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ANTIPROLIFERATIVE EFFECTS OF SOME MEDICINAL PLANTS ON HELA CELLS

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Abstract - Medicinal plants maintain the health and vitality of individuals, and also have potential curative effect on various diseases, including cancer. In this study were investigated the antiproliferative effects of water extracts of previously obtained ethanolic dry extracts of three different medicinal plants (*Echinacea angustifolia, Salvia officinalis* and *Melissa officinalis*) on cell lines derived from human cervix adenocarcinoma (HeLa cells). The best cytotoxic activity (IC50 = 43.52 μg/ml) on HeLa cell lines was exhibited by *Echinacea angustifolia.* The extract of *Salvia officinalis* also showed a good cytotoxic activity against HeLa cell lines; the IC50 value was 70.41 μg/ml. *Melissa officinalis* manifested a slightly weaker cytotoxic activity and an IC50 value of 122.22 μg/ml.

Key words: Antiproliferative effect, *Echinacea angustifolia, Salvia officinalis, Melissa officinalis*

INTRODUCTION

Every year, millions of people are diagnosed with cancer, leading to death. According to the American Cancer Society (ACS, 2006), deaths arising from cancer constitute 2-3% of the annual deaths recorded worldwide. Thus, cancer kills about 3.5 million people annually all over the world. Several chemopreventive agents are used to treat cancer, but they cause toxicity that prevents their usage (Kathiresan et al., 2006). Cancer as the most invasive disease is the object of intensive novel drug development but most of the used drugs have strong side effects, and due to the variety of cancer cells in the same patient and their fast mutation rate, it is very difficult to develop specific drugs (Barbaric et al., 2011).

Because of the high death rate associated with cancer and because of the serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative and/or complementary methods of treatment. Plants have been used for treating various diseases of human beings and animals since time immemorial. They maintain the health and vitality of individuals, and also cure diseases, including cancer without causing toxicity. More than 50% of all modern drugs in clinical use are of natural products, many of which have the ability to control cancer cells (Rosangkima and Prasad, 2004). According to the estimates of the WHO, more than 80% of people in developing countries depend on traditional medicine for their primary health needs. A recent survey shows that more than 60% of cancer patients use vitamins or herbs as therapy (Madhuri and Panday, 2008; Sivalokanathan et al., 2005).

The use of naturally occurring dietary agents is becoming increasingly appreciated in suppressing

cancer growth (Waladkhani and Clemens, 1998; Orsolic and Basic, 2005) and cancer prevention (Pan and Ho, 2008). *Echinacea angustifolia, Salvia officinalis* and *Melissa officinalis* have been widely used and well-documented medicinal plants for centuries (Weiss, 1988; Hansel and Sticher, 2002). However, the anticancer properties of *Echinacea angustifolia, Salvia officinalis* and *Melissa officinalis* have not been fully investigated and proven. In this study, we investigated the antiproliferative properties of extracts of *Echinacea angustifolia, Salvia officinalis* and *Melissa officinalis* in the HeLa cell line derived from human cervix adenocarcinoma.

MATERIALS AND METHODS

Plant material

The biological source of Echinaceae is *Echinacea angustifolia*. The cultivated medicinal plant is from Serbia, Vojvodina, region of Apatin. The biological source of Salviae is *Salvia officinalis* from Herzegovina. The biological source of Melissae is *Melissa officinalis*. The cultivated medicinal plant is from the region of Pančevo in Serbia.

Preparation of extracts

The first extraction was done in a percolator using 70% ethanol. Low-pressure evaporation of the extract was done following extraction. A 2 l glass percolator was first lined with some cotton wool and then filled with the desired amount of precut and sifted (0.75 sift) plant which was then covered with 70% ethanol. When the extract started to flow through the faucet on the percolator, the faucet was closed and the content left to macerate for at least 16 h. Following maceration, the extract was poured out of the percolator at a speed of 2 l/h. The amount of the poured extract was six times the volume of the starting drug (1:6 extract). The extract was then stored for the next 3 to 5 days, filtered through a series of Whatman filters and finally passed through a 0.22 μm filter (Millipore, Billerica, MA). Subsequently, the extract was evaporated in a rotational vacuum evaporator until a dry powder was obtained. The temperature in the evaporator was kept below 65°C under a pressure of 15-25 mbar.

A second extraction was performed in order to obtain water-soluble components. Previously obtained dry ethanolic extracts were weighted and mixed with physiological saline in a final concentration of 10 mg/mL. These suspensions were put in the dark at room temperature for 24 h; they were shaken for 3 h. Supernatants of suspensions were then filtered through 0.22 μm. The obtained water extracts, were used as stock solutions and were diluted with nutrient medium to the various working concentrations.

Cell line

Human cervix adenocarcinoma HeLa cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). HeLa cells were maintained in the recommended nutrition medium: (RPMI 1640 medium supplemented with 100 g/L heat-inactivated (56°C) fetal bovine serum (FBS), 3 mmol/L L-glutamine, 100 μg/mL streptomycin, 100 IU/mL penicillin and 25 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and adjusted to pH 7.2 with bicarbonate solution. Cells were grown in a humidified atmosphere of 5% CO_2 in air at 37°C.

Treatment of the cell line

Neoplastic HeLa (2000 cells per well) cells were seeded into 96-well microtiter plates; 24 h later, after cell adherence, five different doubly diluted concentrations of investigated water extracts were added to the wells. The final concentrations of extracts applied to target cells were 12.5, 25, 50, 100 and 200 μg/mL.

Determination of cell survival

The effect of extracts on target cell survival was determined by the microculture tetrazolium test (MTT) according to Mosmann (1983) with modification by Ohno and Abe (1991), 72 h after addition of the compounds, as described earlier. Briefly, 20 μL of MTT solution (5 mg/mL phosphate-buffered sa-

line) was added to each well. Samples were incubated for a further 4 h under the same conditions. Then 100 μL of 100 g/L sodium dodecyl sulfate was added to dissolve formazan, a product from conversion of MTT dye by viable cells. The number of the viable cells in each well was proportional to the intensity of absorbance at 570 nm, measured in an ELISA plate reader 24 h later. To determine cell survival (%), the *A* of a sample with cells grown in the presence of various concentrations of the investigated extracts was divided by the control optical density (the *A* of control cells grown only in nutrient medium) and multiplied by 100. It was implied that the *A* of the blank was always subtracted from the *A* of the corresponding sample with target cells. The inhibitory concentration (IC50) was defined as the concentration of an agent that inhibits cell survival by 50 % compared with a vehicle-treated control. All IC50's were reported as a mean of two measurements, each done in triplicate.

IC50 were established from dose-dependent data using Graphpad Prism Ver 3.0 software.

RESULTS

In vitro antitumor activity

The cytotoxic action of *Echinacea angustifolia, Salvia officinalis* and *Melissa officinalis* extracts was tested on the HeLa cell line. The IC50 values of the studied extracts are presented in Table 1, while Fig. 1 depicts the cytotoxic curves from MTT assay showing the survival of HeLa cells grown for 72 h in the presence of increasing concentrations of extracts**.** The extract of *Echinacea angustifolia* exhibited the best cytotoxic activity. The IC50 on HeLa cell lines was 43.52 μg/ml. The extract of *Melissa officinalis* also showed a good cytotoxic activity against HeLa cell lines. The IC50 value was 70.41 μg/ml. The *Salvia officinalis* extract manifested a slightly weaker cytotoxic activity. The IC50 value was 122.22 μg/ml.

Light microscopy

Results of microscopic examination (Carl Zeiss inverted microscopy, with total magnification 630) of the investigated HeLa cells after 72 h treatment with *Echinacea angustifolia, Salvia officinalis* and *Melissa officinalis* extracts are shown in Fig. 2. Extracts at a concentration of 200 μg/ml induced rounding, detachment and decreased the number of HeLa cells, as compared to control cells.

Table 1. Concentrations of extracts that induced a 50% decrease in HeLa cell survival

Extracts	HeLa IC50* (μg/ml)
Echinacea angustifolia	43.52 ± 0.01
Melissa officinalis	70.41 ± 0.91
Salvia officinalis	122.22 ± 3.30

Note: *IC50 values were obtained from the filtered extracts suspensions, as described in Materials and Methods. IC_{50} values were expressed as the mean ± SD determined from the results of MTT assay in three independent experiments.

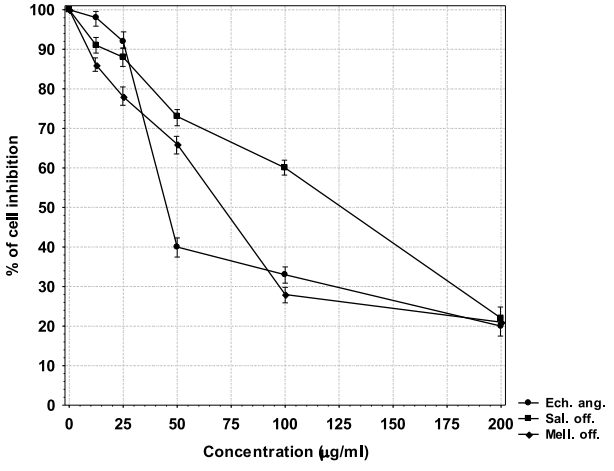

Fig. 1. Representative graph of HeLa cells survival after 72 h cell growth in the presence of increasing concentrations of investigated extracts.

DISCUSSION

In this study were investigated the antiproliferative effects of water extracts of obtained ethanolic extracts of three different medicinal plants (*Echinacea angustifolia, Salvia officinalis* and *Melissa officinalis*) on cell

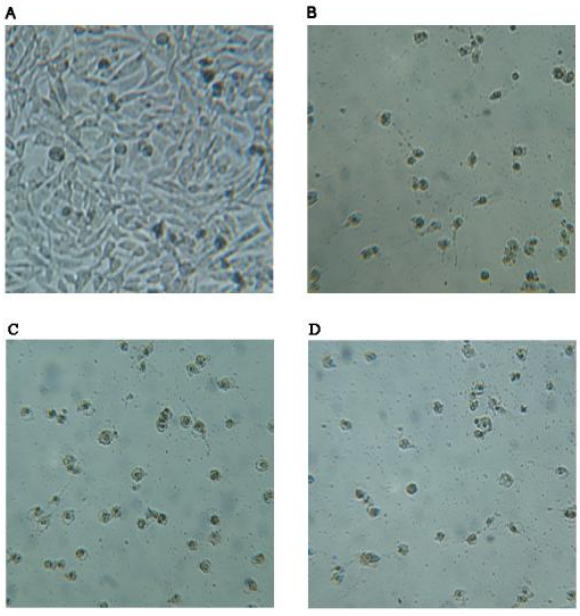

Fig. 2. Light microscopy of HeLa cells cultured with or without the 200μg/ml of extracts: **A)** control; **B)** *Echinacea angustifolia*; **C)** *Salvia officinalis*; and **D)** *Melissa officinalis* as described in Materials and Methods and photographed 72 h after the addition of drugs. (Magnification 12.5X, 1.6X, 6.3/0.2).

lines derived from human cervix adenocarcinoma (HeLa) cells. *Echinacea angustifolia* is a genus of herbaceous flowering plants in the daisy family, Asteraceae. The generic name is derived from the Greek word 'echino', meaning sea urchin, due to the spiny central disk. Some species are used in plant medicines and some are cultivated in gardens for their showy flowers. *Echinacea* was widely used by the North American Plains Indians for its general medicinal qualities (Wishart, 2004). *Echinacea* was one of the basic antimicrobial herbs of eclectic medicine from the mid 19th century through the early 20th century, and its use was documented for snakebite, anthrax and for relief of pain. In the 1930s, *Echinacea* became popular in both Europe and America as a plant medicine, but there was no evidence about its antiproliferative effects. Our results showed that *Echinacea* at a concentration of around 43 μg/ml (43.52 ± 0.01) inhibited HeLa cell survival by 50% compared with a vehicle-treated control. Photometric determination of phenols by spectrophotometer UV VIS HP 8453 showed that the percent of phenols was 9.07%, in the used extract of *Echinacea angustifolia*. Chemical compounds called phenols are common to many other plants. Other chemical constituents that may be important in the health effects of *Echinacea* include alkylamides and polysaccharides. Alkylamides bind particularly to human CB2 and to a much lesser degree to CB1 cannabinoid receptors; as a result they are implicated in a variety of modulatory functions, including immune suppression, induction of apoptosis, cell migration and inhibition of tumor necrosis factor alpha (Raduner et al., 2006).

Salvia officinalis is a member of the family Lamiaceae and is native to the Mediterranean region, though it has naturalized in many places throughout the world. It has a long history of medicinal and culinary use, and in modern times as an ornamental garden plant. The common name "sage" is also used for a number of related species. *Salvia* and sage are derived from the Latin salvere (to save), referring to the healing properties long attributed to the various *Salvia* species (Kintzios, 2004). Modern evidence shows possible uses as an anti-sweating agent, antibiotic, antifungal, astringent, antispasmodic, estrogenic, hypoglycemic and tonic (Sage, 2008). In a double blind, randomized and placebo-controlled trial, sage was found to be effective in the management of mild to moderate Alzheimer's disease (Akhondzadeh, 2003). There is no data on the antiproliferative effects of *Salvia officinalis*. According to the results of this study, *Salvia officinalis* in concentration of around 122 μg/ml (122.22±3.3) inhibits HeLa cell growth by 50% compared with a vehicle-treated control. Photometric determination of phenols by spectrophotometer UV VIS HP 8453 showed that the percent of phenols is 12.5% in the used extract of *Salvia officinalis*. Other chemical constituents are tannic acid, oleic acid, ursonic acid, ursolic acid, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavonoid glycosides and estrogenic substances (Sage, 2008). The concentration of phenols is higher in *Salvia officinalis* (12.5%) than in *Echinacea angustifolia* (9.07%), but the antiproliferative effects of *Echinacea angustifolia* is better

than that of *Salvia officinalis*, which probably means that the other chemical constituents may be important for antiproliferative effects, like alkylamides in *Echinacea angustifolia* and flavones and flavonoids in *Salvia officinalis*.

Melissa officinalis (Lemon balm) is a perennial herb in the mint family Lamiaceae, native to southern Europe and the Mediterranean region. Lemon balm is often used in cuisine, but is also used medicinally as a herbal tea or in extract form. It is claimed to have antibacterial and antiviral properties (it is effective against herpes simplex) (Kucera et al., 2006; Allahverdiyev et al., 2004; Schnitzler et al., 2008). Its antibacterial properties have also been demonstrated scientifically, although they are markedly weaker than those from a number of other plant studies (Nascimento et al., 2000). The extract of lemon balm was also found to have exceptionally high antioxidant activity (Dastmalchi et al., 2008). Results of this study showed that *Melissa officinalis* in a concentration of around 70 μg/ml (70.41 ± 0.91) inhibits HeLa cell survival by 50% compared with a vehicle-treated control. *Melissa officinalis* contains eugenol, which kills bacteria, tannins that contribute to its antiviral effects, as well as terpenes, 1-octen-3-ol, 10-alpha-cadinol, 3-octanol, 3-octanone, alpha-cubebene, alpha-humulene, beta-bourbonene, caffeic acid, caryophyllene, caryophyllene oxide, catechinene, chlorogenic acid, cis-3-hexenol, cis-ocimene, citral A, citral B, citronellal, copaene, delta-cadinene, eugenyl acetate, gamma-cadinene, geranial, geraniol, geranyl acetate, germacrene D, isogeranial, linalool, luteolin-7-glucoside, methyl heptenone, neral, nerol, octyl benzoate, oleanolic acid, pomolic acid, protocatechuic acid, rhamnazine, rosmarinic acid, rosmarinin acid, stachyose, succinic acid, thymol, trans-ocimene and ursolic acid.

CONCLUSION

Medicinal plants maintain the health and vitality of individuals, and also have potential curative effect on various diseases, including cancer. This study investigates the antiproliferative effects of extracts of *Echinacea angustifolia, Salvia officinalis* and *Melissa officinalis* on cell lines derived from human cervix adenocarcinoma (HeLa cells). The obtained results show that the best antiproliferative properties are exhibited by *Echinacea angustifolia*, then *Melissa officinalis*, and least of all, *Salvia officinalis*. An important goal of our future studies will be to investigate the antiproliferative effects and anticancer properties of other medicinal plants and propolis used in Serbian folk medicine.

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