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Original Research Article

Inhibitory effects of methanol extracts of selected plants on proliferation of two human melanoma cell lines

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Abstract

Purpose: *The aim of the current study was to investigate the invitro antiproliferative activity of methanolic extracts of six plants regardless of their claimed ethnopharmacological application.*
Methods: *Methanol extracts of different parts of Glycyrrhizaglabra L. (Licorice),Matricariachamomilla L. (Chamomile), Salvia triloba L. (Sage), Rheum palmatum L. (Rhubarb), Trigonellafoenum-graecumL. (Fenugreek) and Sambucusebulus L. (Dwarf Elder) were prepared. The antiproliferative effects of the extracts were tested on two skin cancer melanoma cell lines namely A375.S2 (low tyrosinase expression) and WM 136.1A (high tyrosinase expression) using MTT assay. The IC_{50} values for the active extracts were determined against the two melanoma cell lines.*
Results: *The methanolic extracts of G.glabra, M. chamomilla, S.triloba, R. palmatum inhibited the melanotic WM1361A proliferation in a dose-dependent manner revealing IC_{50} values of 35.2, 25.2, 20.6, 17.8, µg/ml, respectively but not A375.S2 cell line. However, the extracts of T. foenum-graecum and S. ebulus did not exhibit any significant cytotoxic activity on both melanoma cell lines.*
Conclusion: *The results of these experiments show that methanol extracts of licorice, chamomile, sage and rhubarb have significant antiproliferative activity onWM1361A cell line; a representative human melanotic melanocyte tumor cell line. This renders these plants as potential sources of new lead compounds for the development of new drugs for melanoma cancer.*

Keywords: *Melanoma, Plant extract, tyrosinase, Licorice, Chamomile, Sage, Rhubarb, WM1361A*

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INTRODUCTION

Discovery of new drugs or compounds with antineoplastic activity from natural sources have become popular among scientific communities, presenting new opportunities in the management of different types of cancer. The structural variety, cost effectiveness with their fewer side effects of these new compounds in comparison to their synthetic counterparts makes them much more preferable in cancer treatment.

Based on literature search, folk-lore use and investigational experiments, six popular plants

(Table 1) that are widely distributed in the Mediterranean region and cultivated in Jordan have been reported to exhibit anticancer activity against one or more type of cancer cell line and possessed many medical benefits [1].

Sage (Salvia) species are greatly valued medicinal plants that are widely used in pharmaceutical products and traditional medicine. Aerial extract of Sage has been used in the relief of pain, protecting the body against oxidative stress and free radical damages [2] and also exhibited anti-angiogenic activity [3]. Salvia essential oils have also been reported to exhibit antibacterial [4,5], anti-viral [6], and cytotoxic effects against some cancer cell lines including human larynx epidermoid carcinoma (HEp-2) [7] and breast adenocarcinoma (MCF7) [8].

Chamomile flowers have been used for centuries in the relief of inflammation [9] and colic spasms in young children. Cosmetically, it is used as a rinse for blonde hair. It is also used in topical preparation for the management of eczema and skin disorders [10]. Recently, it was reported that chamomile extracts can induce apoptosis in cancer cells but not in normal cells at similar doses [11].

Various beneficial effects of Fenugreek seeds extract have been reported these include antioxidant and anti-carcinogenic activities [12,13]. The seeds contain several highly desirable biologically active compounds which are used in the food and pharmaceutical industries [14,15]. Rhubarb roots extract has been associated with different medical activities such as laxative, astringent and antibacterial [16,17]. Recent research has shown promising effect in possibly inhibiting angiogenesis, which may be implicated in its antitumor and anti-inflammatory activities [18]. Furthermore, rhubarb roots extract has also been shown to have inhibitory effects against the activity of mushroom tyrosinase [19].
Regardless of the world-wide intake of Licorice roots, their potential effects on endogenous substances and drugs have not been reported. It has been found that the licorice root extract can both improve the impaired function of liver and kidney and also has shown to have hypoglycemic activity in rats [20]. Also, it has an anti-inflammatory action that might inhibit the breakdown of cortisol produced by the body [21].Licorice roots extract has been implicated in skin whitening due to its tyrosinase inhibition activity [22].

Many traditional medicinal uses of Dwarf Elder fruits are known. These fruits are used in the treatment of burns, infectious wounds, eczema and rheumatism and also possesses anti-inflammatory, antimicrobial, anti-neoplastic and antioxidant activities [23-25].

In spite of the numerous folkoric uses and the recorded anticancer or\and antioxidant activities of Sage, Chamomile, Licorice, Fungreek, Rhubard and Dwarf Elder, little is known about their anti-proliferative effect against melanoma. Cancer being the second most common cause of death after cardiovascular and heart diseases in Jordan coupled with the use of traditional topical herbal preparations as an acceptable safe and readily available choice of therapy for skin cancer in Jordan [26] have informed this study. The *in vitro* antiproliferative activities of six plant extracts on two melanoma cell lines that vary in the degree of tyrosinase enzyme expression were investigated. The two melanoma cell lines were a melanotic epithelial-like A375.S2 which shows low tyrosinase expression and the melanotic WM1361A cell line that is characterized with high tyrosinase expression [27].

EXPERIMENTAL

Plant extraction

Plants were obtained from local market were identified by Prof. Kahled Tawaha (Professor of Pharmacognosy, School of Pharmacy, the University of Jordan). Voucher specimens (nos. GLY-GL 2015,MAT-CA 2015,SA-TR 2015,RH-PA 2015,TR-GR 2015,SA-EB 2015) for Licorice, Chamomile, Salvia, Rhubarb, Fenugreek and Dwarf Elder respectively have been deposited at the herbarium of the same institute.

Table 1: The tested plants in this the current study

Plant name	Family	Common Name	Part used
*Glycyrrhizaglabra*L.	Fabaceae	Licorice	Root
*Matricariachamomilla*L.	Asteraceae	Chamomile	Aerial part
*Salvia triloba*L.	Lamiacea	Sage	Aerial part
*Rheum palmatum*L.	Polygonaceae	Rhubarb	Roots
*Trigonellafoenum-graecum*L.	Fabaceae	Fenugreek	Seeds
*Sambucusebulus*L.	Caprifoliaceae	Dwarf Elder	Leaves and fruits

For each plant, methanolic extract was prepared by weighing 100 gm of powdered dried plant, then soaking in 500 ml of 96% methanol solution at room temperature for 7 days and then filtered. The filtrate was then evaporated to dryness using a rotary evaporator (Stuart, UK) and the resultant extracts were stored in dry and cool place until used [28].

Plant extract preparation

The extract residue (2 mg each) was dissolved in 10 ml of RPMI 1640 medium with 10% heat-inactivated fetal bovine serum (FBS) (Euroclone, Italy) to get a final concentration of 200 µg/ml, and then was filtered using a syringe filter with 0.2 µm pore size. The sterile plant solution with the concentration of 200µg/ml was used as a stock solution for preparing further 5-fold dilutions with the concentrations of 6.25 to100 µg/ml to determine their cytotoxic effects and screening their anticancer activities.

Cell culture

The two solid human melanocyte tumor cell lines; A375.S2 and WM1361A were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum, 10 mM HEPES buffer (pH 7.3), 2mM L-glutamine, 50 µg/ml gentamicin, 100U/ml penicillin, and 100 µg/ml streptomycin sulfate. The cultures were maintained at 5% CO_2in humid environment at 37 ºC. Viable cell count was assessed using trypan blue dye exclusion method [28].

Cytotoxicity and MTT cell viability assays

Cytotoxicity of each plant extract was determined by MTT assay. Briefly, the cells were cultured in RPMI 1640 medium which was supplemented with 10% FBS in a humidified 5% CO_2 atmosphere. Cells in their log growth phase were harvested using trypsin-EDTA solution (Euroclone, Italy), washed three times with PBS, and then suspended in 10 ml of culture media with trypsin 0.25%-EDTA 10 mM. The cell suspension was then centrifuged at 5000 rpm for 10 min and the pellets were re-suspended in 10 ml of RPMI 1640 supplement with 10% FBS medium to obtain a single cell suspension. Based on optimization experiments, melanoma cells were seeded at a final count of 4 x 10^4 cells/200µl/well in flat-bottomed 96-well micro plates and were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. Cells were treated with 6.25, 12.5, 25, 50 and100 µg/ml of the plant extracts prepared in the same media as described previously. Proper control experiments were also performed, including human fibroblast cell lines as normal cells. Melanoma cell lines were treated with doxoruibicin as a positive control, while untreated cells were considered as a negative control. Triplicate preparations of each treatment group were made and the cultures were then incubated for another 72 h before measurement of cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT. PBS (20 µl) containing 5 mg/ml MTT was added. After incubation for 3 h at 37°C, the supernatant was discarded and the formazan blue crystals formed in the cells was dissolved in 200 µl dimethyl sulfoxide (DMSO). The optical density at 570 nm was measured (BioteK, USA) and compared with that of the control (untreated). IC_{50}, the concentration at which 50 % of cell viability was inhibited, was then calculated.

Statistical analysis

Data collected were analysed and expressed as mean ± SD (n = 3). Statistical analysis was carried out using one-way ANOVA in Prism (GraphPad, USA). P values less than 0.05 were considered to be significant.

RESULTS

The MTT assay results showed that there were no significant cytotoxic effects of the tested extracts on the proliferation of A375.S2 melanoma cells within the concentration ranges used in these experiments. Similar results also were obtained when they were tested against the normal fibrocytes cell line. On the other hand, Sage, Chamomile, Rhubarb, and Licorice extracts inhibited the melanotic WM1361A proliferation in a dose-dependent manner revealing an IC_{50} of 20.6, 25.2, 17.8, 35.2µg/ml, respectively (Figure 1). The degree of cellular inhibition increased with increasing the concentration of the plant extracts with a high correlation coefficient (Figure 1). However, both Dwarf Elder and Fenugreek extracts did not exhibit any significant cytotoxic activity for both melanoma cell lines. Doxorubicin used as positive standard inhibited bothWM1361A and A375.S2 cell lines in a dose dependent manner with an IC_{50} of 3.5 and 4.7µg/ml respectively.

DISCUSSION

This study has shown that the extracts of Sage, Chamomile, Rhubarb, and Licorice have inhibitory potential against the melanotic WM1361A proliferation in a dose-dependent manner with the degree of cellular inhibition increasing with increased concentration of the extracts.

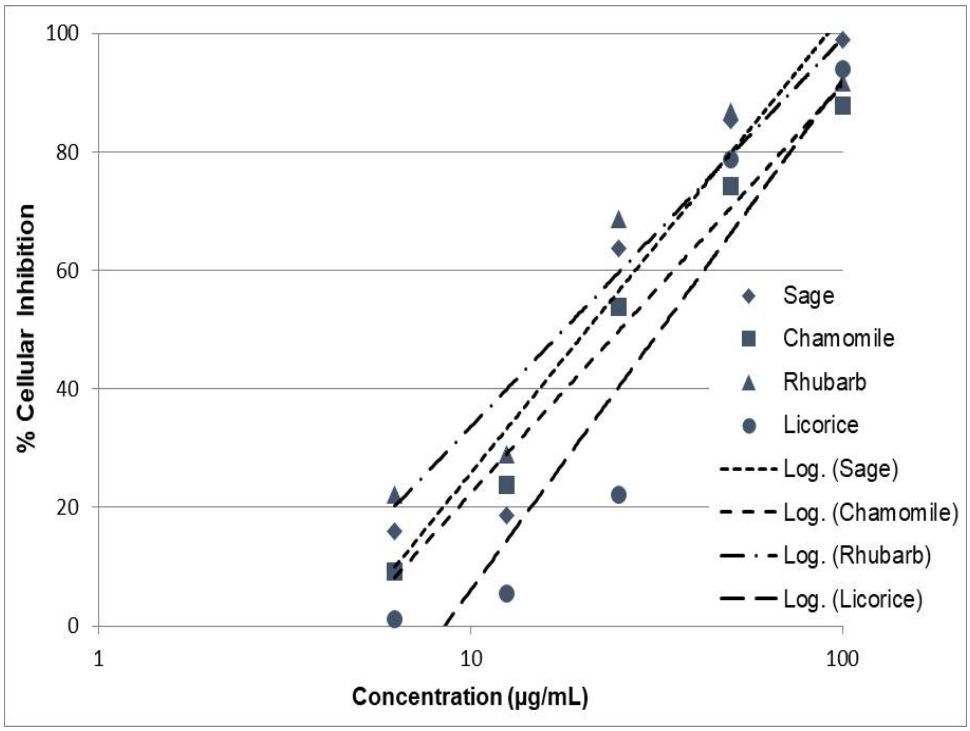

Figure 1: Cellular inhibition of WM 136.1A cell line by the selected plants

Melanotic WM1361A melanoma cell line is characterized with high tyrosinase expression [27] which catalyzes the key step in melanoma synthesis and hence the presence of tyrosinase facilitates the melanin accumulation in these cells. In recent years, several naturally occurring tyrosinase inhibitors which belonged to several chemical classes like phenols, flavanols, terpenes, steroids, flavonoids, alkaloids coumarins, stilbenes, tetra ketones, and many other secondary metabolites have been identified. Many of them have been used in skin whitening, as anti-ageing compounds and in cancer managements [28]. As many tyrosinase inhibitors have shown anti-proliferative activities against melanoma skin cancer cell lines, the inhibitory effects of these plant extracts against melanotic WM1361A can be attributed, at least in part, to the tyrosinase inhibitory activity. Earlier studies have shown that triterpene saponins present in Glycyrrhiza glabra L. (Licorice) possess anti-inflammatory, anti-allergic and anti-tumor activities [29,30]. In addition, the main ingredients of licorice extracts like glycyrrhizin and glycyrrhetinic acid have been shown to modulate melanogenesis in B16 melanoma cell, and tyrosinase inhibition activity has been shown in skin whitening [22]. Anthraquinones, the major active ingredients isolated from of Rhubarb rhizome, are known as tyrosinase enzyme inhibitors [31].

Flavonoids (e.g., apigenin) were described to be the main phytochemical responsible for anticancer activity of chamomile. These have promising effect against skin, prostate, breast and ovarian cancers [32, 33]. Moreover, many of these flavonoids were reported to potentially inhibit the tyrosinase enzyme [28] which can also explain the chamomile effects on the tyrosinase expressing human melanoma cell line WM1361Aunlike A375.S2 cell line where the plant extracts showed no cytotoxic activity. Other compounds associated with cytotoxic activities present in some of the plants are phenols, flavonoids, and terpenoids as the main available secondary metabolites Salvia species [34,35].

CONCLUSION

The data obtained in this study suggest that the methanol extracts of *G. glabra* (Licorice), *M. chamomilla* (Chamomile), *S. triloba* (Sage) and *R. palmatum* (Rhubarb) have potent antiproliferative activity against WM1361A cell line; a representative human melanotic melanocyte tumor cell line. These new insights into the anticancer activity of these plants make them potential source of lead compounds for the development of new, safe and cost effective treatments for skin diseases ranging from rashes to dreadful skin cancer melanoma. Moreover, new natural tryosinase inhibitors from these plants can be introduced into natural skin whiting formulations.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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