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Cytotoxic Activity of Cyclamen Persicum Ethanolic Extract on MCF-7, PC-3 and LNCaP Cancer Cell Lines

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Abstract
It is important to develop new approaches to increase the efficacy of cancer treatments. Nowadays, the uses of natural products to treat cancer are very common. In addition, working with plants that are endemic to Palestine and determining the biological activities of these plant extracts, is extremely important due to the potential for new drug development. *Cyclamen persicum* is used in traditional medicinal to treat anti-rheumatic, diarrhea, abdominal pains, edema, abscesses, eczema, cancer and other ailments. In this study the cytotoxic effect of *C. persicum* tubers and leaves ethanolic extracts were studied against MCF-7, PC-3 and LNCaP cancer cell lines, using mitochondrial dehydrogenase enzyme method. Results showed the remarkable cytotoxic activity of *C. persicum* extracts, against breast and prostate adenocarcinoma. For tubers extract the IC_{50} value was found to be 0.05 mg/ml for the three cell lines. Although the leaves extract the IC_{50} value was found to be 0.25 mg/ml for PC-3 and MCF-7 cell lines, while LNCaP cell inhibition were less than 30% at all tested leaves extract concentrations. MCF-7 cells exhibited the highest sensitivity to the *C. persicum* extracts, compared to PC-3 and LNCaP cell lines evaluated. In contrast, LNCaP cells generally exhibited the lowest sensitivity to extracts. These results displayed that *C. persicum* is a good source for natural products with antitumor compounds that can be further exploited for the development of a potential therapeutic anticancer agent.
Key words: *Cyclamen persicum*, Cytotoxicity, MTT assay, LNCaP, MCF-7, PC-3.
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1. Introduction

Cancer is a genomic disease that appears as a result of dynamic changes of the DNA of an organism's cells during its life time. The burden of cancer is increasing worldwide with 14.1 million new cancer cases yearly and 8.2 million cancer deaths occurring in 2012 according to GLOBOCAN series (Ferlay *et al*., 2014). In Palestine, the most common cause of death out of all cancer types was lung cancer among males (22.8 %) and breast cancer among females (21.5 %) followed by prostate cancer for males (9.5 %) and by colon cancer for females (11.4 %) (Abu-Rmeileh *et al*., 2016).

It is important to develop new approaches to increase the antitumor effects against cancer cells in order to increase the efficacy of cancer treatments. Today, the uses of natural products to treat cancer are very common, in the world. However, some plants have not yet been scientifically proven to exert antitumor activity. Therefore, it is very important to study endemic plant extracts with unknown biological activities to possibly develop new drugs that will guide the intentional use of natural medicines (Ozcan *et al.,* 2016).

Regional traditional medicinal plants and herbs will be served as a potential source of novel therapeutic agents. A large number of regional medicinal plants are claimed to possess anticancer activity. A systematic review conducted in Palestine, by Ali-Shtayeh and Jamous, 2014 identified a total of 368 plant species used in Traditional Arabic Palestinian Herbal Medicine (TAPHM) as part of complementary and alternative medicine (CAM). Some of these plants claimed to be effective in the treatment of cancer such as *Cyclamen persicum, Arum palaestinum, Nigella ciliaris, Matricaria aurea, Salvia fruticosa, Zingiber officinale, Anisum vulgare , Allium sativum , Trigonella berythea, Curcuma longa, Majorana syriaca , Rosmarinus officinalis, Allium cepa ,Olea europaea, Camellia thea ,* and *Teucrium capitatum* (Ali- Shtayeh *et al.,* 2000; Khalilia, 2001; Ali-Shtayeh and Jamous, 2008; Ali- Shtayeh *et al.,* 2011; Jaradat *et al*., 2016). Recently, the ability of medicinal plant extracts to control the proliferation of cancer cells were reported (Yaacob *et al*., 2010; Alzeer *et al*., 2014; Ozcan *et al.,* 2016).

Cyclamen persicum Mill. is a species of flowering herbaceous perennial plant of the *Primulaceae* family, growing from a tuber, native to rocky hillsides, scrublands, and woodlands in the Mediterranean area. *C. persicum* used in traditional medicinal to treat antirheumatic, headache, goiter, antihelmintic, laxative, diarrhea, abdominal pains, eye infections, edema, nerve infections, female infertility, open wounds, abscesses, eczema, skin burns, toothache, cancer and other ailments (Ali- Shtayeh *et al.,* 2000; Khalilia, 2001; Ali- Shtayeh *et al.,* 2011; Fernández-Campos *et al*., 2019; Al-zuabe *et al*., 2019).

To the best of our knowledge *C. persicum* has not yet been studied for their antitumoral activities against PC-3 and LNCaP prostate cancer cells and MCF-7 cervical cancer cells.

The aims of this study, was to demonstrate the antitumor effect of *C. persicum* on various cancer cell lines, to compare the cytotoxicity of tubers and leaves extracts and determining whether their use in folkloric medicine to treat cancer disease is justified.

2. Materials and methods

2.1 Plant Collection

Tubers and leaves of *C. persicum* were collected between March and July from Nablus mountains (West Bank, Palestine) (altitude 600–900 m). Authentification of the plants was conducted by Prof. Dr. Ali-Shtayeh by comparison with plant specimens located at BERC Herbarium and voucher specimens were deposited in the Biodiversity and Biotechnology Research Unit at BERC Herbarium, Til, Nablus, Palestine.

2.2 Preparation of crude extracts

Tubers and leaves of *C. persicum* (100 g) dried in the shade at room temperature, powdered and infused in 70% ethanol (1:5 w/v) at room temperature with periodic shaking for 72h. The filtrates were condensed by evaporating the solvents using rotary evaporator, dried under reduced pressure, and stock solutions of 10 mg/ml in dimethyl sulphoxide (DMSO) were prepared at room temperature and stored at -20°C. (Ali-Shtayeh *et al.,* 2013).

2.3 Cell lines

Prostate cancer PC-3 and LNCaP adenocarcinoma and breast cancer MCF-7 adenocarcinoma cell lines were obtained from American Type Culture Collection, USA (ATCC). MCF-7 and LNCaP cell lines were cultured in RPMI-1640 medium (Sigma), while PC-3 cell line was cultured in Minimum Essential Medium (MEM, Sigma). Medium were supplemented with 10% fetal calf serum (FCS, Sigma), 2 mM L-Glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma). Cell lines were incubated at 37 °C in a humidified incubator containing 5% CO2 (Bahk *et al*., 1998; Kelner *et al.,* 1998; Eilon *et al.,* 2000).

2.4 Cytotoxicity

Cells were seeded in 96 well- plates at a density of $4x10^4$ cell/ 200µl for LNCaP cells, while $3x10^4$ cells/ 200µl for PC-3 and MCF-7cell lines. The cells were allowed to attach in a 5% CO2 incubator at 37° C for 24 h. In order to prepare the 4 different doses of extracts (0.05, 0.125, 0.25 and 0.5 mg/ml), crude extract was serially diluted into supplemented media using a separate 96-well plate and extract doses applied to the cells. After incubation for 24 hours viable cells were quantitated by using 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) (MERCK, Germany) assay. In brief after cells incubation for 24 hours, medium was aspirated carefully and discarded. After that 50 µl of MTT solution (1.5mg/ml) was added to each well and incubated for 3 h, then100 µl of iced acetone-ethanol solution (1:1) were added to dissolve violet crystals. Viability was quantitated by using a 96 well- plate reader at 570 nm wavelength, with a reference wave length of 650 nm. The percentage of cell survival was determined as (mean of treated wells/ mean of untreated control wells) x100%. DMSO was used as a negative control (Srivastava *et al*., 1998; Ozcan *et al*., 2016).

2.5 Statistical analysis

IC_{50} (50% growth inhibition) values were defined as the concentration of the extract where there is a 50% loss of total metabolic activity as compared to untreated controls and are reported as mean ± standard deviation (SD). The data were analyzed and the treatment were compared using analysis of variance (ANOVA) obtained by Duncan's multiple-range test and *P* values less than 0.05 were considered to be significant. All experiments have been conducted in three replicates.

3. Results

In order to evaluate the cytotoxic effect of ethanolic extracts from *C. persicum* (tubers and leaves), MTT assay with three different cancer cell lines (PC3, LNCaP, and MCF-7) were performed. To determine the IC_{50} value the extracts were screened for its cytotoxicity at different concentrations (0.05, 0.125, 0.25 and 0.5 mg/ml).

When cells treated with ethanolic extract of the tubers and leaves of *C. persicum*, there was a concentration dependent cytotoxic effect. As the concentration increased from 0.05- 0.5 mg, percentage of inhibition increases (Table 1).

Table 1. Cytotoxicity of *C. persicum* against PC-3, MCF-7 and LNCaP cells treated with 4 different doses of ethanolic extracts after a 24 h. (MTT assay).

C. persicum parts used	Concentration(mg/ml)	% of cell inhibition ± SD.		
		PC-3	MCF-7	LNCaP
Tubers	0.5	94.6 ± 3.5	95.7 ± 2.1	90.3 ± 3.5
	0.25	77.6 ± 4.6	85.2 ± 6.5	79.1 ± 1.5
	0.125	62.3 ± 8.2	73.4 ± 2.1	65.6 ± 3.2
	0.05	45.2 ± 5.2	68.3 ± 7.4	51.1± 3.4
	0 (Control)	0	0	0
Leaves	0.5	69.4 ± 7.8	63.2 ± 10.6	28.3 ± 7.1
	0.25	60.8 ± 3.9	55.1 ± 8.8	24.9 ± 3.5
	0.125	44.0 ± 7.6	38.3 ± 2.1	23.2 ± 6.4
	0.05	29.3 ± 3.6	28.2 ± 7.4	22.6 ± 5.7
	0 (Control)	0	0	0

This study showed the remarkable cytotoxic activity of *C. persicum* extracts, against breast and prostate adenocarcinoma. For tubers extract the IC_{50} value was found to be 0.05 mg/ml for the three cell lines. While the leaves extract the IC_{50} value was found to be 0.25 mg/ml for PC-3 and MCF-7 cell lines, but LNCaP cell inhibition were less than 30% at all tested leaves extract concentrations (Table 1. & Figure 1.).

MCF-7 cells exhibited the highest sensitivity to the *C. persicum* extracts, with lower IC_{50} values as compared to PC-3 and LNCaP cell lines evaluated. In contrast, LNCaP cells generally exhibited the lowest sensitivity to the plant extracts (Table 1).

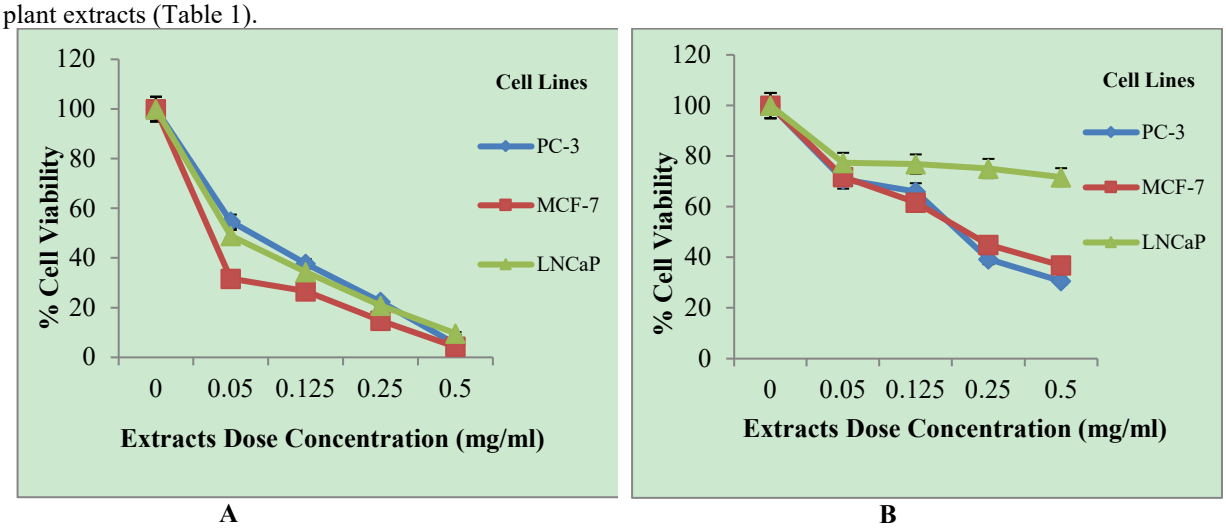

Figure 1. % Cell viability of PC3, LNCaP, and MCF-7 cell lines after 24 hours incubation with ethanolic extracts prepared from *C. persicum* tubers (A) and leaves (B). Cell viability was determined using MTT assay. Results are expressed as mean ± S.D (N= 2).

Figure 1 shows that a significant decrease in cell viability with 0.05, 0.125, 0.25 and 0.5 mg/ml from *C. persicum* tuber extracts after 24 h., and all doses showed viability by 10% at 0.5 mg/ml when compared with the control group. Cell viability decreased with increasing of leaves extracts concentration and all doses showed cell viability less than 30% at 0.5 mg/ml extract concentration for PC-3, MCF-7 cell lines. While LNCaP cell viability were more than 70% with 0.05, 0.125, 0.25 and 0.5 mg/ml from leave extracts after 24 hours compared with the control group (Table 1 & Figure 1).

C. persicum tubers extract appeared to be the most effective extract compared to the leaves extract applied to the PC-3, MCF-7 and LNCaP cells. It can be seen that LNCaP cells were more resistant to the treatment with *C. persicum* tubers extract compared with other cell lines.

4. Discussion

Most of the currently used anticancer drugs are highly toxic, expensive, and resistance mechanisms pose a significant problem (Hait and Hambley, 2009; Alzeer *et al*., 2014). There is a continuing need to identify new drug candidates that are more effective, widely available and less toxic. Plants extracts are an important source of potentially useful compounds for the development of new anticancer drugs.

In our study we investigated ethanolic extraction of *C. persicum* Palestinian medicinal plant for cytotoxic activities against breast and prostate adenocarcinoma cell lines. Results reported that *C. persicum* showed strong

cytotoxic activity against PC-3, MCF-7 and LNCaP cell lines with an inhibition of > 90% at 0.5 mg/ml (Table 1). *C. persicum* is currently used for anticancer treatments in traditional Palestinian medicine (Khalilia, 2001; Ali-Shtayeh and Jamous, 2008; Ali- Shtayeh *et al.,* 2011). The anti-oxidant properties of this species have been previously evaluated, which suggested the possibility of anti-proliferative potential for these plants (Metın *et al.* 2013; Mazouz and Djeddi, 2014 and Jaradat *et al*. 2015). Although the cytotoxicity of *C. persicum* has been previously reported on breast, colon, hepatocellular carcinoma, lung, pancreatic and cervical cancer cell lines (Yildiz *et al*., 2013; El Hosry *et al*., 2014). Therefore, this plant extracts with significant cytotoxic activity should be further assessed using animal models.

Results of this study shows the inhibitory effect against the three cell lines varied significantly between the two *C. persicum* extracts. At concentration of 0.5 mg/ml, tubers extract was the most toxic (> 90% inhibition). While leaves extract showed inhibition effect less than 70%. *Cyclamen* spp (Primulaceae) are rich in toxic saponins, known to have interesting biological activities (Spoerke *et al.,* 1987; Çaliş *et al.,* 1997; Altunkeyik *et al.,* 2012; Sajjadi *et al*., 2016; Stanojević *et al*., 2018). In a study conducted by El Hosry *et al*., 2014, showed the remarkable cytotoxic activity of saxifragifolin B (saponin derived from cyclamen species), against breast adenocarcinoma and lung carcinoma and its chemo protective activity against mitomycin C.

From our results, MCF-7 cells exhibited the highest sensitivity to the *C. persicum* extracts, with lower IC_{50} values as compared to PC-3 and LNCaP cell lines evaluated. These results are agreement with previous studies on various plant extracts (Yaacob *et al*., 2010; Subarnas *et al*., 2012). In order to identify the bioactive component(s) and to further understand the mechanism of action of *C. persicum* extracts should be evaluated.

5. Conclusion

The results of the present study demonstrated that the extracts of *C. persicum* tubers and leaves possessed strong cytotoxic activity and can be easily accessible source of natural anticancer products in cell cultures. *C. persicum* tubers extract appeared to be more effective compared to the leaves extract applied to the PC-3, MCF-7 and LNCaP cells. These results justified the use of *C. persicum* in traditional medicine.

The researcher decaled that the components responsible for antitumor activity of the extracts of *C. persicum* are unclear. Future studies will be aimed at investigating the effects of different parts of *C. persicum* upon isolating and identifying the substances responsible for the anticancer effects of the solvent extracts. We hope that the results of this study will play an important role in designing and developing natural anticancer drugs and will make contributions to other studies that might be conducted in this area.

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