



## Cytotoxic effects of selective species of Caryophyllaceae in Iran

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### Abstract

Cancer is a major cause of death worldwide and causes serious problems in human life. It is developed by uncontrolled growth of a cell or a group of cells. There are many difficulties in treatment of cancer and many researchers are involved in investigating for effective drugs to treat the disease. Caryophyllaceae is a large family of about 86 genera and 2200 herbaceous or subshrub species. The family is known for its ornamental plants and saponin compounds. In the present study, the potential cytotoxic activity of 17 selected species from Caryophyllaceae has been investigated against MCF-7, HepG-2, A-549, HT-29 and MDBK cells using MTT assay. Five species exhibited cytotoxic effects with IC<sub>50</sub> values < 100 µg/mL. *Silene ampullata* and *Acanthophyllum bracteatum* extracts were toxic only against MCF-7 cell line suggesting them as suitable candidates for more investigations of breast cancer studies.

**Keywords:** Caryophyllaceae, cytotoxic activity, MTT assay

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### Introduction

Cancer is known as the second cause of death worldwide and is characterized by the failure in the regulation of the tissue growth that results in uncontrolled multiplication of the normal cells and forming tumors which invade adjacent tissues. However, cancer is considered preventable since the major causes of different cancers are environmental factors and also people's lifestyle [1]. There are many difficulties in the treatment of cancer, among which, drug resistance, toxicity and the low specificity are more common [2]. Many recent investigations have focused on introducing more effective drugs

with lower side effects. Medicinal plants as natural compounds represent a vast potential resource for anticancer researches [3].

Saponins are a large class of natural products present in higher plants [4]. They exhibit a wide variety of both structural diversity and biological activity. They are widespread in medicinal plants and are very often responsible for their pharmacological effects. Saponins isolated from plant sources are usually natural glycosides which possess pharmacological properties including anti-inflammatory, antimicrobial, antispasmodic, antidiabetic, anticancer,

anticonvulsant, anthelmintic, antitussive and cytotoxic activities. Plant saponins have also a number of traditional and industrial applications [5].

Caryophyllaceae is a large family of about 86 genera and 2200 herbaceous or subshrub species [6-8]. The family is known for its ornamental plants and saponin compounds. It represents one of the largest and widespread plant families with some 38 genera in Iran. *Silene*, *Dianthus*, *Gypsophila* and *Acanthophyllum* comprise the largest genera of the family in Iran [9], while *Arenaria* L., *Minuartia* L., *Stellaria* L., *Cerastium* L., *Vaccaria* Medicus, *Bufo* L. and *Paronychia* P.Mill., could be considered as the minor important genera.

In the present study, we have investigated the cytotoxic effects of the crude extracts of 17 species from Caryophyllaceae belonging to the genera *Acanthophyllum*, *Arenaria*, *Dianthus*, *Gypsophila*, *Minuartia*, *Silene* and *Vaccaria*, against MCF-7 (human breast adenocarcinoma), HepG-2 (human hepatocellular carcinoma), MDBK (median- darby bovine kidney), A-549 (non-small cell lung carcinoma) and HT-29 (human colon adenocarcinoma), using Methyl Thiazol Tetrazolium (MTT) assay.

## Experimental

### *Plant material*

Seventeen species of Caryophyllaceae were collected from West of Iran during 2009-2011. The voucher specimens were deposited at the Herbarium of Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

### *Extraction*

The extracts were obtained by macerating the dried plant powder with 80% methanol for 24 h and were dried at 40 °C. The cell lines HepG-2, MCF-7, HT-29, A-549 and MDBK were obtained from Pasteur Institute of Iran. Each cell line was cultured in suitable medium to obtain the desired growth and the growth curve of each

cell line was plotted.

### *MTT assay*

Cytotoxic activity was assessed by MTT assay with different concentrations of the plant extracts [10]. The extracts were dissolved in DMSO (dimethyl sulfoxide) and further diluted with cell culture medium. The cells were seeded in 96-well flat bottom tissue culture plates at a density of approximately  $(0.5-1.5) \times 10^4$  cells/well and incubated for 24 h at 37 °C. Then, the medium was replaced by fresh medium containing different concentrations of the extracts and incubated for 72 h. By removing the supernatant of each well, MTT solution (0.5 mg/mL) was added to each well. After incubation for 4 h, the resultant formazan crystals were dissolved in DMSO (200  $\mu$ L) and the absorbance intensity was measured by a microplate reader at 570 nm. Cytotoxicity was expressed as the concentration of the extract inhibiting cell growth by 50% (IC<sub>50</sub>). Tamoxifen was included as the positive control.

## Results and Discussion

Cytotoxic effect of the species was measured against 5 cell lines including MCF-7, HepG-2, HT-29, A-549 and MDBK by MTT assay. Five methanol extracts of the species of Caryophyllaceae exhibited cytotoxic effects (table 1). Among the extracts, *Acanthophyllum microcephalum* showed activity against MCF-7 and MDBK cell lines (IC<sub>50</sub> value 27.09 and 32.78  $\mu$ g/mL, respectively). Besides, *Acanthophyllum bracteatum* and *Silene ampullata* showed cytotoxic effects against MCF-7 and *Gypsophila bicolor* and *Vaccaria oxyodonta* against MDBK cells.

Medicinal plants have been prescribed and used for centuries with a strong belief for their ability to cure diseases. Over the past 20 years, there has been growing interest in investigations of natural materials as sources of new insecticidal and cytotoxic materials [11].

The family Caryophyllaceae contains triterpenoid saponins with medicinal application and there

**Table 1.** Cytotoxic effects [IC<sub>50</sub> (µg/ml)] of some species of Caryophyllaceae family, up to concentrations of 100 µg/mL

| Plant Name   | IC <sub>50</sub> (µg/mL) |        |       |       |       |
|--|--------------------------|--------|-------|-------|-------|
|  | A-549                    | HepG-2 | HT-29 | MCF-7 | MDBK  |
| <i>Acanthophyllum acerosum</i> Sosn.               | -                        | -      | -     | -     | -     |
| <i>Acanthophyllum bracteatum</i> Boiss.            | -                        | -      | -     | 60.48 | -     |
| <i>Acanthophyllum microcephalum</i> Boiss.         | -                        | -      | -     | 27.09 | 32.78 |
| <i>Arenaria graminea</i> C.A.Mey                   | -                        | -      | -     | -     | -     |
| <i>Dianthus orientalis</i> Adams                   | -                        | -      | -     | -     | -     |
| <i>Gypsophila adenophora</i> Boiss. & Buhse        | -                        | -      | -     | -     | -     |
| <i>Gypsophila bicolor</i> (Freyn & Sint.) Grossh.  | -                        | -      | -     | -     | 7.82  |
| <i>Gypsophila polyclada</i> Fenzl ex Boiss.        | -                        | -      | -     | -     | -     |
| <i>Minuartia glandulosa</i> (Boiss. & Huet) Bornm. | -                        | -      | -     | -     | -     |
| <i>Minuartia lineata</i> (Boiss.) Bornm.           | -                        | -      | -     | -     | -     |
| <i>Silene ampullata</i> Boiss.                     | -                        | -      | -     | 59.08 | -     |
| <i>Silene bupleuroides</i> L.                      | -                        | -      | -     | -     | -     |
| <i>Silene chlorifolia</i> Sm.                      | -                        | -      | -     | -     | -     |
| <i>Silene morganae</i> Freyn                       | -                        | -      | -     | -     | -     |
| <i>Silene peduncularis</i> Boiss.                  | -                        | -      | -     | -     | -     |
| <i>Silene propinqua</i> Schischk.                  | -                        | -      | -     | -     | -     |
| <i>Vaccaria oxyodonta</i> Boiss.                   | -                        | -      | -     | -     | 43.04 |

have been several studies on saponins of different genera of the family [12]. For example, studies on saponins of *Gypsophila* have demonstrated their anticarcinogenic properties, including direct cytotoxicity, immune-modulating effects and normalization of carcinogen induced cell proliferation [13]. *Gypsophila oldhamiana*, rich in triterpenoid saponins, has exhibited antitumor properties [14]. A triterpenoid saponin isolated from the methanol extract of *Gypsophila pilulifera* has displayed significant cytotoxicity by XTT assay [15] against A-549 cell line with IC<sub>50</sub> values <16 µM [16]. In our study, the cytotoxic activity of *Gypsophila bicolor* has been investigated by MTT assay which has shown IC<sub>50</sub> value of 7.82 µg/mL against MDBK cell line, however it did not show cytotoxic effect in other cell lines.

Two new triterpenoid saponins have been isolated from *Acanthophyllum gypsophiloides* roots that have suggested it as a prospective source of immunoactive agents [17]. Four cyclopeptide dianthins C, D, E, and F have been isolated from *Dianthus superbus*. Dianthin E has significantly inhibited the growth of the human liver cancer cell line, (HepG-2, IC<sub>50</sub> 2.37 µg/ml)

[18]. However, in the present study, *Dianthus orientalis* did not show any cytotoxicity to the cell lines up to concentrations of 100 µg/mL. The Phytoecdysteroids from the aerial parts of *Silene guntensis* have exhibited substantial inhibition of cell growth against human cervix adenocarcinoma (HeLa), hepatocellular carcinoma (HepG-2), and breast adenocarcinoma (MCF-7) cells [19] while *Silene ampullata* in our research indicated cytotoxic activity only against MCF-7 cells (IC<sub>50</sub> value of 59.08 µg/mL).

It could be concluded that the evaluated species contain potential bioactive compounds and can be proper candidates for further natural anti-proliferative and antitumor compound studies.

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**References**

- [1] Dashora N, Sodde V, Bhagat J, Kirti S, Lobo PR. Antitumor Activity of *Dendrophthoe falcata* against Ehrlich Ascites Carcinoma in Swiss Albino Mice. *Topharmac J*. 2011, 2: 1-7.
- [2] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011; 61(2): 69-90.
- [3] Evans WC, Trease E. *Pharmacognosy*. London: Saunders, 2002.
- [4] Sparg SG, Light ME, Staden J. Biological activities and distribution of plant saponins. *J Ethnopharmacol*. 2004; 94: 219-243.
- [5] Niaz A, Syed Wadood A, Ismail Sh, Ghayour A, Mehreen Gh, Imran Kh. Cytotoxic and anthelmintic potential of crude saponins isolated from *Achillea Wilhelmsii* C. Koch. *Teucrium Stocksianum* boiss. *Complement Altern Med*. 2011; 11:106
- [6] Smissen RD, Clement JC, Garnock-Jones PJ, Chambers GK. Subfamilial relationships within Caryophyllaceae as inferred from 5' *ndhF* sequences. *Am J Bot*. 2002; 89: 1336-1341.
- [7] Fior S, Karis PO, Casazza G, Minuto L, Sala F. Molecular phylogeny of the Caryophyllaceae (Caryophyllales) inferred from chloroplast *matK* and nuclear rDNA ITS sequences. *Am J Bot*. 2006; 93: 399-411.
- [8] Greenberg AK, Donoghue MJ. Molecular systematics and character evolution in Caryophyllaceae. *Taxon*. 2011; 60(6): 1637-1652.
- [9] Rechinger KH. *Flora Iranica* 163. Graz: Akademische Druch-u, Verlagsanstalt, 1988.
- [10] Hamzeloo-Moghadam M, Naghibi F, Atoofi A, Asgharian Rezaie M, Irani M, Mosaddegh M. Cytotoxic activity and apoptosis induction by gaillardin. *Z Naturforsch C*. 2013; 68(4):108-112.
- [11] Sindhu S, Manorama S. Antimicrobial activity of *Polycarpaea corymbosa* Lam. (Caryophyllaceae) against human pathogens. *J Chem Pharm Res*. 2012; 4(8): 4014-4019.
- [12] Gevrenova R, Stancheva T, Voynikov Y, Laurain-Mattar D, Henry M. Root in vitro cultures of six *Gypsophila* species and their saponin contents. *Enzyme Microb Technol*. 2010; 47: 97-104.
- [13] Krasteva IN, Popov IS, Balabanova VI, Nikolov SD. Phytochemical study of *Gypsophila trichotoma* Wend. (Caryophyllaceae). *Quim. Nova*. 2008; 31(5): 1125-1126.
- [14] Zhang W, Luo JG, Zhang C, Kong LY. Different apoptotic effects of triterpenoid saponin-rich *Gypsophila oldhamiana* root extract on human hepatoma SMMC-7721 and normal human hepatic L02 cells. *Biol Pharm Bull*. 2013; 36(7):1080-1087.
- [15] Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH. Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res*. 1988; 48: 4827-4833.
- [16] Arslan I, Celik A, Chol JHA. Cytotoxic triterpenoid saponin from under-ground parts of *Gypsophila pilulifera* Boiss. & Heldr. *Fitoterapia*. 2012; 83(4):699-703.
- [17] Khatuntseva EA, Vladimir MM, Alexander SS, Tsvetkov YE, Stepanenko RN, Vlasenko RY, Shults EE, Tolstikov GA. Triterpenoid saponins from the roots of *Acanthophyllum gypsophiloides* Regel. *Beilstein J Org Chem*. 2012; 8: 763-775.
- [18] Hsieh P, Chang F, Wu C. New cytotoxic cyclic peptide and dianthramide from *Dianthus superbus*. *J Nat Prod*. 2004; 67:1522-1527.
- [19] Mamadaliyeva NZ, El-Readi MZ, Janibekov AA, Tahrani A, Wink M. Phytoecdysteroids of *Silene guntensis* and their in vitro cytotoxic and antioxidant activity. *Z Naturforsch C*. 2011; 66(5-6):215-224.