cells at a dose of 100 μg/mL. But the combination of three substances was cytotoxic in all tumor cell lines when used at a dose of 200 μg/mL.

The antiproliferative effect of the EO and the mixture of its main compounds was probably related to its ability to penetrate through the cell wall and cytoplasmic membrane. Morphological observations of the cells using an inverted microscope showed modification of the cell membrane after 48 hours of treatment.

Many reports suggest that the major components of an oil reflect the biological activities of the EOs from which they were isolated[32]. In this study, although the whole oil showed antiproliferative effect, the cytotoxic activity of the three main components seemed to suggest they had specific toxic activity that was independent of any modulation by minor components of the oil or any synergic effect from the rest of the oil. This possibility has previously been discussed in the literature, where it was pointed out that diverse substances of EOs can affect cell growth in a different manner[6].

In light of these results and the data reported in the literature, these findings are interesting and should encourage further investigations on the mechanism of action of alternative low-toxicity molecules from Sicilian *S. officinalis* EO. The cytostatic and cytotoxic effects of the mixture (of the three main components) on breast, prostate and cervical cancer cells are clear and support the hypothesis that this could be used alone or in addition to conventional therapy for certain types of cancer[33].

In conclusion, EOs from aromatic plants showed anticancer properties against blood, mouth, breast, lung, prostate, liver, colon and brain cancer[9,11,34–38], and information about the antitumoral effect of *S. officinalis* EO on tumor cells is poor, if not absent, for hormone-dependent cancers. Therefore these results could shed light on the development of new therapeutic agents. An additional studies are necessary to investigate the molecular mechanisms of the anticancer properties of this species of *Salvia* in tumor cells and to obtain evidence of its *in vivo* antitumor effects.

Conflict of interest statement

We have no conflict of interest.

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