Jundishapur J Nat Pharm Prod. 2020 May; 15(2):e65760. doi: 10.5812/jjnpp.65760.

Published online 2019 November 27. **Research Article**

Cytotoxic Constituents and Molecular Docking Study of the Active Triterpenoids from *Tripleurospermum disciforme* (C. A. Mey.) Schultz-Bip

Salar Hafez Ghoran [1,*], Esmaeil Babaei [2], Hasan Rezaei Seresht [3] and Zahra Karimzadeh [4]

[1]Department of Chemistry, Faculty of Basic Sciences, Golestan University, Gorgan, Iran
[2]Department of Animal Biology, School of Natural Sciences, University of Tabriz, Tabriz, Iran
[3]Traditional and Complementary Medicine Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran
[4]Department of Organic Chemistry, School of Chemistry, University of Tabriz, Tabriz, Iran

[*]*Corresponding author*: Department of Chemistry, Faculty of Sciences, Golestan University, P.O. Box: 4913815759, Gorgan, Iran. Tel: +98-9144425047, Email: s_hafezghoran@yahoo.com

Received 2017 December 31; **Revised** 2018 August 14; **Accepted** 2018 September 05.

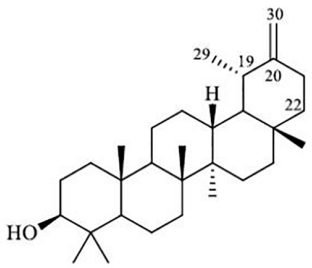
Abstract

Background: Terpenoids are produced by a wide variety of plants, animals and microorganisms, which effectively plays a role in the survival of the organisms by means of functional, defensive and communicational attitudes.
Objectives: The main purpose of the present study was isolation and elucidation of the natural terpenoids from the aerial parts of *Tripleurospermum disciforme* (Compositae/Asteraceae family).
Methods: The phytochemical investigation of the dichloromethane extract of *T. disciforme* was carried out by various chromatographical methods such as column chromatography and thin layer chromatography. The major compounds were purified and their structures were established by using nuclear magnetic resonance and electron impact mass spectroscopic data. Moreover, the cytotoxic ability of the isolated compounds were measured on the human gastric carcinoma (AGS) and the mouse skin fibrosarcoma (WEHI-164) cell lines by using MTT assay. Molecular docking studies of the specialized metabolites were performed with Bcl-2, Bcl-xl, CDK2 accompanied by tubulin proteins using AutoDock Vina.
Results: Three triterpenoids including (1) taraxasterol; (2) lupeol; and (3) betulinic acid were isolated and elucidated. Our cytotoxic results exhibited that compound 2 could be considered as an anti-tumor component with an IC_{50} value of 3.2 μM on WEHI-164 cell lines. Likewise, 3 displayed the potent cytotoxic activity, with an IC_{50} value of 5.6 μM on AGS cell lines. It is noteworthy to mention that the triterpenes 1 - 3, newly reported in *T. disciforme* impacted on the prevention of tubulin polymerization because of the strong interaction with the vinblastine binding site of tubulin.
Conclusions: Our data suggested that the isolated triterpenoides from *T. disciforme* possess anti-tumor properties, and may be included among the effective natural anti-tumors.

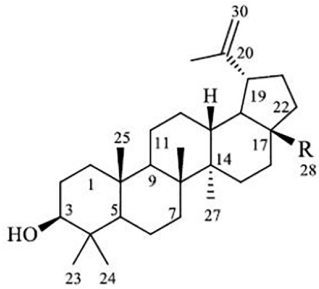
Keywords: Betulinic Acid, Compositae, Lupeol, MTT Assay, *Tripleurospermum disciforme*, Molecular Docking

1. Background

Tripleurospermum disciforme (C. A. Mey.) Schultz-Bip. belongs to the Compositae (Asteraceae) family. Known as "Iranian Babouneh", it is a perennial herb that grows wildly to about 10 - 70 cm in height. The plant synonyms are *Chrysanthemum disciforme* (C. A. Mey.), *Matricaria disciforme* (C. A. Mey.) DC., *Chamaemelum disciforme* (C. A. Mey.) Vis. as well as *Chamaemelum disciforme* var. *quadrilobum* Boiss (1). It is believed that in Iranian traditional medicine (particularly in Hamedan and Mashhad provinces), decoction of *T. disciforme* is used for treatment of kidney stone (2), treatment of cough and febrifuge (3) and acting as antispasmodic and anti-inflammatory agent (4). According to the previous studies on various *T. disciforme* extracts and essential oils, several pharmacological and biological activities such as anti-oxidant (5, 6), anti-inflammatory (7), anti-ulcer (8), anti-bacterial (9, 10) and anti-fungal (11) have been reported. Meanwhile, recent phytochemical analysis of *Tripleurospermum* species extracts have revealed the presence of dioxaspirans (6), flavonoids (10), melatonins (12), triterpenes (13), acetylenes (14), and saponins (15). Herein, the isolation and structural identification of one taraxastane triterpene 1 and two lupane triterpenes 2 and 3 (Figure 1) are reported for the first time from the species mentioned. Among different kinds of studies evaluated in vitro, (1) taraxasterol; (2) lupeol; and (3) betulinic acid had cytotoxic effects on the various cancerous cell lines such as breast, cervix, colon, prostate, lung, ovary, skin, hematopoietic and lymphoid tissue (16-18).



Taraxasterol (1)



$R = CH_3$, Lupeol (2)
R = COOH, Betulinic Acid(3)

Figure 1. Chemical structures of isolated triterpenoids 1 - 3 from the aerial parts of *T. disciforme*

2. Objectives

The present study was designed to isolate and elucidate the natural terpenoids form the dichloromethane (DCM) extract of *Tripleurospermum disciforme* aerial parts (Compositae/Asteraceae). Moreover, not only the cytotoxic activity of the obtained compounds were assayed on two cancerous cell lines, AGS and WEHI-164, but the molecular duking of isolated compounds were also studied computationally by using AutoDock Vina.

3. Methods

3.1. Plant Material

The aerial parts of *Tripleurospermum disciforme* were collected in July 2014 from Taleghan (Tehran province) and were dried in the air. The plant was identified by Hamid Moazzeni (Traditional Medicine and Materia Medica Research Center), TMRC, Shahid Beheshti University of Medical Sciences, Tehran, Iran), and a voucher specimen (no. 3245) was deposited in the herbarium of the same institute.

3.2. Extraction and Isolation

The air-dried powdered aerial parts (200 g) of *T. disciforme* (C. A. Mey.) were extracted at room temperature with DCM by maceration method (3 × 2 L, rt for 24 h) and the combined extracts evaporated to give a dark green gummy residue (15 g). Five g of the extract was subjected on a silica gel (70 - 230 mesh) column chromatography (CC) and eluting with n-hexane:EtOAc:MeOH [100:0:0, 95:05:0, 90:10:0, 80:20:0, 70:30:0, 60:40:0, 50:50:0, 30:70:0, 10:90:0 and 0:80:20 (v/v), respectively] to yield ten fractions, A-J. Fraction E (277 mg) was re-chromatographed by Et_2O:EtOAc in order of increasing polarities to yield 1 (43 mg, with R_f = 0.7 for Et_2O:EtOAc, 5:5) and 2 (25 mg, with R_f = 0.6 for Et_2O:EtOAc, 7:3). Compound 3 (19 mg, with R_f = 0.65 for $CHCl_3$:EtOAc, 15:5) was purified from the fraction G (326 mg) by twice silica gel column chromatography. The first silica gel column (230 - 400 mesh) was prepared by stepwise gradient eluting with $CHCl_3$:EtOAc, 10:0 - 8:2, and 7 subfractions were obtained. Consequently, the fifth subfraction loaded over second silica gel column (230 - 400 mesh) and isocratically eluted by $CHCl_3$:MeOH, 98:2. The 1H-13C NMR along with mass spectroscopic data for (1) taraxasterol (19); (2) lupeol (20); and (3) betulinic acid (21) have previously been reported.

3.3. General Experimental Procedures

1H and 13C-NMR spectra (in $CDCl_3$) were measured on a Bruker Avance TM 400 DRX spectrometer with TMS as an internal standard. Electron ionization mass spectra (EI-MS) were acquired using a Bruker Apex II mass spectrometer (Bruker, Bremen, Germany). Silica gel (70 - 230 and 230 - 400 mesh, Merck) were used for column chromatography. TLC was performed on Merck F_{254} silica gel plates (10 × 10 cm).

3.4. Cell Lines and Reagents

Two cell lines including AGS and WEHI-164; both from Pasteur Institute, Tehran, Iran, were grown in Dulbecco's modified Eagle's medium (DMEM; GIBCO, USA) containing 10% fetal bovine serum (FBS; GIBCO, USA) at 37°C in a humidified atmosphere of 5% CO_2.

3.5. MTT Cytotoxicity Assay

Cell viability was measured by MTT assay according to the manufacturer's instructions (Sigma-Aldrich, USA). Dendrosmal curcumin as an anti-proliferative compound was also employed as a positive control (22, 23). The relative cell viability was determined at 540 nm by a 96-well plate reader (Biorad-USA) and the concentration at which cell growth was inhibited by 50% (IC_{50}) was determined by standard curve method (24, 25).

3.6. Computational Chemistry

3.6.1. Ligand preparation

The structures of isolated compounds were drawn by using ChemDraw Ultra (version 8.0 CambridgeSoft Corporation) and the 3D structures were optimized by Gaussian W90 software using semi empirical, PM3 level. The ligand structures were prepared by PyRx software and saved as pdbqt using autodock menu.

3.6.2. Receptors Preparation

The 3D X-ray structure of proteins Bcl-2, Bcl-xl, CDK2 and tubulin were obtained from RCSB database (PDB codes: 4aq3, 3spf, 2wlh and 4eb6, respectively). The presence of Vina plugin in PyRx software was used for the docking studies.

3.6.3. Docking

The grid boxes were constructed around the binding site of all proteins mentioned. The number of grid points in the three dimensions [npts] and coordinates by way of three dimensions [gridcenter] were in the following order;

Bcl-2 protein; [npts]: X: 40.63, Y: 34.76, Z: 39.13; [gridcenter]: X: -13.929, Y: 14.177, Z: -10.172.

Bcl-xl protein; [npts]: X: 42.81, Y: 36.67, Z: 37.65; [gridcenter]: X: 35.054, Y: 14.895, Z: -13.164.

CDK2 protein; [npts]: X: 52.49, Y: 40.53, Z: 63.72; [gridcenter]: X: 28.513, Y: 5.046, Z: 49.971.

Tubulin protein; [npts]: X: 17.07, Y: 15.67, Z: 17.78; [gridcenter]: X: 11.357, Y: 89.419, Z: 107.420.

The spacing and exhaustiveness ranges for all cases were determined 1.000 and 15, respectively. Hydrogen atoms and Gasteiger partial charges were added using AutoDock software (V. 4.2).

4. Results and Discussion

The DCM extract of *T. disciforme* aerial parts was fractioned by a normal phase-column chromatography (NP-CC) system, and subsequently three major triterpenoids were isolated. The triterpenoids exhibited violet spots on TLC plates by spraying Anisaldehyde-H_2SO_4 reagent followed by heating. The structure elucidations of (1) taraxasterol (19); (2) lupeol (20); and (3) betulinic acid (21) were acquired based on comparing their spectroscopic data (NMR and EI-MS) with those described in the literature (Table 1). The NMR elucidation procedures have been described in the supplementary file.

The taraxastane and lupane triterpenes have exhibited the potential cytotoxicity (26-29). Hence, to determine the cytotoxic activities of isolated triterpenoids (1-3), cellular toxicity was evaluated by MTT assay on two cancerous cell lines - the human gastric carcinoma (AGS) cell line as well as the mouse skin fibrosarcoma (WEHI-164) cell line. The toxicity effect at 0 - 200 μM concentrations was detected in a time- and dose-dependent manner. As shown in Table 2, the results of cytotoxicity revealed that compounds 2 and 3 with lupane structures possess stronger activity than compound 1 with taraxastane framework. Interestingly, compounds 1 - 3 in particular were more active than the positive control compound, dendrosmal curcumin, with an expectation for compound 1 on WEHI-164 cell line behaving a less actively than that of dendrosmal curcumin (IC_{50} values of 23.8 and 18.0 μM, respectively). Although some other similar structures have been previously reported from other *Chrysanthemum* species, for instance, arnidiol, faradiol (taraxastane-type), calenduladiol and heliantriol B_2 (lupane-type) from *C. morifolium*. Arnidiol had remarkably exhibited a wide range of cytotoxicity against sixty human cancer cell lines (GI_{50} values of mostly < 6 μM) (13).

Molecular docking studies of three metabolites-taraxasterol, lupeol and betulinic acid were carried out with Bcl-2, Bcl-xl, CDK2 accompanied by tubulin proteins using AutoDock Vina, to recognize the binding mode of ligands and the intramolecular hydrogen bond and other interactions between the target proteins and ligands. The outcomes revealed that the best interactions were related to lupeol and/or taraxasterol with Bcl-xL. On the other hand, the weakest interaction was related to betulinic acid with Bcl-2. The interactions of isolated compounds with tubulin recorded by computational docking study have been presented in Figure 2A. The schematic 2D plots were displayed intermolecular interactions of the representative taraxasterol with tubulin (Figure 2B). The Bcl-2 protein functions to prevent apoptosis through protection of mitochondrial external membrane integrity via binding to Bax/Bak (30). The high binding affinity of compounds 1 - 3 indicated the ability to either inhibit the Bcl-2 or induce the apoptosis trend (mentioned in the Appendix 1 in Supplementary File).

Note that overexpression of the antiapototic protein, Bcl-xL, provides a public mechanism bringing about the

Table 1. NMR Spectroscopic Data for (1) Taraxasterol; (2) Lupeol; and (3) Betulinic Acid[a]

	Taraxasterol		Lupeol		Betulinic Acid	
Number	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	-	39.1	-	38.9	-	39.1
2	-	27.5	-	25.6	-	27.8
3	3.21, dd (6.4, 13.1)	79.3	3.24, dd (4.8, 11)	79.0	2.98, dd (5.9, 10)	78.3
4	-	38.9	-	39.6	-	39.1
5	-	55.4	-	55.4	-	55.6
6	-	18.5	-	18.3	-	18.6
7	-	34.3	-	34.0	-	34.7
8	-	50.0	-	41.2	-	40.3
9	-	50.5	-	50.5	-	50.0
10	-	37.2	-	37.1	-	37.5
11	-	21.7	-	21.5	-	21.0
12	-	26.4	-	27.4	-	25.8
13	-	39.1	-	39.4	-	38.3
14	-	42.3	-	42.9	-	42.5
15	-	26.7	-	27.6	-	30.7
16	-	38.6	-	38.3	-	32.6
17	-	34.5	-	44.1	-	56.5
18	-	47.1	-	48.7	-	47.0
19	2.37 m	40.1	2.25 m	47.6	2.82 m	49.5
20	-	154.9	-	151.1	-	150.3
21	-	25.9	-	29.7	-	30.0
22	-	39.0	-	40.0	-	37.5
23	0.78 s	28.4	1.00 s	28.0	0.68	28.0
24	0.84 s	15.6	0.78 s	15.4	0.74	15.4
25	0.86 s	17.1	0.88 s	16.4	0.72	16.3
26	0.90 s	16.2	1.02 s	15.9	0.75	16.5
27	0.94 s	14.9	0.93 s	14.8	0.78	14.7
28	0.85 s	18.7	0.86 s	17.8	-	179.6
29	1.02 s	26.0	1.62 s	19.5	1.50	19.7
30	4.62 s	107.4	4.63 s	107.2	4.40 s	109.2
	4.64 s		4.65 s		4.53 s	

[a] 1H-13C NMR data recorded in $CDCl_3$; (400 MHz for δ_H, 100 MHz for δ_C)

survival of cancerous cells along with special resistance to the conventional chemotherapy. Therefore, an attractive strategy for cancer therapy was proposed as the specific enzyme inhibition (31). In a respective manner, taraxasterol, lupeol and betulinic acid have had potent interaction with Bcl-xL along with the high binding affinity -8.9, -9.3 and -9.3 kcal/mol (Figure 3).

Additionally, the cycling-dependent kinase-2 (CDK2) plays a key role in regulating several events of eukaryotic cell division cycle. The evidence demonstrated that over expression of CDK2 might be a main reason of the abnormal regulation of cell-cycle, which would be straightly related to hyperproliferation in cancer cells. The microtubules formed during polymerization of α- and β-tubulins; however, the most important role of microtubules is to create the mitotic spindle involved in cell division (32). It has been

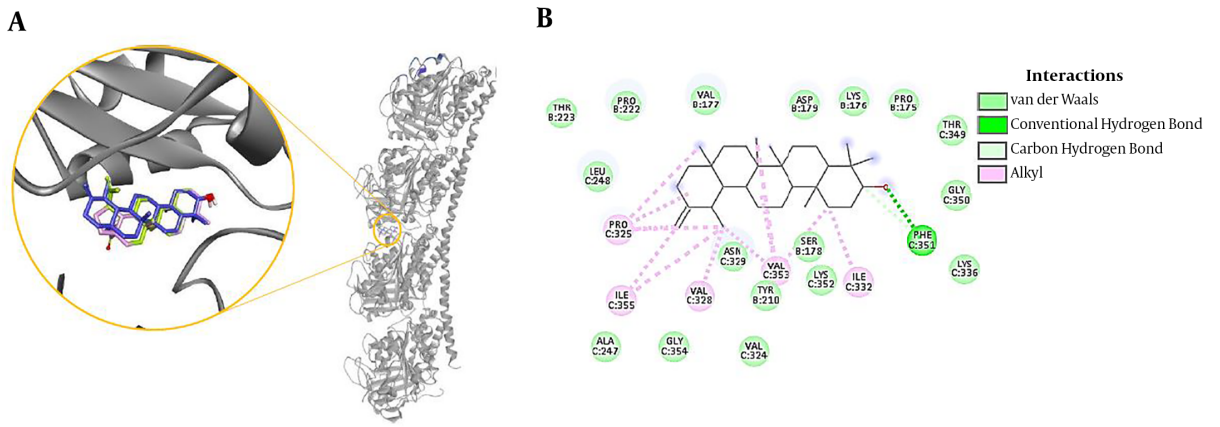


Figure 2. A, Binding modes at: vinblastine binding site of tubulin (PDB: 4eb6), betulinic acid (green), lupeol (pink), taraxasterol (blue); B, hydrophobic interactions and hydrogen bonding between taraxasterol and tubulin.

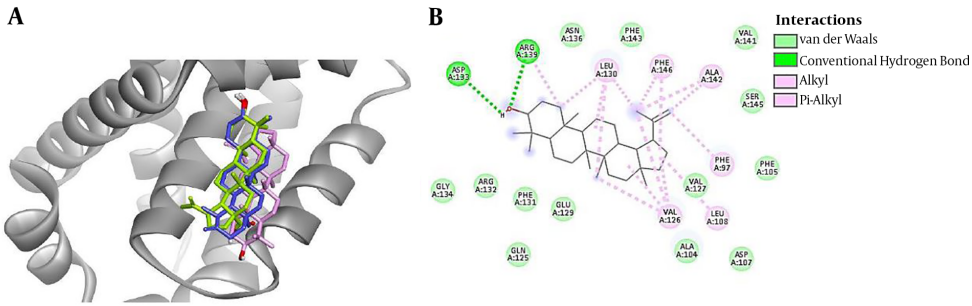


Figure 3. A, Proposed binding model of betulinic acid (green), lupeol (pink), taraxasterol (blue) within the Bcl-xL (PDB: 3spf); B, hydrophobic interactions and hydrogen bonding between lupeol and Bcl-xL.

Table 2. Cytotoxic Activity of Compounds 1 - 3 Isolated from the *T. disciforme* Aerial Parts

Natural Compounds	IC_{50}, μM[a]	
	AGS	WEHI-164
Taraxasterol (1)	16.1 ± 0.4	23.8 ± 2.1
Lupeol (2)	10.4 ± 1.7	3.2 ± 0.9
Betulinic acid (3)	5.6 ± 1.3	7.7 ± 1.6
Dendrosmal curcumin[b]	16.6 ± 0.6	18.0 ± 0.8

[a]These data represent the average values of three repeated experiments and expressed as mean ± SD.
[b]Dendrosmal curcumin was used as a positive control; the values are checked in every experiment.

reported that the afforded triterpenoids not only could effectively decrease the levels of Bcl-2 expression but upregulate the box gene in various cells as previously approved (27, 31, 33). Other studies have discussed how these compounds could be act as the main factor of downregulation in Bcl-xl and CDK2 expression (34, 35). In the present study, our data indicated that the compounds 1 - 3 could computationally inhibit Bcl-2 protein activity and impact on the prevention of tubulin polymerization because of the strong interaction existing between all compounds with the vinblastine binding site of tubulin.

4.1. Conclusions

To the best of our knowledge, this is the first study of isolation and structure elucidation of three known triterpenes (taraxasterol, lupeol and betulinic acid) from dichloromethane extract of the *Tripleurospermum disciforme* (C. A. Mey.) Schultz-Bip. aerial parts, elucidated by spectroscopic analyses in comparison with spectroscopic and physical data mentioned in the literature. As these isolated compounds exert the ability of cytotoxic activity studied by computational technique (molecular docking), its future pharmacological application is of particular im-

portance because it may be consequential in the prevention of cancer.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

Acknowledgments

We are grateful to Golestan University and Tabriz University Research Council for partial support of the work.

Footnotes

Authors' Contribution: Study concept and design: Salar Hafez Ghoran. Study supervision: Salar Hafez Ghoran. Analysis and interpretation of data: Salar Hafez Ghoran and Esmaeil Babaei. Acquisition of data: Hasan Rezaei Seresht, Zahra Karimzadeh.

Conflict of Interests: All authors declare that there is no conflict of interest.

Funding/Support: The work was partially supported by Golestan University and Tabriz University Council Research.

References

1. Mozaffarian V. *Identification of medicinal and aromatic plants of Iran*. Tehran, Iran: Frahang Moaser Pub; 2013. p. 289–90.
2. Amiri MS, Joharchi MR. Ethnobotanical investigation of traditional medicinal plants commercialized in the markets of Mashhad, Iran. *Avicenna J Phytomed*. 2013;**3**(3):254.
3. Naghibi F, Esmaeili S, Malekmohammadi M, Hassanpour A, Mosaddegh M. Ethnobotanical survey of medicinal plants used traditionally in two villages of Hamedan, Iran. *Res J Pharmacogn*. 2014;**1**:7–14.
4. Dehkordi Ghasemi NA, Amin GHR, Rahiminezhad MR, Salehi MH, Jafarpisheh A. Morphological and phytochemical study of *Tripleurospermum disciforme* (CA Mey) Schultz Bip. *Pajouhesh Sazandegi*. 2003;**16**:42–6.
5. Cavar Zeljkovic S, Ayaz FA, Inceer H, Hayirlioglu-Ayaz S, Colak N. Evaluation of chemical profile and antioxidant activity of *Tripleurospermum insularum*, a new species from Turkey. *Nat Prod Res*. 2015;**29**(3):293–6. doi: 10.1080/14786419.2014.968156. [PubMed: 25299590].
6. Souri E, Sarkhail P, Kaymanesh P, Amini M, Farsam H. Antioxidant activity of extract and a new isolated dioxaspiran derivative of *Tripleurospermum disciforme*. *Pharm Biol*. 2008;**43**(7):620–3. doi: 10.1080/13880200500302009.
7. Parvini S, Hosseini MJ, Bakhtiarian A. The study of analgesic effects and acute toxicity of *Tripleurospermum disciforme* in rats by formalin test. *Toxicol Mech Methods*. 2007;**17**(9):575–80. doi: 10.1080/15376510701580864. [PubMed: 20020884].
8. Minaiyan M, Ghassemi-Dehkordi N, Mohammadzadeh B. Anti-ulcer effect of *Tripleurospermum disciforme* (CA Mey) Shultz Bip on pylorus ligated (Shay) rats. *Res Pharm Sci*. 2007;**1**(1):15–21.
9. Chehregani A, Mohsenzadeh F, Mirazi N, Hajisadeghian S, Baghali Z. Chemical composition and antibacterial activity of essential oils of *Tripleurospermum disciforme* in three developmental stages. *Pharm Biol*. 2010;**48**(11):1280–4. doi: 10.3109/13880201003770143. [PubMed: 20795784].
10. Tofighi Z, Molazem M, Doostdar B, Taban P, Shahverdi AR, Samadi N, et al. Antimicrobial activities of three medicinal plants and investigation of flavonoids of *Tripleurospermum disciforme*. *Iran J Pharm Res*. 2015;**14**(1):225–31. [PubMed: 25561928]. [PubMed Central: PMC4277635].
11. Amin GR, Dehmoobed Sharifabadi A, Salehi Surmaghi MH, Yasa N, Aynechi Y, Emami M, et al. Screening of Iranian plants for antifungal activity: Part 1. *DARU J Pharm Sci*. 2002;**10**(1):34–7.
12. Ansari M, Rafiee K, Yasa N, Vardasbi S, Naimi SM, Nowrouzi A. Measurement of melatonin in alcoholic and hot water extracts of *Tanacetum parthenium, Tripleurospermum disciforme and Viola odorata*. *DARU J Pharm Sci*. 2010;**18**(3):173.
13. Ukiya M, Akihisa T, Tokuda H, Suzuki H, Mukainaka T, Ichiishi E, et al. Constituents of Compositae plants III. Anti-tumor promoting effects and cytotoxic activity against human cancer cell lines of triterpene diols and triols from edible *Chrysanthemum* flowers. *Cancer Lett*. 2002;**177**(1):7–12. doi: 10.1016/s0304-3835(01)00769-8. [PubMed: 11809525].
14. Sorensen JS, Bruun T, Holme D, Sörensen NA. Studies related to naturally occurring acetylene compounds. XIII. The occurrence of trans-methyl-n-dec-2-en-4:6:8-triynoate in the Genus *Tripleurospermum* schultz bipontinus. *Acta Chemica Scandinavica*. 1954;**8**:26–33. doi: 10.3891/acta.chem.scand.08-0026.
15. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour HR. Phytochemical screening of some species of Iranian plants. *Iran J Pharm Res*. 2010:77–82.
16. Saleem M. Lupeol, a novel anti-inflammatory and anti-cancer dietary triterpene. *Cancer Lett*. 2009;**285**(2):109–15. doi: 10.1016/j.canlet.2009.04.033. [PubMed: 19464787]. [PubMed Central: PMC2764818].
17. Amico V, Barresi V, Condorelli D, Spatafora C, Tringali C. Antiproliferative terpenoids from almond hulls (*Prunus dulcis*): Identification and structure-activity relationships. *J Agric Food Chem*. 2006;**54**(3):810–4. doi: 10.1021/jf052812q. [PubMed: 16448187].
18. Lee SM, Min BS, Lee CG, Kim KS, Kho YH. Cytotoxic triterpenoids from the fruits of *Zizyphus jujuba*. *Planta Med*. 2003;**69**(11):1051–4. doi: 10.1055/s-2003-45155. [PubMed: 14735446].
19. Khalilov LM, Khalilova AZ, Shakurova ER, Nuriev IF, Kachala VV, Shashkov AS, et al. PMR and 13C NMR spectra of biologically active compounds. XII. Taraxasterol and its acetate from the aerial part of *Onopordum acanthium*. *Chem Nat Compd*. 2003;**39**(3):285–8. doi: 10.1023/a:1025478720459.
20. Jamal AK, Yaacob WA, Din LB. A Chemical study on *Phyllanthus reticulatus*. *Phys Sci*. 2008;**19**(2):45–50.
21. Ayatollahi AM, Ghanadian M, Afsharypour S, Abdella OM, Mirzai M, Askari G. Pentacyclic triterpenes in *Euphorbia microsciadia* with their T-cell proliferation activity. *Iran J Pharm Res*. 2011;**10**(2):287–94. [PubMed: 24250356]. [PubMed Central: PMC3828914].
22. Hafez Ghoran S, Saeidnia S, Babaei E, Kiuchi F, Dusek M, Eigner V, et al. Biochemical and biophysical properties of a novel homoisoflavonoid extracted from *Scilla persica* HAUSSKN. *Bioorg Chem*. 2014;**57**:51–6. doi: 10.1016/j.bioorg.2014.08.001. [PubMed: 25181677].
23. Babaei E, Sadeghizadeh M, Hassan ZM, Feizi MA, Najafi F, Hashemi SM. Dendrosomal curcumin significantly suppresses cancer cell proliferation in vitro and in vivo. *Int Immunopharmacol*. 2012;**12**(1):226–34. doi: 10.1016/j.intimp.2011.11.015. [PubMed: 22155627].
24. Hafez Ghoran S, Saeidnia S, Babaei E, Kiuchi F, Hussain H. Scillapersicene: A new homoisoflavonoid with cytotoxic activity from the bulbs of *Scilla persica* HAUSSKN. *Nat Prod Res*. 2016;**30**(11):1309–14. doi: 10.1080/14786419.2015.1054286. [PubMed: 26140544].

25. Malich G, Markovic B, Winder C. The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. *Toxicology*. 1997;**124**(3):179–92. doi: 10.1016/s0300-483x(97)00151-0. [PubMed: 9482120].
26. Hata K, Hori K, Ogasawara H, Takahashi S. Anti-leukemia activities of Lup-28-al-20(29)-en-3-one, a lupane triterpene. *Toxicol Lett*. 2003;**143**(1):1–7. doi: 10.1016/s0378-4274(03)00092-4. [PubMed: 12697374].
27. Rzeski W, Stepulak A, Szymanski M, Sifringer M, Kaczor J, Wejksza K, et al. Betulinic acid decreases expression of bcl-2 and cyclin D1, inhibits proliferation, migration and induces apoptosis in cancer cells. *Naunyn Schmiedebergs Arch Pharmacol*. 2006;**374**(1):11–20. doi: 10.1007/s00210-006-0090-1. [PubMed: 16964520].
28. Kessler JH, Mullauer FB, de Roo GM, Medema JP. Broad in vitro efficacy of plant-derived betulinic acid against cell lines derived from the most prevalent human cancer types. *Cancer Lett*. 2007;**251**(1):132–45. doi: 10.1016/j.canlet.2006.11.003. [PubMed: 17169485].
29. Saleem M, Maddodi N, Abu Zaid M, Khan N, bin Hafeez B, Asim M, et al. Lupeol inhibits growth of highly aggressive human metastatic melanoma cells in vitro and in vivo by inducing apoptosis. *Clin Cancer Res*. 2008;**14**(7):2119–27. doi: 10.1158/1078-0432.CCR-07-4413. [PubMed: 18381953].
30. Zhu Y, Tchkonia T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, et al. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell*. 2016;**15**(3):428–35. doi: 10.1111/acel.12445. [PubMed: 26711051]. [PubMed Central: PMC4854923].
31. Bruncko M, Oost TK, Belli BA, Ding H, Joseph MK, Kunzer A, et al. Studies leading to potent, dual inhibitors of Bcl-2 and Bcl-xL. *J Med Chem*. 2007;**50**(4):641–62. doi: 10.1021/jm061152t. [PubMed: 17256834].
32. Dong M, Liu F, Zhou H, Zhai S, Yan B. Novel natural product- and privileged scaffold-based tubulin inhibitors targeting the colchicine binding site. *Molecules*. 2016;**21**(10). doi: 10.3390/molecules21101375. [PubMed: 27754459]. [PubMed Central: PMC6273505].
33. Chintharlapalli S, Papineni S, Lei P, Pathi S, Safe S. Betulinic acid inhibits colon cancer cell and tumor growth and induces proteasome-dependent and -independent downregulation of specificity proteins (Sp) transcription factors. *BMC Cancer*. 2011;**11**:371. doi: 10.1186/1471-2407-11-371. [PubMed: 21864401]. [PubMed Central: PMC3170653].
34. Pitchai D, Roy A, Ignatius C. In vitro evaluation of anticancer potentials of lupeol isolated from *Elephantopus scaber* L. on MCF-7 cell line. *J Adv Pharm Technol Res*. 2014;**5**(4):179–84. doi: 10.4103/2231-4040.143037. [PubMed: 25364696]. [PubMed Central: PMC4215481].
35. Reiner T, Parrondo R, de Las Pozas A, Palenzuela D, Perez-Stable C. Betulinic acid selectively increases protein degradation and enhances prostate cancer-specific apoptosis: Possible role for inhibition of deubiquitinase activity. *PLoS One*. 2013;**8**(2). e56234. doi: 10.1371/journal.pone.0056234. [PubMed: 23424652]. [PubMed Central: PMC3570422].