Journal of Applied Pharmaceutical Science Vol. 12(11), pp 091-096, November, 2022 Available online at http://www.japsonline.com DOI: 10.7324/JAPS.2022.121110 ISSN 2231-3354



# Evaluation of the antiproliferative effect of selected plant extracts on colon and skin cancer cell lines

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#### ARTICLE INFO

Received on: 23/05/2022 Accepted on: 10/08/2022 Available Online: 05/11/2022

Key words: Ajuga chia, Micromeria myrtifolia, Micromeria nervosa, Origanum dayi, Salvia palaestina, Varthemia iphionoides, Bongardia chrysogonum, antiproliferation.

# ABSTRACT

The aim of this study was to assess the antiproliferative effect of extracts obtained from medicinal plants that grow wild in Jordan against the human melanoma (WM136-1A) and colon cancer (Caco-2 cancer) cell lines using fibroblast cell lines as control cells. The selected plants are *Varthemia iphionoides* (Asteraceae), *Micromeria myrtifolia* (Lamiaceae), *Micromeria nervosa* (Lamiaceae), *Origanum dayi* (Lamiaceae), *Ajuga chia* (Lamiaceae), *Salvia palaestina* (Lamiaceae), and *Bongardia chrysogonum* (Berberidaceae). The results showed that *M. nervosa* and *O. dayi* exhibited antiproliferative activity against Caco-2 cancer cell line, with half-maximal inhibitory concentrations (IC<sub>50</sub>) that equal 98  $\pm$  2.5 and 73.6  $\pm$  3.4 µg/ml, respectively. Furthermore, *A. chia, B. chrysogonum*, and *O. dayi* exhibited antiproliferative activity against the WM136-1A cancer cell line, with IC<sub>50</sub> that equals 400  $\pm$  5.1, 98.1  $\pm$  3.2, and 245  $\pm$  3.6 µg/ml, respectively. None of the extracts had an effect on fibroblast cells at any used concentration. In conclusion, the extracts of *M. nervosa*, *O. dayi*, *A. chia*, and *B. chrysogonum* showed promising antiproliferative potential and can be good candidates for the development of novel anticancerous agents.

#### **INTRODUCTION**

Cancer is one of the health problems that impose huge economic and health burdens on the world (Ma and Yu, 2006). Cancer is considered the second cause of death in the world (Ma and Yu, 2006). There are several types of cancer. Melanoma is a malignant tumor that occurs in melanocytes, the cells that make the melanin pigment. Melanoma is considered a problem that affects the homeostatic balance of skin cells. In the skin, there are components that play key roles in tumor development, such as dermal fibroblasts, epidermal keratinocytes, inflammatory and endothelial cells, thus contributing to the control of melanocyte proliferation in normal skin cells (Serrone and Hersey, 1999).

Colorectal adenocarcinoma is another type of cancer that generates from epithelial cells (Le Marchand et al., 1997). It was noted that the invasion of carcinoma occurs through muscular mucosa into the submucosa and is characterized by the presence of desmoplasia represented by fibrous proliferation around tumor cells and necrotic debris in the lumina (Le Marchand et al., 1997). The causes of colorectal cancer pathogenesis are diverse and can include lifestyle, diet, genetic factors, and several diseases such as inflammatory bowel diseases (Niederreiter et al., 2018). Notably, genetic alterations are the direct leader of colorectal cancer, causing the neoplastic formation of the epithelium and stimulating the malignant stages (Niederreiter et al., 2018). Unfortunately, cancer treatment is associated with several side effects urging the need to find natural sources for treating cancer or alleviating its consequences. In this regard, plants have been considered a powerful source of therapeutics since ancient times. Plants produce a large number of chemical compounds that can have different medicinal values (Gurib-Fakim, 2006). Jordan has significant numbers of medicinal plants; many of these plants are used in the pharmaceutical industry and folk medicine (Oran and

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Al-Eisawi, 1998; Oran, 2014). According to Oran (2014), there are 363 medicinal plants in Jordan. Several researches were conducted to screen the medicinal herbs that have potential against cancer in Jordan. For instance, cooked lentils demonstrated enhanced chemoprevention against colorectal carcinogenesis (Afifi-Yazar et al., 2011), while Ononis hirta caused apoptosis in different cancerous cell lines (Afifi-Yazar et al., 2011). Furthermore, different Salvia species exerted antiproliferation against cancerous cell lines (Afifi-Yazar et al., 2011). These medicinal plants that have antineoplastic agents are examples of some precious plants from the Jordanian flora. More studies are needed to assess the effect of medicinal plants that were not previously explored to discover and benefit from their therapeutic values. Ajuga chia, Micromeria myrtifolia, Micromeria nervosa, Origanum dayi, Salvia palaestina, Varthemia iphionoides, and Bongardia chrysogonum are some of the plants that grow in Jordan (Oran and Al-Eisawi, 1998). These plants contain valuable therapeutic constituents and are used in Jordanian traditional medicine (Oran and Al-Eisawi, 1998). Several studies were conducted on these plants and proved multiple biological activities in different cell lines (Abdelwahab et al., 2015; Abdollahi-Ghehi et al., 2019; Abuhamdah et al., 2017; Formisano et al., 2014; Yacob et al., 2016; Yarmolinsky et al., 2015; Yousef et al., 2018). However, none of the previous studies, to the best of our knowledge, determined the effect of the extracts from these plants on colorectal cancer cell lines. Thus, the aim of this study was to prepare the ethanolic extract from these plants and assess their effect on Caco2, WM136-1A, and control cell lines.

## MATERIALS AND METHODS

## **Collection of plants**

The aerial parts of *V. iphionoides*, *M. myrtifolia*, *M. nervosa*, *O. dayi*, *A. chia*, and *S. palaestina* in addition to the corms of *B. chrysogonum* were collected (2019–2020) and were completely dried. The plant species were taxonomically identified by Prof. Sawsan Oran, Department of Biological Sciences, University of Jordan, Amman, Jordan. The voucher specimens were deposited at the Jordan University Herbarium, Amman, Jordan, and were labeled as follows: voucher specimens for *V. iphionoides* (JU-31), *M. myrtifolia* (JU-23), *M. nervosa* (JU-27), *O. dayi* (JU-29), *A. chia* (JU-11), *S. palaestina* (JU-30), and *B. chrysogonum* (JU-37).

#### **Preparation of plant extracts**

Plants were allowed to dry for 5 weeks at room temperature ( $24^{\circ}C-26^{\circ}C$ ) and then were powdered. 100 g of each powdered plant was dissolved in 1,000 ml of 100% ethanol separately. The mixture was placed in a shaker for 72 hours at 40°C. After that, the extracts were filtered using Whatman filter papers. The solutions were concentrated using a rotary evaporator at 50°C. Then, the extracts were stored at  $-20^{\circ}C$ .

# **Cell culture**

Fibroblast, colorectal cancer (Caco-2), and human melanoma (WM136-1A) cell lines from The National Cancer

Institute (NCI) of United States were used in this study. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum, 50 IU/ml penicillin, 50 µg/ml streptomycin, and 20 µM *L*-glutamine. The cells were incubated at 37°C under conditions of 5% carbon dioxide (CO<sub>2</sub>) and 95% humidity conditions.  $1 \times 10^4$  cells/ml were seeded in 24-well plates. Each of the seven plants' extracts was dissolved in a growth medium to treat the cells with 200, 100, 50, 25, 12.5, and 6.25 µg/ml concentrations. After treatment, the cells were incubated for 72 hours at 37°C, 5% CO<sub>2</sub>, and 95% humidity conditions. Each experiment was repeated at least thrice.

#### Cell viability assay

The 3-(4,5-dimethyl-thiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich, St. Louis) was used to assess the viability of the cells. Control cells were treated with dimethylsulfoxide (*DMSO*) or media (Lau *et al.*, 2004). 40 µl of MTT was added to every well and was incubated for 2 hours at 37°C. Then, the MTT solution was removed and DMSO was added. A plate reader was used to measure the absorbance at 570 and 630 nm. Data were collected from three independent experiments. The percentage of surviving cells was detected by using the following formula:

Survival  $\% = 100 - [(AC - AT) / AC] \times 100\%$ 

where AC represents the absorbance of the control group and AT represents the absorbance of the treated group. Doxorubicin was used as a positive control. Then,  $IC_{50}$  values were calculated for all groups.

# Statistical analysis

Data are shown as mean  $\pm$  standard deviation (SD). The one-way analysis of variance was used for the statistical analysis using Dunnett's test as a *post-hoc* test. *p* < 0.05 was considered significant. GraphPad Prism, version 5.02, was used to perform the statistical analysis and draw the figures.

### **RESULTS AND DISCUSSION**

Drugs derived from plants can be good candidates for the treatment of cancer because they are natural, available, and nontoxic (Greenwell and Rahman, 2015). In the present study, the cytotoxicity of the crude ethanolic extracts of selected medicinal plants from Jordan was evaluated. The present study was conducted to evaluate the antiproliferative effect of A. chia, B. chrysogonum, M. myrtifolia, M. nervosa, O. davi, S. palaestina, and V. iphionoides medicinal plants that grow wild in Jordan. The cytotoxicity of the selected plant extracts on the fibroblast, human melanoma, and colon cancer cell lines was determined by the cell viability assay using the MTT assay. Varthemia iphionoides is a plant in the family Asteraceae. In Jordanian folk medicine, the aqueous extract of V. iphionoides is used to treat gastrointestinal disorders and diabetes mellitus (Oran and Al-Eisawi, 1998). Our findings showed that the ethanolic extract of V. iphionoides did not exhibit antiproliferative activity against Caco-2 and WM1361-A cell lines (data not shown). The results are in agreement with an earlier report that revealed the weak

antiproliferative effect of *V. iphionoides* on the adenocarcinomic human alveolar basal epithelial cells (A549) and melanoma cells (BG) cancer cells (Yarmolinsky *et al.*, 2015). In fact, it is well known that the cytotoxic effect of an extract can differ according to the type of cell line and solvent that was used in extraction. For instance, a previous study documented slight cytotoxicity for *V. iphionoides* extract (ethyl acetate extract) compared to the high cytotoxic effect of the hexane extract of *V. iphionoides* against the HL-60 human leukemia cell line (Al-Dabbas *et al.*, 2006). Additionally, *V. iphionoides* from the Judea region showed a moderate antiproliferative effect against the SKOV3 cell line (ovarian carcinoma cells). Notably, the most active components of the ethanolic extract of *V. iphionoides* against leukemia were flavonoids and polyphenols (Yarmolinsky *et al.*, 2015).

*M. myrtifolia* plant belongs to the family Lamiaceae. Earlier reports proved that the essential oils of *M. myrtifolia* have demonstrated antifungal, antioxidant, and antiviral activity (Formisano *et al.*, 2014). The current research showed that the ethanolic extract of *M. myrtifolia* did not have any toxicity on the proliferative rate of different cancer cells (data not shown). To the best of our knowledge, there are no publications regarding the effect of *M. myrtifolia* extract on the proliferation of cancer cell lines.

*M. nervosa* plant belongs to the family Lamiaceae. Our results show that the ethanolic extract of *M. nervosa* exhibited good antiproliferative activity against Caco-2 with  $IC_{50} = 98 \pm 4.7$  (Fig. 1) but not the melanoma or fibroblast cell lines. According to Abdelwahab *et al.* (2015), the acetone extract of *M. nervosa* showed cytotoxicity against the human liver hepatocellular carcinoma (SNU-398, HepG2), ovary adenocarcinoma (OVCAR-3, SK-OV-3), human colon cancer (COLO 205, HCT-116), and stomach gastric carcinoma (MKN-28, NCI-N87) cell lines. It is suggested that the cytotoxicity of *M. nervosa* can be attributed to diterpenes and flavonoids (Abdelwahab *et al.*, 2015).

*Origanum dayi* is a plant that belongs to the Lamiaceae family (Dudai *et al.*, 2003). This plant is rich in 50 volatile components such as α-terpineol, 1,8-cineole, and terpinen-4-ol and has a wide margin of biological effects (Dudai *et al.*, 2003). The data of the present study demonstrated that the ethanolic extract of *O. dayi* had an antiproliferative effect against the against Caco2 cell line after 72 hours cell line after 72 hours of treatment compared to the nontreated cells (control group) with IC<sub>50</sub> = 73.6 ± 3.4 µg/ml and WM1361A with IC<sub>50</sub> = 245 ± 3.6 µg/ml (Figs. 2 and 3). Previously, we found that treating the MCF7 and T47D cell lines with *O. dayi* ethanolic extract had a promising anticancerous effect on these cell lines (Yousef *et al.*, 2018). Additionally, the use of *O. dayi* extract had antitumor activity against HepG2 human hepatocellular carcinoma cells, as reported in earlier studies (Thoppil *et al.*, 2013).

*Ajuga chia* belongs to the Lamiaceae family and includes many species. In this study, the ethanolic extract of *A. chia* showed an antiproliferative effect against the WM1361A cell line with IC<sub>50</sub> = 400 ± 5.2 µg/ml (Fig. 4). This result is in agreement with the work of Sadati *et al.* (2012) who documented the cytotoxic effect of *Ajuga chamaecistus* spp. *tomentella* on the T47D, Caco-2, and HT-29 cancer cell lines. It is worth mentioning that many compounds were isolated from Ajuga species, such as neo-clerodane-diterpenes, phytoecdysteroids, diterpenoids, triterpenes, specific sterols, flavonoids, and essential oils (Yacob *et al.*, 2016, Jaffal *et al.*, 2019). Phytoecdysteroids that are found in Ajuga species have demonstrated various pharmacological and biological activities (Yacob *et al.*, 2016, Jaffal *et al.*, 2019). (Yacob *et al.*, 2016).

Salvia species, which belong to the Lamiaceae family, are commonly used for the treatment of a large number of diseases. Salvia species are characterized by two main bioactive compounds, namely flavonoids and terpenoids (Irtegun Kandemir et al., 2022). Salvia palaestina is a common plant that is used in



Figure 1. Effect of *M. nervosa* ethanolic extract on Caco-2 cell line. Error bars represent SD. \*Significant compared to the control group.



Figure 2. Effect of *O. dayi* ethanolic extract on Caco-2 cell line. Error bars represent SD. \*Significant compared to the control group.



Figure 3. The cytotoxic effect of the crude ethanolic extract of *O. dayi* on WM1361A cell line. Error bars represent SD. \*Significant compared to the control group.



Figure 4. The cytotoxic effect of the crude ethanolic extract of *A. chia* on WM1361A cell line. Error bars represent SD. \*Significant compared to the control group.

folk medicine by Jordanian people (Oran and Al-Eisawi, 1998). Previous studies reported many active compounds in *S. palaestina* such as flavonoids, ursolic acid, and modified abietane diterpenoids (Irtegun Kandemir *et al.*, 2022). Our data demonstrated that the ethanolic extract of *S. palaestina* did not exhibit antiproliferative activity against Caco-2 (data not shown) but was effective against the WM1361-A cell lines (Fig. 5). In this regard, Yildirim and



Figure 5. The cytotoxic effect of the crude ethanolic extract of *S. palaestina* on WM1361A cell line. Error bars represent SD. \*Significant compared to the control group.



Figure 6. The cytotoxic effect of the crude ethanolic extract of *B. chrysogonum* on WM1361A cell line. Error bars represent SD. \*Significant compared to the control group.

Kutlu (2015) revealed that *S. absconditiflora* had a moderate cytotoxic effect against the MCF7 and MDA-MB-231 breast cancer cell lines.

*B. chrysogonum* plant belongs to the Berberidaceae family. *B. chrysogonum* is widely used in traditional medicine in Jordan as a remedy for epilepsy (Abuhamdah *et al.*, 2017). In folk medicine in Lebanon, the corms of *B. chrysogonum* were used in the treatment of prostate hypertrophia (Baydoun *et al.*, 2015). In our research, the ethanolic extract of *B. chrysogonum* corms demonstrated a cytotoxic effect against the WM1361A cell line with IC<sub>50</sub> = 98.1 ± 3.2 µg/ml (Fig. 6). It was reported that the methanolic extract of *B. chrysogonum* exhibited a cytotoxic effect on the U266 multiple myeloma and BJAB Burkitt's lymphoma cell lines, with IC<sub>50</sub> = 126.3 and 114.4 µg/ml, respectively (Assaf *et al.*, 2013). Importantly, none of the extracts had an effect on fibroblast cells at any used concentration (data not shown).

On the other hand, the ethanolic extracts of the selected medicinal plants were screened for their cytotoxicity in Caco-2, WM1361A, and fibroblast cell lines at various concentrations to detect  $IC_{s0}$  values (µg/ml). The results of  $IC_{s0}$  are illustrated in Table 1. The most potent extracts in the present study are the extracts that scored the lowest  $IC_{s0}$  in the *in vitro* MTT assay.

In conclusion, the results of this research showed that M. nervosa, O. dayi, A. chia, and B. chrysogonum can be good

**Table 1.**  $IC_{50}$  of different groups.

IC50 value (µg/ml) for ethanolic extract of plants								
Cell line	Varthemia iphionoides	Varthemia iphionoides	Micromeria myrtifolia	Origanum dayi	Ajuga chia	Salvia palaestina	Bongardia chrysogonum	Doxorubicin
Caco-2	_	$98 \pm 2.5$	$73.6\pm3.4$	_	_	_	_	16±1.7
WM1361A	—	—	—	$245\pm3.6$	$400\pm5.1$	$\textbf{98.1} \pm \textbf{3.2}$	—	2±2.9
Fibroblast	0.9±1.1	_	_	_	_	_	—	_

candidates as anticancerous compounds. Notably, *O. dayi* was the most potent plant in antiproliferation effect.

## ACKNOWLEDGMENTS

The authors are thankful to the Deanship of Scientific Research at the University of Jordan for the financial support.

# **CONFLICT OF INTEREST:**

The authors declare no conflicts of interest.

# **AUTHOR CONTRIBUTIONS**

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

## ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## DATA AVAILABILITY

All data generated and analyzed are included within this research article.

# **PUBLISHER'S NOTE**

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How to cite this article:

Oran SA, Yousef I, Jaffal SM. Evaluation of the antiproliferative effect of selected plant extracts on colon and skin cancer cell lines. J Appl Pharm Sci, 2022; 12(11): 091–096.