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Original Article

Cytotoxic effects of chloroform and hydroalcoholic extracts of aerial parts of *Cuscuta chinensis* and *Cuscuta epithymum* on Hela, HT29 and MDA-MB-468 tumor cells

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Abstract

Previous studies have indicated that some species of *Cuscuta* possess anticancer activity on various cell lines. Due to the lack of detailed researches on the cytotoxic effects of *Cuscuta chinensis* and *Cuscuta epithymum*, the aim of the present study was to evaluate cytotoxic effects of chloroform and hydroalcoholic extracts of these plants on the human breast carcinoma cell line (MDA-MB-468), human colorectal adenocarcinoma cell line (HT29) and human uterine cervical carcinoma (Hela). Using maceration method, different extracts of aerial parts of *C .chinensis* and *C. epithymum* were prepared. Extraction was performed using chloroform and ethanol/water (70/30). Total phenolic contents of the extracts were determined according to the Folin-Ciocalteu method. Using MTT assay, the cytotoxic activity of the extracts against HT29, Hela and MDA-MB-468 tumor cells was evaluated. Extracts were considered cytotoxic when more than 50% reduction on cell survival was observed. The poly-phenolic content of the hydroalcoholic and chloroform extracts of *C. chinensis* and *C. epithymum* were 56.08 ± 4.11, 21.49 ± 2.00, 10.64 ± 0.86 and 4.81 ± 0.38, respectively. Our findings showed that the chloroform extracts of *C. chinensis* and *C. epithyum* significantly reduced the viability of Hela, HT-29 and MDA-MB-468 cells. Also, hydroalcoholic extracts of *C. chinensis* significantly decreased the viability of HT29, Hela and MDA-MB-468 cells. However, in the case of hydroalcoholic extracts of *C. epithymum* only significant decrease in the viability of MDA-MB-468 cells was observed (IC50 = 340 µg/ml). From these findings it can be concluded that *C. chinensis* and *C. epithymum* are good candidates for further study to find new possible cytotoxic agents.

Keywords: *Cuscuta chinensis; Cuscuta epithymum;* Cytotoxicity; MTT assay

INTRODUCTION

Cancer is one of the most important factors of mortality in the world. It is the third leading cause of death in Iran and more than 30,000 of Iranian lose their lives from cancer annually. It is estimated more than 70,000 new cases of cancer occur in Iran and it is expected that we encounter with increase of cancer incidence in the elderly population in the next two decades (1). During the last decades there have been numerous researches for anti-cancer compounds with plant origin (2). Herbs have an elongated history of use in the treatment of cancer in the world. It has been shown that more than 3000 plant species possess anti-cancer effects (3). Traditionally in many countries such as Iran, plants are used to cure many diseases. In some of the old medicinal sources such as Avicenna's Canon of Medicine a detailed section is devoted to the use of medicinal plants (4). Recently, in the developing countries, all over the world, about 80% of the populations are using traditional medicinal plants (5).

Cuscuta spp. or dodder is one of the medicinal herbs that belong to the Convolvulaceae plant family and there are over 150 species of dodders in the world (6). They are annual parasitic plants that reproduce by seed and are distributed on every area except Antarctica (7). These parasitic plants do

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not have any roots, leaves or chlorophyll to produce their own food. Dodders live by attaching to a host plant with small appendages (called "haustoria") and extract its necessary growth elements (8). Seeds of *Cuscuta* have rough coats and vary in size, depending on the species, and may be able to survive more than 20 years in the soil (9). The active compounds of *Cuscuta* species include flavonoids, lignans, quinic acid and poly-saccharides. Flavonoids are kinds of effective antioxidants and polysaccharides are the effective constituents to improve the immune system (10). *Cuscuta chinensis* is an important herbal medicine that is effective in the treatment of liver and kidney failure, sexual impotence and vision weakness. It also prevents abortion senescence and aging. Previous studies have indicated that *C. chinensis* possesses anticancer, immuno-stimulatory, antioxidant, and anti-osteoporotic activities and in rats have shown hepato-protective against acetaminophen and H_2O_2 toxicities (11-14). In addition, its glycoside compounds enhance memory in rats by inducing the PC12 cells differentiation (15).

Cuscuta epithymum or common dodder is a holoparasitic plant that has known as an indicator species of waterless european heathland. like *C. chinensis,* this plant having limited photosynthetic activity, is able to produces hundreds to thousands seeds with average diameter and mass of 0.9 mm and 0.3 mg, and has been used in different traditional remedies (16,17).

Recently extract of *C. epithymum* had shown temperate antimicrobial properties and cytotoxic effects (18). Some species of *Cuscuta* such as *C. reflexa* are known as anti-tumor and anti-inspiring agent and are used in the treatment of prostate cancer (19).

Based on the previous studies on the assessment of the effects of different species of *Cuscuta* on various cell lines including lymphoblastic-like tumor cell line (HL60), epidermoid carcinoma cell line (MCF-7), human breast cancer cell line (T47D), human Caucasian acute lymphoblastic leukemia (CCRF-CEM) and Jurkat (JM) cell line (20-22), and the lack of detailed researches on the cytotoxic effects of *C .chinensis* and *C. epithymum*, the aim of the present study was to evaluate the cytotoxic effects of chloroform and hydroalcoholic extracts of these plants on human breast carcinoma cell line (MDA-MB-468), human colorectal adenocarcinoma cell line (HT29) and human uterine cervical carcinoma (Hela).

MATERIALS AND METHODS

Materials

Compounds used were: Ethanol, chloroform, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), sodium chloride, potassium chloride, sodium hydroxide, hydrochloric acid, sulfuric acid, sodium bicarbonate, sodium phosphate dibasic (Merck, Germany), penicillin/streptomycin, trypan blue (Sigma, USA), Roswell Park Memorial Institute medium (RPMI-1640), fetal calf serum (FCS), sodium pyruvate, trypsine, L-glutamine (Gibco, Scotland), dimethyl sulfoxide (DMSO) (Fluka, Italy) and doxorubicin (Farmitalia, Italy).

Preparation of plant materials

The aerial parts of *C. chinensis* was collected in the early summer 2010 from area around Qazvin, and identified systematically, by Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences. A voucher specimen (NO: 6737) was deposited in the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

The aerial parts of *C. epithymum* was collected in the early autumn 2011 from Ashtian (Markazi province) and identified by Department of Biology, University of Isfahan. A voucher specimen (NO: 2668) was deposited in herbarium of Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences.

37 g of C. *chinensis* and 33 g of C *epithymum* were pulverized (Moulinex, France), mixed with 260 ml of ethanol/water (70/30) solution and shaked at room temperature for 3 h separately, then it macerated for 1 day and filtered via Büchner funnel three times. Then the extracts were evaporated in several stages by rotary evaporator and stored at 4 °C until use (23).

Determination of total phenolic contents

Total phenolic contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu method (23). The total phenolic content was calculated from the calibration curve of gallic acid (as a reference for phenolic compounds). Gallic acid stock solution was prepared by dissolving 0.5 g of dry gallic acid and diluting in 10% hydro-alcoholic solutions to prepare concentrations of 0, 50, 100, 150, 250 and 500 mg/L. The absorbance of each standard and sample were determined at 765 nm by a spectrophotometer (Jenway, UK). The absorbances of the standard solutions were plotted against gallic acid concentrations. Results are given as gallic acid equivalent per gram of the extracts. Data are presented as mean of three separate measurements.

Cell lines

HT29, Hela and MDA-MB-468 cell lines were purchased from Pasture Institute of Iran, Tehran. They were cultured in 75 cm^2 flasks (Cell Star, Germany) in RPMI-1640 (Gibco, Scotland) supplemented with 10% fetal bovine serum, penicillin/streptomycin (100 U/ml)/ (100 µg/ml), L-glutamine (200 mM), sodium pyruvate (1 mM), NaHCO3 (1 g). The media was sterilized by 0.22 microbiological filters and the cells were grown at 37 °C in an environment of 5% CO_2. Cell counts were performed by Trypan blue exclusion method using haemocytometer slide.

Methyl tetrazolium bromide cytotoxicity assay

Cell viability was assessed using MTT assay. This test is based on the metabolic reduction of soluble MTT by mitochondrial enzyme activity of viable tumor cells, into an insoluble dyed formazan product, which can be measured spectrophotometrically after dissolving in DMSO (25). Briefly, 200 µl of cells (5×10^4 cells/ml of media) were seeded in 96 microtiter plates and incubated for 24 h (37 °C, 5% CO_2, humidified air). Then 20 µl of the prepared concentrations of each extract was added to get final concentration of 1, 10, 50, 100 and 500 µg/ml in the plates containing cells and extracts were incubated for another 48 h in the same condition. Doxorubicin was used as a positive control and DMSO 1% was considered as a negative control (24). To assess cell survival, 20 µl of MTT solution (5 mg/ml in phosphate buffer solution) was added to each well and the cells were incubated for 3 h.

Then the plates were removed from the incubator and gently 150 µl of old medium was replaced by DMSO and pipetted to dissolve any formed formazan crystals and the absorbance was measured by an enzyme-linked immunosorbent assay (ELISA) microtitrer plate reader (StatFax- 2100, USA) at 540 nm.

Each extract concentration was examined in 8 wells and repeated 6 times. Standard curves (absorbance against number of cells) for each cell line were plotted. Intraday and inter-day variations were determined. Based on standard curves, cell survival was calculated. Cell survival in the negative control was assumed 100%.

Statistical Analysis

All data were expressed as means ± SD. Analysis of variance (ANOVA) followed by Student-Newman-Keuls test was used to explore the differences among groups. Significance was assumed at the 5% level.

RESULTS

Using maceration method for extraction, the extract yield of dried masses after evaporation and solvent removal of chloroform and hydroalcohoic extracts of *C. chinensis* and *C. epithymum* were 29.6%, 1.3% and 33.1%, and 0.8%, respectively.

Poly-phenol contents of C. chinensis and C. epithymum extracts

The total amount of poly-phenolic compounds of concentrated plant extracts was determined using Folin-ciocalteu method. The results are shown in Table 1.

The absorbance of each standard and samples were determined at 765 nm using Folin-Ciocalteu method. Results are given as gallic acid equivalent per g of the concentrated extracts. Data are presented as mean ± SD of three separate measurements.

Table 1. Poly-phenolic content of the extracts of *C. chinensis* and *C. epithymum.*

Extracts	Total poly-phenolic content (mg/g of concentrated extract)
Hydroalcholic extract of *C. chinensis*	56.08 ± 4.11
Hydroalcholic extract of *C. epithymum*	21.49 ± 2.00
Chloroform extract of *C. chinensis*	10.64 ± 0.86
Chloroform extract of *C. epithymum*	4.81 ± 0.38

Table 2. Equations and regression parameters of the calibration curve generated for Hela, HT-29 and MDA-MB-468 cells.

Cell line	Equation	R^2
Hela	Y = 0.007X + 0.009	0.9822 ± 0.0008
HT-29	Y = 0.008X - 0.049	0.9824 ± 0.0006
MB-468 cell	Y = 0.014X - 0.101	0.9883 ± 0.0009

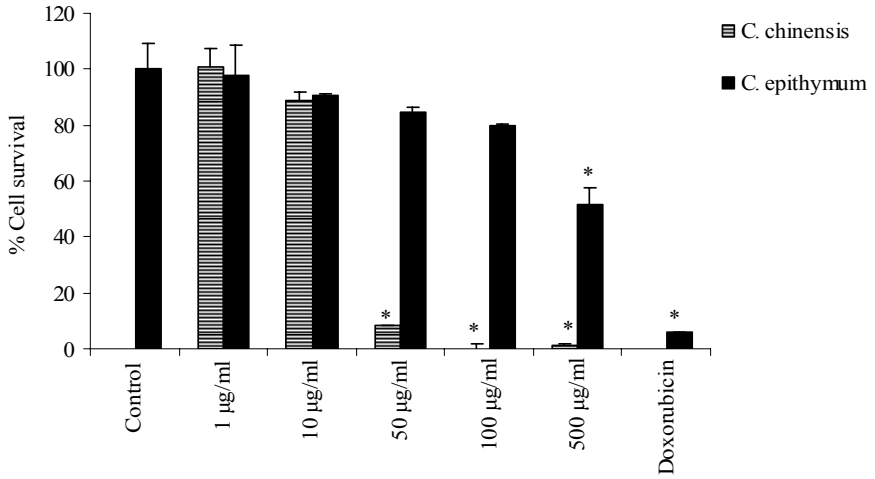

Fig. 1. The cytotoxic effect of the chloroform extracts of *C. chinensis* and *C. epithymum* on Hela cells. Viability of the cells was determined by MTT assay. Each extract concentration was examined in 8 wells. Percent cell survival in the control group was assumed 100. *=p<0.05, n=3.

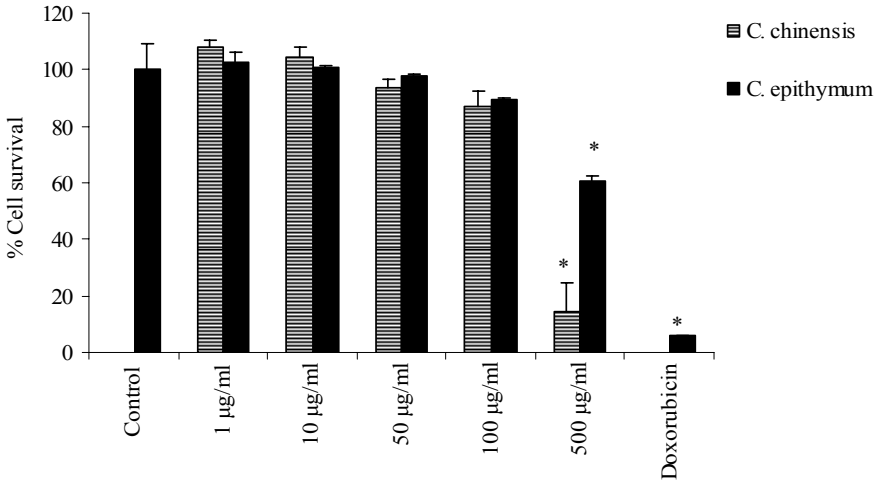

Fig. 2. The cytotoxic effect of the hydroalcoholic extracts of *C. chinensis* and *C. epithymum* on Hela cells. Viability of the cells was determined by MTT assay. Each extract concentration was examined in 8 wells. Percent cell survival in the control group was assumed 100. *=p<0.05, n=3.

Cytotoxic activities of C. chinensis and C. epithymum extracts

To evaluate the relationship between number of cells and absorbance, standard curves for Hela, HT-29, and MDA-MB-468 cell lines were prepared. Findings showed a good relationship between number of the cells and absorbances for three tested cell lines (Table 2). Intra-day and inter-day variations for all standard curves were acceptable (%CV<15). Doxorubicin (20 µg/ml) as a positive control significantly inhibited the proliferation of all tested cell lines to less than 25%. Extracts were considered cytotoxic when they reduced cell viability to less than 50%.

To investigate the cytotoxic effects of plant extracts on the cell lines, the cells were exposed to various concentrations of chloroform and hydroalcoholic extracts of *C. chinensis* and *C. epithymum* for 48 h and their viability was assessed by MTT assay. Our findings showed that the chloroform extracts of *C. chinensis* and *C. epithyum* significantly reduced the viability of Hela, HT-29 and MDA-MB-468 cells (Figs. 1-3).

In the case of HT-29 and MDA-MB-468 cells concentration-dependent effects were seen. Also, Hydroalcoholic extracts of *C. chinensis* and *C. epithymum* significantly decreased the viability of Hela and MDA-MB-468 cells (Figs. 4-5). However, only hydroalcoholic extracts of *C. chinensis* significantly decreased the viability of HT29 cells (Fig, 6). Chlorofom extracts of *C. chinensis* and *C. epithymum* were more cytotoxic than that of hydroalcoholic extracts in all tested cell lines (Table 3). In adition our data showed that extracts of *C. chinensis* were more cytotoxic than that of *C. epithymum* extracts.

Table 3. IC50 of the extracts of *C. chinensis* and *C. epithymum*.

Plant	***C. chinensis***			***C. epithymum***		
	IC_{50} (µg/ml)			**IC_{50} (µg/ml)**		
Cell line	**Hela**	**HT-29**	**MDA-MB-468**	**Hela**	**HT-29**	**MDA-MB-468**
Chloroform extract	45	470	25	520	50	230
Hydroalcholic extract	310	530	95	-	-	340

-= did not get to IC_{50} up to the concentrations tested.

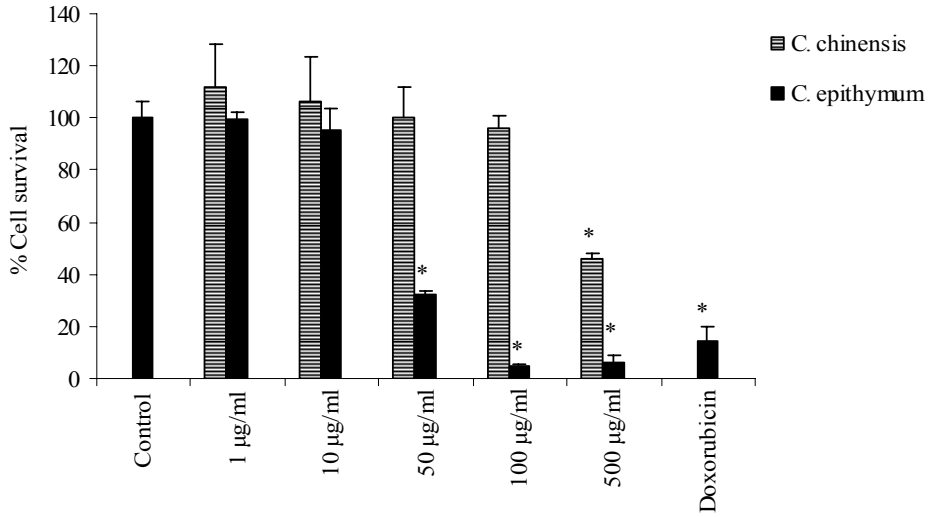

Fig. 3. The cytotoxic effect of the chloroform extracts of *C. chinensis* and *C. epithymum* on HT29 cells. Viability of the cells was determined by MTT assay. Each extract concentration was examined in 8 wells. Percent cell survival in the control group was assumed 100. *=p<0.05, n=3.

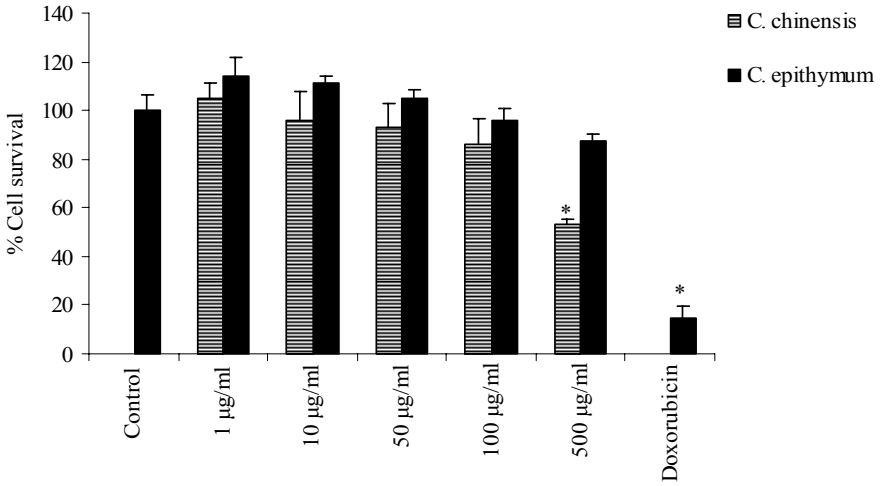

Fig. 4. The cytotoxic effect of the hydroalcoholic extracts of *C. chinensis* and *C. epithymum* on HT29 cells. Viability of the cells was determined by MTT assay. Each extract concentration was examined in 8 wells. Percent cell survival in the control group was assumed 100. *=p<0.05, n=3.

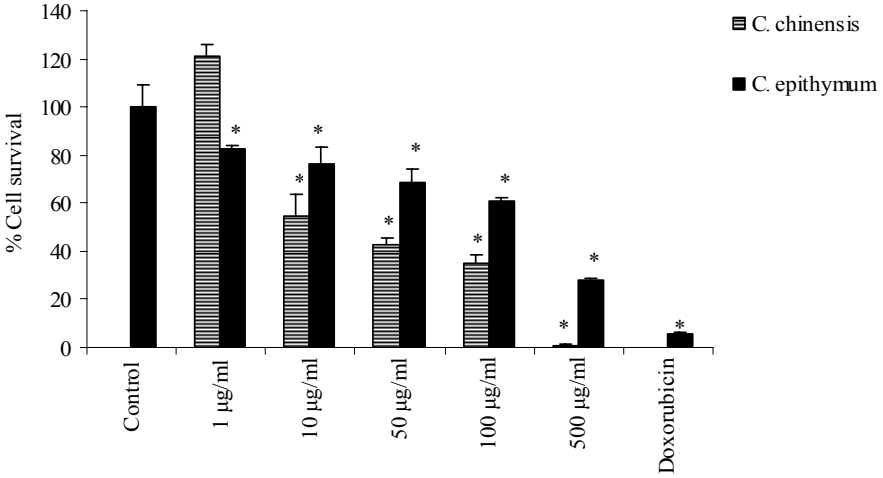

Fig. 5. The cytotoxic effect of the chloroform extracts of *C. chinensis* and *C. epithymum* on MDA-MB-468 cells. Viability of the cells was determined by MTT assay. Each extract concentration was examined in 8 wells. Percent cell survival in the control group was assumed 100. *=p<0.05, n=3.

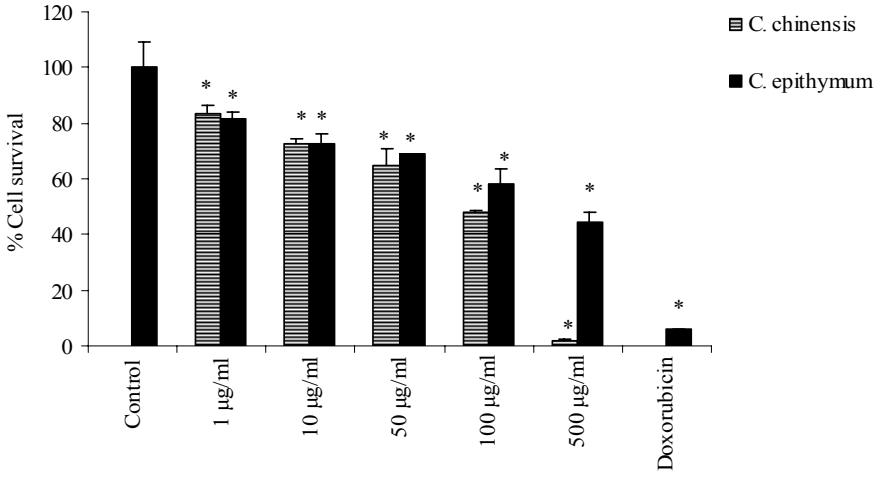

Fig. 6. The cytotoxic effect of the hydroalcoholic extracts of *C. chinensis* and *C. epithymum* on MDA-MB-468 cells. Viability of the cells was determined by MTT assay. Each extract concentration was examined in 8 wells. Percent cell survival in the control group was assumed 100. *=p<0.05, n=3.

DISCUSSION

Based on the discovery of vinblastine and vincristine, and the isolation of the cytotoxic podophyllotoxins in early 1950s, the United States National Cancer Institute (NCI) started extensive search for anticancer agents with plant origin in 1960. In this program several compounds with cytotoxic effect such as the taxanes and camptothecins were found (26). In the current study, we looked at the cytotoxic effects of *C. chinensis* and *C. epithymum* using MTT assay. Doxorubicin, a known cytotoxic drug (27), as a positive control significantly reduced viability of Hela, HT-29 and MDA-MB-468 cells indicating accuracy of the method used in this experiment. Our findings showed that the chloroform extracts of the aerial parts of *C. chinensis* and *C. epithyum* had significant cytotoxic effect on Hela, HT-29 and MDA-MB-468 cells. Also, the hydroalcoholic extracts of *C. chinensis* and *C. epithymum* significantly decreased the viability of Hela and MDA-MB-468 cells but they had no significant effect on HT29 cells. Chlorofom extracts of *C. chinensis* and *C. epithymum* were more cytotoxic than that of hydroalcoholic extracts in all tested cell lines (Table 3). In adition our data showed that extracts of *C. chinensis* were more cytotoxic than that of *C. epithymum* extracts.

Several studies have demonstrated that the pharmacological effects of *C. chinensis* and *C. epithymum* can be attributed to their main constituents including flavonoids, saccharids, alkaloids, lignans, saponins, and resin glycosides (18-22). Also, it has been shown that many flavonoids significantly decrease cell viability via the rise in caspase activity (20). As hydroalcoholic extract of *C. chinensis* and *C. epithymum* with more poly-phenolic content showed lower cytotoxic activity against all tested cell lines, it can be concluded that the role of other constituents in these plants in the cytotoxicity is higher than that of the poly-phenolic compounds of these plants. As chloroform has lower polarity than that of ethanol-water, it is expected that chloroform extracts of *C. chinensis* and *C. epithymum* contain components with lower polarity, so that the cytotoxicity of *C. chinensis* and *C. epithymum* may be attributed to less polar components in this plants.

According to our data, MDA-MB-468 and Hela cell lines were more sensitive to the choloroform extract of *C. chinensis* than HT-29 cell line, whereas the choloroform extract of *C. epithymum* was more cytotoxic on HT-29 cell line. These differences in susceptibility of tumor cell lines have been shown by several studies (24,28).

CONCLUSION

From the findings of this study, it can be concluded that extracts of the aerial parts of *C. chinensis* and *C. epithymum* possess cytotoxic activity against Hela, HT-29 and MDA-MB-468 cells. Also, the data represent that chloroform extracts are more potent than hydroalcoholic extracts .

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REFERENCES

1. Darafarin H. National program of cancer registries and pathology specialists play a role in the success of the program. Iranian J Pathol. 2009;24:2-4.
2. Chatterjee D, Sahu RK, Jha AK, Dwivedi J. Evaluation of antitumor activity of *Cuscuta refiexa* Roxb against ehrlich ascites carcinoma in Swiss albino mice. Tropical J Pharm Res. 2011;10:447-454.
3. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. Ethnopharmacology. 2003;100:72-79.
4. Avicenna, Canon on medicine. 1st ed. Morsal Publication, 2007.
5. Saha P, Mazumder UK, Haldar PK. Acute and subchronic toxicity of *C. maxima* aerial parts. Int J Res Pharmac Biomed Sci. 2011;2:634-639.
6. MC Neal JR, Arumugunathan K, Kuehl J, Boore JL, Depamphilis CW. Systematics and plastid genome evolution of the cryptically photosynthetic parasitic plant genus *Cuscuta*. BMC Biol. 2007;5:1-19.
7. Costea M, Spence I, Stefanović S. Systematics of *Cuscuta chinensis* species complex (subgenus Grammica, Convolvulaceae): evidence for long-distance dispersal and one new species. Organisms Divers Evol. 2011; 11:373-386.
8. Nice, G., B. Johnson, and T. Bauman. 2004. The infamous giant hogweed. Purdue University Extension Weed Science. Bulletin WS-32-W

(http://www.btny.purdue.edu/weedscience/2004/articles/gianthogweed04.pdf)
9. Ashigh J, Marquz E. Dodder *(Cuscuta spp.)* biology and management. NM State University, aces.nmsu.edu/pubs/_a/A-615,Guide A-615, p;1-4. 2007.
10. http://www.mdidea.com, Research Update Dodder Seed, 2003.
11. Feng-Lin Y, Tzu-Hui W, Liang-Tzung L, Chun-Ching L. Hepatoprotective and antioxidant effects of *Cuscuta chinensis* against acetaminophen-induced hepatotoxicity in rats. J Ethnopharmacol. 2007;111:123-128.
12. Feng-Lin Y, Tzu-Hui W, Liang-Tzung L, Chun-Ching L: nanoparticles formulation of *Cuscuta chinensis* prevents acetaminophen-induced hepatotoxicity in rats. Food Chemical Toxicol. 2008;46:1771-1777.
13. Yang L, Chen Q, Wang F, Zhang G. Anti osteoporotic compounds from seeds of *Cuscuta chinensis*. J Ethnopharmacol. 2011:135:553-560.
14. Amin G, Mokhtari Kondori B, Vazirian M, Abdi L, Arabshahi G. Evaluation of chemical composition and antioxidant activity of total exteract of *Cuscuta chinensis* Lam. used in traditional medicine. J Plant Med. 2010;76:147-150.
15. Jian-Hui L, Bo J, Yong-Ming B, Li-Jia A. Effect of *Cuscuta chinensis* glycoside on the neuronal differentiation of rat pheochromocytoma PC12 cells. Int J Devl Neurosci. 2003;21: 277-281.
16. Meulebrouck K, Brys R, Tanghe L, Verheyen K, Hermy M. Hidden in the host–unexpected vegetative hibernation of the holoparasite *Cuscuta epithymum* (L.) and its implications for population persistence. Flora. 2009;204:306-315.
17. Abdel Khalik KN. Seed morphology of *Cuscuta* L. (Convolvulaceae) in Egypt and its systematic significance. Wiley Inter Science, 2006;117:217-224.
18. Biswas SK, Chowdhury A, Das J, Karamkar UK, Raihan SZ, Das AC, *et al*. Phytochemical investigation and chromatographic evaluation with antimicrobial and cytotoxic potential of *Cuscuta epithymum.* Int J Pharm. 2012;8:422-427.
19. Suresh V, Sruthi V, Padmaja B, Asha VV. In vitro anti-inflamatory and anti-cancer activities of *Cuscuta reflexa Roxb*. J Ethnopharmacol. 2011;134:872-877.
20. Farzami Sepehr M, Jameie SB, Hajijafari B. The *Cuscuta kotschyana* effects on breast cancer cells line MCF7. J MPR. 2011;5:6344-6351.
21. Zeraati F, Zamani A, Goodarzi MT, Hashjin SMM, Razzaghi K. In vitro cytotoxic effects of *Cuscuta chinensis* whole extract on human acute lymphoblastic leukemia cell line. Iranian J Med Sci. 2010;35:310-314.
22. Ghazanfari T, Naseri M, Shams J, Rahmati B. Cytotoxic effects of *Cuscusta* extract on human cancer cell line. Food Agric Immunol. 2013;24:87-94
23. Minaiyan M, Ghannadi A, Etemad M, Mahzouni P. A study of the effects of *Cydonia oblonga* miller (quince). on TNBS-induced colitis in rats. Res Pharm Sci. 2012;7:103-110.
24. Jafarian-Dehkordi A, Emami SA, Saeidi M, Sadeghi H. Cytotoxicologic studies of the extracts of iranian *Juniperus sabina* and *Platycladus orientalis*on Cancer Cells. J Res Med Sci. 2004;5:205-209.
25. Feng-Lin Y, Tzu-Hui W, Liang-Tzung L, Chun-Ching L, Thau-Ming C. Concordance between antioxidant activities and flavonol contents in different extracts and fractions of *Cuscuta chinensis*. Food Chem. 2008;108:455-462.
26. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol. 2005;100:72-79.
27. Germain1 E, Chajès V, Cognault S, Lhuillery C, Bougnoux P. Enhancement of doxorubicin cytotoxicity by polyunsaturated fatty acids in the human breast tumor cell line MBA-MB-231: Relationship to lipid peroxidation. Int J Cancer. 1998;75:578-583.
28. Monajemi R, Oryan S, Haeri-Roohani A, Ghannadi A, Jafarian A. Cytotoxic effects of essential oils of some Iranian Citrus peels. Iran J Pharm Res. 2005;3:183-187.