Cytotoxic Potential of *Centaurea bruguierana* ssp. *belangerana*: The MTT Assay

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Abstract- The genus *Centaurea* with an extensive background in Iranian traditional medicine represents more than 70 species in Iran that some of them are endemic to Iran. A variety of secondary metabolites has been isolated from this genus thus far. Sesquiterpene lactones and flavonoids have been reported as main compounds of *C.bruguierana*. Methanolic extract and different fractions of the whole fruiting samples of *Centaurea bruguierana* ssp.*belangerana* (*Asteraceae*) were examined for cytotoxicity against various cell lines using MTT cytotoxicity assay in order to identify active fraction(s). The chloroform and ethyl acetate fractions of plant have demonstrated significant cytotoxicity against colon adenocarcinoma and breast ductal carcinoma cell lines, which chloroform fraction, exhibited the most potent in vitro cytotoxic activity against colon adenocarcinoma cell line, and therefore, can be considered as the potential fraction through main compounds of plant against the adenocarcinoma colon cancer cell line. Moreover, only chloroform fraction was moderately active against Swiss embryo fibroblast cell lines while the other fractions were nontoxic. To summarize, the chloroform fraction of C.*bruguierana* demonstrated better cytotoxic activity against all of the tested cell lines compared with the other fractions; and promisingly in some cases represented moderate to very good cytotoxicity that suggests more investigations about its phytochemical properties.

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Keywords: *Centaurea bruguierana* ssp.*belangerana*; Asteraceae; Cytotoxicity; MTT assay; IC_{50}

Introduction

The genus *Centaurea* (*Asteraceae*, tribe *Cardueae*, subtribe *Centaureinae*) comprises ca.600 species widely distributed from Asia, Europe, Tropical Africa to North America as aggressively invading weeds (1). This genus consists of 88 species in the Flora Iranica (2) and represents approximately 74 species in Iran that some of them are endemic (3). *Centaurea bruguierana* (DC.) Hand.-Mzt. ssp.belangerana (DC.) Bornm. (Sect. Tetramorphaea), a 5-50 cm annual herb with purple spiny flowers, is distributed in Iran, Transcaucasia, Afghanistan, Pakistan, and Central Asia (2).

Many species of the genus *Centaurea* have long been used in traditional medicines to cure various ailments, e.g. diabetes, diarrhea, rheumatism, malaria, and used against coughs, as liver-strengthening, itch-eliminating and, ophthalmic remedies (4,5).

Several biological activities have been reported for *Centaurea spp.*including anti-inflammatory, antipyretic, antiplatelet, wound healing, analgesic, anti-plasmodial, cytotoxic, antioxidant, anti-peptic ulcer and anti-*Helicobacter pylori*, antiprotozoal, antifungal and anticancer (6-15).

To our knowledge, chemical constituents of *C.bruguierana* comprising two sesquiterpene lactones, cnicin (a germacranolide), dehydromelitensin-8-acetate (an elemanolide) and four flavonoids including tetramethoxyflavone (16,17). Moreover, a variety of secondary metabolites has been reported from different other species of this genus including sesquiterpene lactones (1,9,11,13,18-21), flavonoids (4,5,22-25), lignans (1,4,22), alkaloids (4,22), acetylenes (26), triterpenes (27), acetophenone and neolignan (28).

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Pharmacological effects of these compounds have been of interest in various studies for example sesquiterpenoids with different skeleton have been studied for their cytotoxic and antitumor activities (29,30). Germacranes, elemanes, and eudesmanes isolated from the aerial parts of various species of the genus *Centaurea* were examined for their in vitro cytostatic activity against five human cell lines including DLD1, SF268, MCF-7, H460 and OVCAR3 and showed significant effects on most of them (31). Cnicin as the main compound of *C.bruguierana* and other *Centaurea* species was tested against nine different cancer cell lines and demonstrated considerable activities on 1A9, KB and KB-VIN (32).

Previous studies on *C.bruguierana* extract have potentially demonstrated various biological effects such as larvicidal activity on malaria vector, primary cytotoxic effect, anti-peptic ulcer activity and hypoglycemic effect on rats (33-37).

In this regard and also as a part of our ongoing cytotoxicity screening of native Iranian plants, in this research, we describe a thorough investigation on cytotoxic activity of *Centaurea bruguierana* ssp.*belangerana*.

Materials and Methods

Plant material

The whole fruiting samples of *Centaurea bruguierana ssp.belangerana* were collected by Mr. R. Khademi from Bushehr, in the south of Iran, at an elevation of 70 m, in June and identified by Dr. Gholamreza Amin, Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran where voucher speciemen is deposited (6683-TEH).

Extraction and fractionation through solvent-solvent extraction

Dried whole fruiting samples (300 g) were extracted with 80% methanol (MeOH, 6×1.5 l) in a percolator at room temperature for 2 weeks. The combined extract was concentrated to dryness under reduced pressure at 40° C (38). The MeOH extract was dissolved in 100 ml MeOH/H_2O (7:1) and extracted with petroleum ether (4×200 ml), chloroform ($CHCl_3$, 4×200 ml), H_2O saturated ethyl acetate (EtOAc, 4×200 ml) and H_2O saturated *n*-butanol (*n*-BuOH, 4×200 ml) successively. Each fraction and the remaining MeOH part after solvent fractionation were then evaporated to dryness under reduced pressure at 40° C to give petroleum ether, CHCl3, EtOAc, *n*-BuOH and remaining MeOH fractions. All solvents were purchased from Merck, Darmstadt, Germany.

Cell culture

Human tumor cell lines (HT-29 (colon carcinoma), Caco-2 (colorectal adenocarcinoma) and T47D (breast ductal carcinoma) and normal cell line (NIH-3T3 (Swiss embryo fibroblast) were obtained from Pasteur Institute, Tehran, Iran. Human tumor cell lines were maintained in RPMI-1640 cell culture medium (PAA, Germany) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) for HT-29 cells and 15% FBS for Caco-2 and T47D cells, 25 mM N-2-Hydroxyethylpiperazone-n-2-Ethanesulfonic Acid (HEPES, Sigma, USA), 0.25% sodium bicarbonate (Sigma, USA), 100 IU/ml penicillin, and 100 µg/mL streptomycin (Boehringer, Germany). The NIH-3T3 cell line was maintained in Dulbecco's modified Eagle's medium (DMEM; PAA, Germany) supplemented with 10% FBS. All stock cultures were grown in T-25 flasks (Nunc, Denmark) in a humidified atmosphere of 95% air and 5% CO_2 at 37° C.

MTT cytotoxicity assay

Cytotoxicity studies were performed using the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay (39). Exponentially growing cells were seeded into 96-well microtiter plates (Nunc, Denmark) at a density of 1×104 cells per well in 100 µl of the medium. After 24 h of incubation at 37° C, stock solutions of test samples dissolved in Dimethyl sulfoxide (DMSO, Sigma, USA) were added to wells at serially diluted concentrations of 750, 500, 300, 150, 75, 37.5, and 18.75 µg/ml in culture medium. After 72 h of incubation for HT-29 cells, 96 h for T47D and NIH-3T3 cells and 120 h for Caco-2 cells, the medium was removed and 20 µL of 5 mg/ml MTT reagent (Sigma, USA) in phosphate-buffered saline (PBS, Sigma, USA) in serum free medium was added to each well. The plates were incubated at 37° C for 4 h. At the end of the incubation period, the medium was removed, and 100 µl of pure DMSO was added to each well to dissolve formazan crystals which were quantified by reading the absorbance at 570 nm on a microplate reader (Anthos Lab Tech Instruments, Austria) and used as a measure of cell viability. Six wells were assayed for each condition, and mean as well as standard deviations were determined using Microsoft Excel. The IC_{50} values (concentrations inducing a 50% inhibition of cell growth) were calculated from the equation of the logarithmic line determined by fitting the best line to the

curve formed from the data by using Sigma Plot 10.0. The IC_{50} value was obtained from the equation y=50 (50% value). In all experiments, the final concentration of DMSO did not exceed 1% (v/v), a concentration that was nontoxic to the cells and cells with no treatment and methotrexate (Sigma, USA) treatment were used as a negative and positive control, respectively.

Results

The yellowish brown total extract (32.0 g) with a yield of 10.67% was fractioned with several solvents to give petroleum ether fraction (0.976 g), $CHCl_3$ fraction (4.268 g), EtOAc fraction (3.394 g), *n*-BuOH fraction (3.485 g) and remaining MeOH fraction (13.077 g). Figure 1 shows the viability of cell lines when treated with total extract and fractions of *Centaurea bruguierana*. The dose-dependent cytotoxicity has been observed in the T47D cell line.

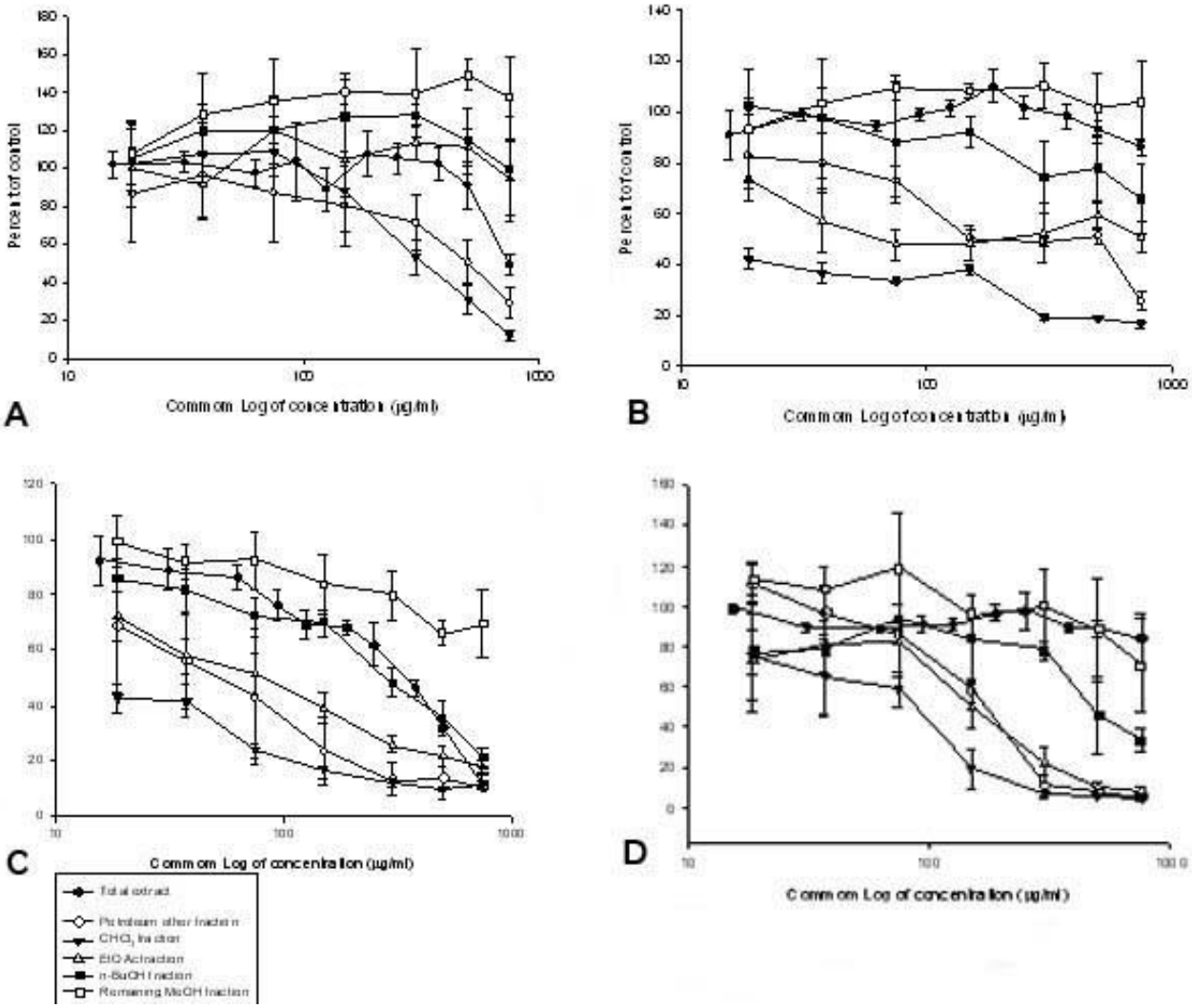

Figure 1. The effect of total extract and solvent fractions obtained from the whole fruiting plant of *C.bruguierana ssp.belangerana* on cell viability. Cells (1×10^4 cells/well) were submitted to different concentrations of test samples. After the indicated time of incubation, cell viability was determined by MTT assay. (A) HT-29 cell line, (B) Caco-2 cell line, (C) T47D cell line, (D) NIH-3T3 cell line.

Table 1 summarizes the cytotoxic activity of total extract and fractions of *Centaurea bruguierana*. None of the test samples were effective on the HT-29 cell line. In Caco-2 cell line, the EtOAc fraction showed moderate cytotoxic activity (IC_{50}<100 µg/ml) while the $CHCl_3$ fraction exhibited the most potent cytotoxic activity (IC_{50}<10 µg/mL) obtained in this study. In T47D cell line, the petroleum ether, EtOAc, and $CHCl_3$ fractions showed moderate cytotoxic activity (IC_{50}<100 µg/ml) with higher potency for $CHCl_3$ fraction. Only $CHCl_3$ fraction was moderately active against NIH-3T3 cell line (IC_{50}<100 µg/ml) while the other fractions were

nontoxic. In none of the cell lines, total extract, *n*-BuOH and remaining MeOH fractions exhibited cytotoxicity activity while the $CHCl_3$ fraction was the most active. According to the IC_{50} values obtained by in vitro MTT cytotoxicity assay (Table 1), total MeOH extract and fractions exhibited the best cytotoxic activity on T47D cell line and the lowest cytotoxic activity on the HT-29 cell line. Among the five fractions obtained by solvent-solvent fractionation of the total MeOH extract, $CHCl_3$ and EtOAc fractions showed significant cytotoxic activity on T47D and Caco-2 cell lines (IC_{50}<100 µg/ml), which $CHCl_3$ fraction exhibited the most potent in vitro cytotoxic activity against Caco-2 cell line with an IC_{50} value of <10 µg/ml, and therefore, can be considered to have active compound(s) against the Caco-2 colon cancer cell line.

Table 1. Cytotoxic activity of total extract and solvent fractions obtained from the whole fruiting plant of *C.bruguierana* ssp.*belangerana*[a]

Sample	Cell Lines[b]			
	HT-29	**Caco-2**	**T47D**	**NIH-3T3**
Total methanolic extract	> 1000	> 1000	320.81±2.54[c]	174.21±1.23
Petroleum ether fraction	506.12±34.2	144.23±23.1	52.89±34.26	> 1200
Chloroform fraction	327±23	< 10	29.54±2.33	87.13±2.11
Ethyl acetate fraction	> 1000	63.73±12.36	83.67±0.84	152.4±11.2
n-Butanol fraction	> 1000	> 1000	280.3±4.13	469.02±84.5
Remaining methanolic fraction	> 1000	> 1000	> 1000	> 1000
Methotrexate	0.23±0.02	0.32±0.04	0.16±0.09	0.24±0.013

[a] Results are expressed as IC_{50} values in µg/ml.
[b] HT-29, colon carcinoma; Caco-2, colon adenocarcinoma; T47D, breast ductal carcinoma; NIH-3T3, Swiss embryo fibroblast.
[c] Mean±SD (*n*=6).

Discussion

On the basis of the presence of the sesquiterpene lactones in $CHCl_3$ fraction of MeOH extract obtained from aerial parts of *Centaurea* species (9,11), and attracted significant interest of sesquiterpene lactones due to their antitumor and cytotoxic activities (40-44), we can consider that the potent cytotoxic activity of $CHCl_3$ fraction on Caco-2 cell line may be due to the presence of antitumor sesquiterpene lactones.

Similar to our study, the cytotoxicity in chloroform fraction of *C.bruguierana*, resulted in the lowest IC50 (ranging from 14.44 to 23.03 µg/mL) against MCF-7 (human breast adenocarcinoma), WEHI-164 (mouse fibrosarcoma), HepG-2 (human hepatocellular liver carcinoma) and MDBK (Madin-Darby bovine kidney) cell lines. On the other hand, the methanol extract showed an IC_{50} less than 100 µg/mL against MCF-7, WEHI-164, HepG-2 and MDBK cells (IC_{50} from 47.30 to 84.70 µg/mL), unlike our study where no cytotoxicity activity was observed in none of the cell lines (37).

In another report, cnicin and its derivatives were determined against A549 and MCF-7 tumor cell lines. Cnicin showed significantly a cytotoxic effect against MCF-7 breast cancer cell line with IC_{50}=4.2 µM (45). Bach *et al*., study in 2011 showed that chloroform extract from the aerial parts of *C.tweediei* and *C.diffusa*, and their main sesquiterpene lactones onopordopicrin and cnicin, carried out high cytotoxicity against human-derived macrophages (46). Cnicin also has demonstrated an inhibitory effect on NF-jB and iNOS activity with IC_{50} values of 1.8 and 6.5 µM, respectively. Moreover, cytotoxic activity of cnicin was observed toward pig epithelial (LLC-PK11), human malignant melanoma (SK-MEL) and human ductal carcinoma (BT-549) (47). Furthermore, it has shown a moderate antiproliferative effect against HeLa, and A431 human tumor cell lines (48). Although some species of *Centureae* genus are reported frequently for their isolated sesquiterpene lactones with remarkable cytotoxicity, lack of adequate studies on major compounds of C.*bruguierana* prevents accurate comments on any other cytotoxic compounds which may be involved in the cytotoxic activity of the plant extract. For instance, the phytochemical study of the aerial parts of *C.deflexa* exhibited two sesquiterpene lactones, aguerin B and 15-nor-guaianolide with anti-proliferative activity against human pancreatic and colonic cancer cells (49).

Evaluation of the cytotoxic activity of arctigenin as a lignan, and schischkinin, a novel indole alkaloid, obtained from the MeOH extract of *C.schischkinii* seeds against Caco-2 colon cancer cell lines exhibited potent activity for arctigenin and a moderate level of cytotoxicity with schischkinin (IC_{50}=7 and 76 µM) (22). Montamine, a novel dimeric indole alkaloid, obtained from the MeOH extract of *C.montana* seeds

showed significant in vitro anticancer activity against Caco-2 colon cancer cell lines (IC_{50}=43.9 µM), whereas the other monomer, moschamine, showed a moderate level of efficacy (IC_{50}=81.0 µM) (4).

The chloroform extract of the aerial parts of *C.musimomum* exhibited a cytotoxic activity on KB human epidermoid tumor cell line with growth inhibition of 89% at 10 µg/ml and 26% at 1 µg/ml (21). Among different fractions of the whole plant of *C.arenaria*, the chloroform extract showed high tumor cell proliferation inhibitory activity by MTT cytotoxicity assay against breast adenocarcinoma (MCF7), cervix adenocarcinoma (HeLa), and skin epidermoid carcinoma (A431) cells. By applying a bioassay-guided multistep separation procedure the flavonoids apigenin, eupatilin, eupatorin and the sesquiterpene cnicin, and the lignans arctigenin, arctiin and matairesinol were separated and found to be an anti-tumor compound of the active fractions (48).

In present study, it is worth mentioning that although the observed IC_{50} values of $CHCl_3$ and EtOAc fractions on T47D and Caco-2 cell lines (IC_{50}<100 µg/ml), are much higher than IC_{50} of methotrexate as an anticancer drug, but this may be due to the impurity of fractions consisting of so many constituents other than active compounds. Interestingly, the IC_{50} values of these two fractions on normal NIH-3T3 cell line are significantly higher than that of T47D and Caco-2 cell lines, which can be considered as inactive on normal cells while active on cancer cells. Finally, the isolation and structure elucidation of the active compounds of $CHCl_3$ and EtOAc fractions as well as the determination of IC_{50} values and understanding the mechanism of inhibition would be of interest.

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References

1. Celik S, Rosselli S, Maggio AM, Russo A. Guaianolides and lignans from the aerial parts of Centaurea ptosimopappa. Biochem Syst Ecol 2006;34:349-52.
2. Rechinger KH. Flora iranica: Akademische Druck-u. Verlagsanstalt: Graz, 1987.
3. Mozaffarian V, eds. A dictionary of Iranian plant names: Latin, English, Persian. Tehran, Iran: Farhang Mo'aser, 1996.
4. Shoeb M, MacManus SM, Jaspars M, Trevidu J, Nahar L, Kong-Thoo-Lin P, et al. Montamine, a unique dimeric indole alkaloid, from the seeds of Centaurea montana (Asteraceae), and its in vitro cytotoxic activity against the CaCo2 colon cancer cells. Tetrahedron 2006;62:11172-7.
5. Flamini G, Pardini M, Morelli I, Ertugrul K, Dural H, Bagci Y, et al. Flavonoid glycosides from Centaurea pseudoscabiosa subsp. pseudoscabiosa from Turkey. Phytochemistry 2002;61:433-7.
6. Koca U, Süntar IP, Keles H, Yesilada E, Akkol EK. In vivo anti-inflammatory and wound healing activities of Centaurea iberica Trev. ex Spreng. J Ethnopharmacol 2009;126:551-6.
7. Khammar A, Djeddi S. Pharmacological and Biological Properties of some Centaurea Species. Eur J Sci Res 2012;84:398-416.
8. Akkol EK, Arif R, Ergun F, Yesilada E. Sesquiterpene lactones with antinociceptive and antipyretic activity from two Centaurea species. J Ethnopharmacol 2009;122:210-5.
9. Özçelik B, Gürbüz I, Karaoglu T, Yeşilada E. Antiviral and antimicrobial activities of three sesquiterpene lactones from Centaurea solstitialis L. ssp. solstitialis. Microbiol Res 2009;164:545-52.
10. Yesilada E, Gürbüz I, Shibata H. Screening of Turkish anti-ulcerogenic folk remedies for anti-Helicobacter pylori activity. J Ethnopharmacol 1999;66:289-93.
11. Yesilada E, Gürbüz I, Bedir E, Tatli I, Khan IA. Isolation of anti-ulcerogenic sesquiterpene lactones from Centaurea solstitialis L. ssp. solstitialis through bioassay-guided fractionation procedures in rats. J Ethnopharmacol 2004;95:213-9.
12. Gürbüz İ, Yesilada E. Evaluation of the anti-ulcerogenic effect of sesquiterpene lactones from Centaurea solstitialis L. ssp. solstitialis by using various in vivo and biochemical techniques. J Ethnopharmacol 2007;112:284-91.
13. Karamenderes C, Khan S, Tekwani BL, Khan IA. Antiprotozoal and Antimicrobial Activities of Centaurea. Species Growing in Turkey. Pharm Biol 2006;44:534-9.
14. Shoeb M, Jaspars M, MacManus SM, Celik S, Nahar L, Kong-Thoo-Lin P, et al. Anti-colon cancer potential of phenolic compounds from the aerial parts of Centaurea gigantea (Asteraceae). J Nat Med 2007;61:164-9.
15. Sarker S, Kumarasamy Y, Shoeb M, Celik S, Yucel E, Middleton M, et al. Antibacterial and antioxidant activities of three Turkish species of the gemus. Centaurea 2005;5:246-50.
16. Rustaiyan A, Niknejad A, Aynehchi Y. Chemical Constituents of Centaurea brugueriana. Planta Med 1982;44:185-6.

17. Harraz F, Kassem F, El-Shaer N. Sesquiterpene lactones and flavonoids from Centaurea bruguierana. Alex J Pharm Sci 1994;8:219.
18. Marco JA, Sanz-Cervera JF, Yuste A, Sancenón F, Carda M. Sesquiterpenes from Centaurea aspera. Phytochemistry 2005;66:1644-50.
19. Robles M, Wang N, Kim R, Choi BH. Cytotoxic effects of repin, a principal sesquiterpene lactone of Russian knapweed. J Neurosci Res 1997;47:90-7.
20. Bicha S, Chalard P, Hammoud L, Benayache F. Maroccanin: A New γ-lactone and Other Constituents from Centaurea maroccana Ball. (Asteraceae). Rec Nat Prod 2013;7:114-8.
21. Medjroubi K, Benayache F, Bermejo J. Sesquiterpene lactones from Centaurea musimomum. Antiplasmodial and cytotoxic activities. Fitoterapia 2005;76:744-6.
22. Shoeb M, Celik S, Jaspars M, Kumarasamy Y, MacManus SM, Nahar L, et al. Isolation, structure elucidation and bioactivity of schischkiniin, a unique indole alkaloid from the seeds of Centaurea schischkinii. Tetrahedron 2005;61:9001-6.
23. Flamini G, Pardini M, Morelli I. A flavonoid sulphate and other compounds from the roots of Centaurea bracteata. Phytochemistry 2001;58:1229-33.
24. Akkal S, Benayache F, Medjroubi K, Tillequin F. Flavonoids from Centaurea furfuracea (Asteraceae). Biochem Syst Ecol 2003;31:641-3.
25. Formisano C, Rigano D, Senatore F, Bancheva S, Maggio A, Rosselli S, et al. Flavonoids in Subtribe Centaureinae (Cass.) Dumort. (tribe Cardueae, Asteraceae): Distribution and 13C-NMR Spectral Data. Chem Biodivers 2012;9:2096-158.
26. Christensen LP, Lam J. Acetylenes and other constituents from Centaurea species. Phytochemistry 1991;30:3289-92.
27. Ulubelen A, Öksüz S. Cytotoxic flavones from Centaurea urvillei. J Nat Prod 1982;45:373.
28. Baykan-Erel S, Bedir E, Khan IA, Karaalp C. Secondary metabolites from Centaurea ensiformis PH Davis. Biochem Syst Ecol 2010;38:1056-8.
29. Bruno M, Rosselli S, Maggio A, , Raccuglia RA, Bastow KF, Wu CC, et al. Cytotoxic activity of some natural and synthetic sesquiterpene lactones. Planta Med 2005;71:1176-8.
30. Bruno M, Bancheva S, Rosselli S, Maggio A. Sesquiterpenoids in subtribe Centaureinae (Cass.) Dumort (tribe Cardueae, Asteraceae): distribution, 13 C NMR spectral data and biological properties. Phytochemistry 2013;95:19-93.
31. Koukoulitsa E, Skaltsa H, Karioti A, Demetzos C, Dimas K.. Bioactive sesquiterpene lactones from Centaurea species and their cytotoxic/cytostatic activity against human cell lines in vitro. Planta Med 2002;68:649-52.
32. Bruno M, Rosselli S, Maggio A, Raccuglia RA, Bastow KF, Lee KH. Cytotoxic activity of some natural and synthetic guaianolides. J Nat Prod 2005;68:1042-6.
33. Khanavi M, Ahmadi R, Rajabi A, Jabbari Arfaee S, Hassanzadeh G, Khademi R, et al. Pharmacological and histological effects of Centaurea bruguierana ssp. belangerana on indomethacin-induced peptic ulcer in rats. J Nat Med 2012;66:343-9.
34. Khanavi M, Rajabi A, Behzad M, Hadjiakhoondi A, Vatandoost H, Abaee MR. Larvicidal Activity of Centaurea bruguierana ssp. belangerana against Anopheles stephensi Larvae. Iran J Pharm Res 2011;10:829-33.
35. Rajabi A, Taheri M, Khanavi M, Fallah-Bonekohal S. Hypoglycemic effect of Centaurea bruguierana ssp. belangerana in streptozotocin/alloxan-induced diabetic rats through stimulation of hepatic glycogenolysis and inhibition of gluconeogenesis. National conference on natural products and medicinal plants; Tehran, Iran; 2013.
36. Rajabi A, Khanavi M, Khademi R. Investigation on cytotoxic activity of Centaurea bruguierana ssp. belangerana. 57th International congress and annual meeting of the society for medicinal plant and natural product research; 2009 Aug 16-20; Geneva, Switzerland. Stuttgart: Thieme; 2009.
37. Ghafari S, Naghibi F, Esmaeili S, Sahranavard S, Mosaddegh M. Investigating the cytotoxic effect of some medicinal plants from northern parts of Iran. RJP 2015;2:47-51.
38. Khanavi M, Vatandoost H, Dehaghi NK, Dehkordi AS, Sedaghat MM, Hadjiakhoondi A, et al. Larvicidal activities of some Iranian native plants against the main malaria vector, Anopheles stephensi. Acta Med Iran 2013;51:141-7.
39. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
40. Lee K-H, Huang E-S, Piantadosi C, Pagano JS, Geissman TA. Cytotoxicity of sesquiterpene lactones. Cancer Res 1971;31:1649-54.
41. Lee K-H, Furukawa H, Huang E-S. Antitumor agents. 3. Synthesis and cytotoxic activity of helenalin amine adducts and related derivatives. J Med Chem 1972;15:609-11.
42. Lee K-H, Meck R, Piantadosi C, Huang ES. Antitumor agents. 4. Cytotoxicity and in vivo activity of helenalin esters and related derivatives. J Med Chem 1973;16:299-301.
43. Lee K-H, Hall IH, Mar E-C, Starnes CO, ElGebaly SA, Waddell TG, et al. Sesquiterpene antitumor agents: inhibitors of cellular metabolism. Science 1977;196:533-6.
44. Vanhaelen–Fastré R, Vanhaelen M. Activite antibiotique et

cytotoxique de la cnicine et de ses produits d'hydrolyse. Planta Med 1976;29:179-89.

45. Rosselli S, Maggio AM, Raccuglia RA, Morris-Natschke SL, Bastow KF, Lee KH, et al. Acid rearrangment of epoxy-germacranolides and absolute configuration of 1beta, 10alpha-epoxy-salonitenolide. Nat Prod Commun 2010;5:675-80.
46. Bach SM, Fortuna MA, Attarian R, de Trimarco JT, Catalán CA, Av-Gay Y, et al. Antibacterial and cytotoxic activities of the sesquiterpene lactones cnicin and onopordopicrin. Nat Prod Commun 2011;6:163-6.
47. Erel SB, Karaalp C, Bedir E, Kaehlig H, Glasl S, Khan S, et al. Secondary metabolites of Centaurea calolepis and evaluation of cnicin for anti-inflammatory, antioxidant, and cytotoxic activities. Pharm Biol 2011;49:840-9.
48. Csapi B, Hajdú Z, Zupkó I, Berényi A, Forgo P, Szabó P, et al. Bioactivity-guided isolation of antiproliferative compounds from Centaurea arenaria. Phytother Res 2010;24:1664-9.
49. Chicca A, Tebano M, Adinolfi B, Ertugrul K, Flamini G, Nieri P. Anti-proliferative activity of aguerin B and a new rare nor-guaianolide lactone isolated from the aerial parts of Centaurea deflexa. Eur J Med Chem 2011;46:3066-70.