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Phytochemical Analysis and Cytotoxic Activity of *Ferulago carduchorum*

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Abstract: *Ferulago carduchorum* Boiss and Hausskn (Apiaceae) is an endemic plant in West of Iran and it are used in dairy and oil ghee as a natural preservative. The aerial parts of *F. carduchorum* were extracted by percolation method with MeOH/H_2O (80/20) and the fractions were provided respectively with hexane, ethyl acetate, methanol, methanol/H_2O (50/50). The fractions have been investigated their phytochemical screening and the cytotoxic activity against the colon carcinoma (HT29), the breast ducal carcinoma (T47D), the hepatocellular carcinoma (HepG2) and Swiss mouse embryo fibroblast (NIH 3T3) cell lines by MTT (3-(4, 5-di methyl thiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. Phytochemical screening revealed the presence of phytosteroids, flavonoids, coumarins and saponins. The cytotoxicity of ethyl acetate and hexane fractions showed more efficacy than the other fractions on T47D and HepG2 cell lines (IC_{50}<100 μg mL^{-1}) could be attributed to their content of coumarins and phytosteroids. The hexane fraction was selected for phytochemical study and suberosin (a coumarin) isolated from hexane fraction as an active compound. The Selective Index (SI) value of ethyl acetate fraction was lower in HepG2, T47D and HT29 cell lines than hexane fraction that indicated the selective effect of ethyl acetate fraction on this cell lines.

Key words: *Ferulago carduchorum*, cytotoxic activity, MTT assay, fraction, phytochemical analysis

INTRODUCTION

Ferulago genus which commonly known as chavil in folk medicine, belongs to the Apiaceae family and includes about 40 species in the world that eight of them exist in Iran (Rechinger and Hedge, 1987; Mozaffarian, 2007). *Ferulago carduchorum* Boiss and Hausskn is khnown as an endemic plant in West of Iran, grows in altitude of 1900-3200 m above the sea level. *F. carduchorum* is a perennial shrub with yellow color flowers and the height of about 60-150 cm (Mozaffarian, 2007). In West of Iran, aerial parts of chavil are used in meat, dairy and oil ghee as a natural preservative. Furthermore, different *Ferulago* species are used traditionally for ulcer, snake bit and headache (Demetzos *et al*., 2000). Literature reviews have been reported coumarins from *F. bernardii* (Khalighi-Sigaroodi *et al*., 2006), *F. meoides* (Ognyanov and Bocheva, 1969), *F. turcomanica* (Andrianova *et al*., 1975; Serkerov *et al*., 1976), *F. sylvatica* (Sklyar *et al*., 1982), *F. granatensis* (De Pascual *et al*., 1979), *F. aucheri* (Doganca *et al*., 1991), *F. asparagifolia* (Doganca *et al*., 1992), *F. nodosa* (Ruberto *et al*., 1994), *F. capillaris* and *F. brachyloba* (Jimenez *et al*., 2000). In one study, it was found that *F. angulata* had a mild stimulating activity on human Peripheral Blood Lymphosytes (PBLs) at low concentration (<1 μg mL^{-1}) (Amirghofran *et al*., 2009). In addition, cytotoxic effects of the isolated coumarins from *F. campestris* have been investigated (Basile *et al*., 2009). Chemical composition of *F. carduchorum* essential oil has been studied, the major constituents were identified as (z)-β-ocimene (21.2%), terpinolene (13.1%)

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and α-phellandrene (12.7%) (Samiee *et al*., 2006). The preservative effect of *F. carduchorum* in oil ghee in folk uses possibly due to antioxidant activity and it suggested that this plant may be have anticancer effect. Cancer is one of the most life threatening diseases now-a-days. Currently there is considerable scientific and commercial interest in discovering of new anticancer agent from natural products because of their compatibility to human body and less adverse effects in comparison with synthetic chemotherapeutic agents (Rahimifard *et al*., 2009).

The goal of the survey is to evaluate the cytotoxic activity of *F. carduchorum* toward four cell lines by MTT (3-(4, 5-di methyl thiazol-2-yl)-2, 5-di phenyltetrazolium bromide) assay and to carry out phytochemical investigation for first time.

MATERIALS AND METHODS

Instruments: The ^{1}H and ^{13}C-NMR spectra were recorded on a Brucker Avance TM 500 DRX (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer with tetramethylsilane, as an internal standard and chemical shifts were given in δ (ppm) and the coupling constants (J) are in Hz. The UV spectra were obtained using a Shimadzu 160A spectrophotometer. The silica gel $60F_{254}$ precoated plates (Merck TM) were used for thin layer chromatography (TLC). The spots were detected under UV (254 and 365 nm) and by spraying anisaldehyde-H_2SO_4 reagent followed by heating. Silica gel for column chromatography (Mesh 70-230) was purchased from Merck Company (Germany).

Plant material: The aerial parts of *Ferulago carduchorum* were collected from mountains of Ilam Province at June, 2011. The plants have been identified and a voucher specimen is deposited in Herbarium Institute of Medicinal Plants (ACECR).

Extraction and isolation: Plant material (1500 g) was dried for even days at room temperature (17-22°C) and was cut into small pieces. The air-dried and ground aerial parts of *F. carduchorum* were extracted by percolation method with MeOH/H_2O (80/20) 3 times at room temperature and the fractions were provided, respectively with hexane, ethyl acetate (AcOEt), methanol, methanol/H_2O (50/50). The extract and fractions were evaporated by rotary evaporator and freeze-dried. The extract and fractions were stored in refrigerator to investigate the phytochemical screening and cytotoxic activity. The hexane fraction (15.24 g) was selected for phytochemical studies. Then, it was subjected to silica gel (mesh 230-400) Column Chromatography (CC) with hexane: AcOEt (19:1-0:20) and MeOH as eluent, to give several fractions (A-U). The fraction K was the pure compound (500 mg).

Pure compound: Suberosin, White crystal, UV λmax nm: 220, 240, 275, 330; ^{1}H-NMR (500 Hz, $CDCl_3$): δ ppm: 7.63 (d, 1H, J = 9.6 Hz, H-4), 7.19 (s, 1H, H-5), 6.78 (s, 1H, H-8), 6.24 (d, 1H, J = 9.6 Hz, H-3), 5.29 (t, 1H, j = 7.2, H-2×), 3.91 (s, 3H, OCH_3), 3.31 (d, 2H, J = 7.2, H-1×), 1.71, 1.78 (s, each 3H, H-4×, H-5×). ^{13}C-NMR ($CDCl_3$): 161.5 (C-2), 160.6 (C-7), 154.5 (C-9), 143.6 (C-4), 133.6 (C-3×), 127.5 (C-6), 127.4 (C-5), 121.3 (C-2×), 112.7 (C-3), 111.9 (C-10), 98.5 (C-8), 55.8 (OCH_3), 27.8 (C-1×), 25.8 (C-4×), 17.74 (C-5×) (De Melo Cazal *et al*., 2009).

Phytochemical screening: Phytochemical screening were performed on the crude extract and fractions for the presence of alkaloids, phytosteroids, flavonoids, anthocyanins, coumarins, tannins and saponins by using prescribed standard methods (Sharifzadeh *et al*., 2006; Saeidnia and Gohari, 2012).

Cell culture: The colon carcinoma (HT29) and the breast ducal carcinoma (T47D) were cultured in RPMI 1640 medium (Gibco, USA) that supplemented with 10% fetal bovine serum (FBS, Gibco, USA). The hepatocellular carcinoma (HepG2) were cultured in Dulbecco's modified Eagle's medium (DMEM; PAA, Germany) supplemented with 10% FBS. Also, the Swiss mouse embryo fibroblast (NIH 3T3) cell line used as a normal cell line and it were maintained in DMEM medium supplemented with 5% FBS. About 100 IU mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (Gibco, USA) were added to the media and all cell lines were kept at 37°C in an air/carbon dioxide (95:5) atmosphere (Mosmann, 1983).

Determination of cell viability by MTT assay: All cell lines were plated at 1×10^{4} cell/well of a 96-well tissue culture plate (Nunc, Denmark) for 24 h, then media was replaced with fresh media containing different concentration of extract and fractions (10-1000 μg mL^{-1}) with 4-well repeat of each concentration and a control group with no extract in media. After 48 h incubation for HT29, HepG2 and NIH 3T3 and 96 h for T47D cells the media was aspirated off and 20 μL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylte-tetrazolium bromide (MTT, Sigma, USA) that was dissolved in Phosphate Buffered Saline (PBS) at a concentration of 5 mg mL^{-1} was added to each well. Plates were incubated for 4 h to allow the viable cells reduce the yellow MTT into blue formazan crystals. In the next step 100 μL dimethysulfoxide (DMSO, Sigma, USA) was added to each well and agitated to dissolve formazan crystals completely. Absorbance was read at wavelenghts of 570 nm by a microplate reader (Anthos, Austria). Cytotoxicity was expressed as the concentration of

extract inhibiting cell growth with 50% (IC_{50}±SD). All tests and analysis were run in triplicate (Mosmann, 1983; Khanavi *et al*., 2010).

Statistical analysis: IC_{50} (the median growth inhibitory concentration) values were calculated from the IC_{50} of dose-response curve in the sigma plot 11 software. Data representative of three independent experiments with similar results were presented as mean±SD. The Selectivity Index (SI) (the ratio of IC_{50} of cancer cell to the IC_{50} of normal cell) was reported.

RESULTS AND DISCUSSION

The obtained results from the phytochemical screening of *F. carduchorum* crude extract and fractions are reported in Table 1. It reveals the presence of phytosteroids, flavonoids, coumarins and saponins in crude extract and polar fractions. The high amounts of phytosteroids and coumarins were present in hexane and ethyl acetate fractions. The effects of *F. carduchorum* crude extract and fractions on the proliferative response of the HT-29, HepG2 and T47D cell lines have been investigated by treating the cells with different concentrations of the extracts and significant decrease in cell lines proliferation were observed. IC_{50}±SD are reported in Table 2. The Selectivity Index (SI) of each fraction on three cancerous cell lines is shown in Table 3.

The cytotoxicity of nonpolar (ethyl acetate and hexane) fractions showed more efficacy than the polar fractions and crude extract. So, hexane fraction was selected for isolation and characterization of active compounds and subjected to silica gel columnchromatography, resulting in isolation of a major compound. Isolated compound was identified as suberosin (Fig. 1) by comparison of its NMR and UV spectral data with those reported in literature (De Melo Cazal *et al*., 2009). A number of studies showed cytotoxicity activity of suberosin to various tumor cells lines with IC_{50}>100 μM (Lin *et al*., 2003) and anti-inflammatory activity of this compound (Chen *et al*., 2007). The results showed that cytotoxic activity of hexane and ethyl acetate fractions on HepG2 and T47D cell lines were much stronger than that of HT-29 (IC_{50}<100 μg mL^{-1}). The hexane fraction showed highest anti-proliferative effect on all cell lines and it showed good cytotoxicity on T47D, HT-29 (IC_{50}<50 μg mL^{-1}).

Table 1: Phytochemical screening of *Ferulago carduchorum* crude extract and fractions

	Samples				
Active compounds	Crude extract	Hexane fraction	Ethyl acetate fraction	Methanol fraction	Hydroalcohol fraction
Alkaloids	-	-	-	-	-
Phytosteroids	+++	++++	++	+	+
Flavonoids	+++	-	+	++++	++
Anthocyanins	-	-	-	-	-
Coumarins	+++	++++	++++	+++	++
Tannins	-	-	-	-	-
Saponins	+	-	+	+	++

+++ = High amount; ++ = Moderate amount; + = Low amount; - = Absent of active compound

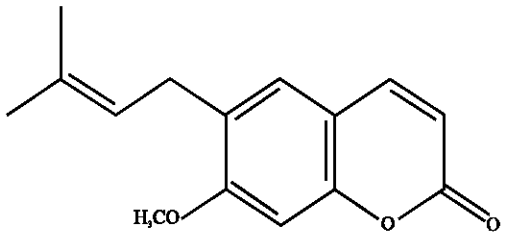

Fig. 1: Molecular structure of isolated coumarin (suberosin) from hexane fraction of *Ferulago carduchorum*

Table 2: Cytotoxic activity of crude extract and fractions of *Ferulago carduchorum* toward HT-29, T47D, HepG2 and NIH3T3 cell lines

	Cell lines[a] (MTT assay)			
Samples	HepG2	T47D	HT29	NIH/3T3
Crude extract	469.91±35.8	181.85±9.090	382.64±8.02	422.96±3.14
Hexane fraction	87.84±3.48	48.32±1.080	43.46±7.36	20.3±2.11
Ethyl acetate fraction	66.06±0.98	63.51±1.090	136.88±0.05	65.6±1.93
Methanol fraction	463.58±8.06	332.85±4.670	586.78±0.09	548.01±0.09
Hydroalcohol fraction	>1000	588.77±111.2	>1000	>1000
Methotrexate	-	0.16±0.090	0.23±0.02	0.24±0.013
Doxorubicin	1.04±0.07	-	-	0.21±0.03
Crude extract of *Vinca rosea*	-	195.78±17.96	412.09±43.55	>900

[a]Results are expressed as IC_{50} values (μg mL^{-1}); Key to cell lines employed: HT-29 = Colon Adenocarcinoma; T47D = Breast carcinoma; HepG2 = Hepatocellular carcinoma; NIH3T3 = Swiss embryo fibroblast

Table 3: Selectivity Index (SI) of crude extract and fractions of *Ferulago carduchorum*

	Cell lines[a]		
Samples	HepG2	T47D	HT29
Crude extract	1.111	0.429	0.904
Hexane fraction	4.327	2.380	2.140
Ethyl acetate fraction	1.007	0.968	2.086
Methanol fraction	0.845	0.607	1.070
Hydroalcohol fraction	-	-	>0.588
Methotrexate	-	0.666	0.958
Doxorubicin	4.950	-	-
Crude extract of *Vinca rosea*	-	>0.216	>0.457

[a]Results are expressed as Selectivity Index: SI values = The Selectivity Index is the ratio of IC50 of cancer cell to the IC50 of normal cell; Key to cell lines employed: HT-29 = Colon Adenocarcinoma; T47D = Breast carcinoma; HepG2 = Hepatocellular carcinoma

Hydroalcoholic fraction had no noticeable effect on cell lines. In comparison with crude extract of *Vinca rosea* as a positive control that comprises anticancer compounds (vinblastine and vincristine), crude extract, hexane and ethyl acetate fractions of *F. carduchorum* was active on T47D and HT-29 cell lines but the SI value of *Vinca rosea* was lower than the Methotrexate, crude extract and fractions of *F. carduchorum* that indicated the selective effect of *Vinca rosea* on this cell lines and less effect on normal cells (Table 2 and 3) (Sadati *et al*., 2012).

The hexane fraction had equal IC_{50} to the ethyl acetate fraction on HepG2 cell line but it has high Selectivity Index (SI). On the other hand, there is no significant difference between the SI value of hexane fraction in HepG2 cells line and Doxorubicin (the positive control). The SI value of ethyl acetate fraction was lower in HepG2 cells line than Doxorubicin that indicated the selective effect of ethyl acetate fraction on this cell line and less effect on normal cells. The IC_{50} value of crude extract and polar fractions in all cell lines was higher than nonpolar fractios but the SI value of them was low therefore, the crude extract and methanol fraction had the selective effect on this cell lines and less effect on normal cells that it may be due to content of flavenoids. The SI value of crude extract was lower in T47D and HT29 cell lines than the Methotrexate (the positive control) and might have less adverse effect than synthetic chemotherapeutic drugs on normal cells. Based on the National Cancer Institute (NCI) guideline, an extract that inhibited cell proliferation with $IC_{50} \leq 20$ μg mL is indicated as an active anticancer extract and extracts with $20<IC50<100$ and $IC_{50} \geq 100$ are mentioned as moderately and inactive compounds (Tanamatayarat *et al*., 2003; Mutee *et al*., 2012), respectively. According to the results of this study, the hexane fraction is moderately effective on all three tested cancerous cell lines but based on Table 2 results, it has no selective activity on cancer cell lines. The IC_{50} value of ethyl acetate fraction was shown moderately active on T47D and HepG2 cell lines according to the NCI guidline. The IC_{50} values indicated that the growth and proliferation of HT-29, HepG2 and T47D cell lines were most affected by hexane and ethyl acetate fractions could be attributed to their content of coumarins and phytosteroids. Literature from previous studies confirms the presence of phytosteroids, coumarins and flavonoids in *Ferulago* genus (Doganca *et al*., 1991; De Pascual *et al*., 1979). The activity of the plant fractions is confirmed to be due to secondary metabolites coumarins, phytosteroids and flavonoids. In previous study, the isolated coumarins from *F. campestris* showed cytotoxic effect (Basile *et al*., 2009). There was a report about the cytotoxic activity of steroids ($IC_{50}<50$ μM) (Kim *et al*., 2012). The cytotoxic activity of *F. carduchorum* may be due to the high content of coumarins and phytosteroids.

CONCLUSION

In this study, hexane and ethyl acetate fractions of *F. carduchorum* have been exhibited cytotoxic activity toward cell lines due to coumarins and phytosteroids. Suberosin (a coumarin) isolated from hexane fraction as an active compound. Knowing the cytotoxic activity of pure compounds may help to improve new synthetic compounds for treatment of cancers.

REFERENCES

Amirghofran, Z., M. Bahmani, A. Azadmehr, K. Javidnia and R. Miri, 2009. Immunomodulatory activities of various medicinal plant extracts: Effects on human lymphocytes apoptosis. Immunol. Invest., 38: 181-192.

Andrianova, V.B., Y.E. Sklyar and M.G. Pimenov, 1975. Coumarins of *Ferulago turcomania* roots. Khimiya Prirodnykh Soedinii, 11: 514-514.

Basile, A., S. Sorbo, V. Spadaro, M. Bruno, A. Maggio, N. Faraone and S. Rosselli, 2009. Antimicrobial and antioxidant activities of coumarins from the roots of *Ferulago campestris* (Apiaceae). Molecules, 14: 939-952.

Chen, Y.C., W.J. Tsai, M.H. Wu, L.C. Lin and Y.C. Kuo, 2007. Suberosin inhibits proliferation of human peripheral blood mononuclear cells through the modulation of the transcription factors NF-AT and NF-κB. Br. J. Pharmacol., 150: 298-312.

De Melo Cazal, C., V. de Cassia Domingues, J.R. Batalhao, O.C. Bueno and E.R. Filho *et al*., 2009. Isolation of xanthyletin, an inhibitor of ants symbiotic fungus, by high-speed counter-current chromatography. J. Chromatogr. A, 1216: 4307-4312.

De Pascual, T.J., B. Jimenez, B. Corrales and M. Grande, 1979. Coumarins from *Ferulago granatensis* group: Isovaleryl marmesin. Anales de Quimica, 75: 175-176.

Demetzos, C., D. Perdetzoglou, M. Gazouli, K. Tan and C. Economakis, 2000. Chemical analysis and antimicrobial studies on three species of *Ferulago* from Greece. Planta Med., 36: 560-563.

Doganca, S., A. Ulubelen and E. Tuzlaci, 1991. 1-acetylhydroquinone 4-galactoside from *Ferulago aucheri*. Phytochemistry, 30: 2803-2805.

Doganca, S., E. Tuzlaci and A. Ulubelen, 1992. Constituents of *Ferulago asperigifolia*. Fitoterapia, 63: 552-552.

Jimenez, B., M.C. Grande, J. Anaya, P. Torres and M. Grande, 2000. Coumarins from *Ferulago capillaris* and *F. brachyloba*. Phytochemistry, 53: 1025-1031.

Khalighi-Sigaroodi, F., A. Hadjiakhoondi, A. Shafiee, V.A. Mozaffarian, A.R. Shahverdi and S.H.R. Alavi, 2006. Phytochemical analysis of *Ferulogo bernardii* Tomk and M. Pimen. DARU J. Pharm. Sci., 14: 214-221.

Khanavi, M., M. Nabavi, N. Sadati, M.S. Ardekani and J. Sohrabipour *et al*., 2010. Cytotoxic activity of some marine brown algae against cancer cell lines. Biol. Res., 43: 31-37.

Kim, K.H., S.U. Choi, C.H. Kim and K.R. Lee, 2012. Cytotoxic steroids from the trunk of *Berberis koreana*. Biosci. Biotechnol. Biochem., 76: 825-827.

Lin, L.C., L.L. Yang, and C.J. Chou, 2003. Cytotoxic naphthoquinones and plumbagic acid glucosides from *Plumbago zeylanica*. Phytochem, 62: 619-622.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55-63.

Mozaffarian, V., 2007. A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran.

Mutee, A.F., S.M. Salhimi, F.C. Ghazali, F.M. Al-Hassan, C.P. Lim, K. Ibrahim and M.Z. Asmawi, 2012. Apoptosis induced in human breast cancer cell line by *Acanthaster planci* starfish extract compared to tamoxifen. Afr. J. Pharm. Pharmacol., 6: 129-134.

Ognyanov, I. and D. Bocheva, 1969. Natural coumarins. II. Coumarins in *Ferulago meoides* (L.) Boiss. Planta Med., 17: 65-70.

Rahimifard, N., S.R. Pakzad, S. Shoeibi, M.H. Hedayati and H. Hajimehdipour *et al*., 2009. Effects of essential oil and extract of *Thymus vulgaris*, *Zataria multiflora* and *Eugenia caryophilata* on Vero, Hela, HepII cell lines by MTT assay. J. Med. Plants, 8: 152-157.

Rechinger, K.H. and I.C. Hedge, 1987. Flora Iranica. Vol. 162, Akademische Druck and Verlagsanstalt, Graz, Austria, pp: 140-142.

Ruberto, G., S. Cannizzo, V. Amico, M. Bizzini and M. Piattelli, 1994. Chemical constituents of *Ferulago nodosa*. J. Nat. Prod., 57: 1731-1733.

Sadati, N., S.N. Ostad, Z. Karimian, M.R. Shams Ardekani, T. Akbarzadeh, A. Hajiakhoondi and M. Khanavi, 2012. Phytochemical study and *in vitro* cytotoxic effect of *Ajuga chamaecistus* ssp. *tomentella*. Asian J. Chem., 24: 2871-2874.

Saeidnia, S. and A.R. Gohari, 2012. Pharmacognosy and Molecular Pharmacognosy in Practice: A Laboratory Desk Reference of Pharmacognosy for Researchers and Students. Lambert Academic Publishing, Saarbrucken, Germany, ISBN-13: 9783848409273, Pages: 96.

Samiee, K., M.R. Akhgar, A. Rustaiyan and S.H. Masoudi, 2006. Composition of the Volatiles of *Ferulago carduchorum* Boiss. et Hausskn. and *Levisticum officinale* Koch. obtained by hydrodistillation and extraction. J. Essent. Oil Res., 18: 19-22.

Serkerov, S.V., A.A. Kagramanov and R.M. Abbasov, 1976. Coumarins of *Ferulago turcomanica*. Chem. Natl. Compounds, 12: 82-82.

Sharifzadeh, M., A. Hadjiakhoondi, M. Khanavi and M. Susanabadi, 2006. Effects of aqueous, methanolic and chloroform extracts of rhizome and aerial parts of *Valeriana officinalis* L. on naloxone-induced jumping in morphine-dependent mice. Addiction Biol., 11: 145-151.

Sklyar, Y.E., V.B. Andrianova and M.G. Pimenov, 1982. Coumarins of the roots of *Ferulago sylvatica*. Chem. Nat. Compd., 18: 488-488.

Tanamatayarat, P., P.N. Limtrakul, S. Chunsakaow and C. Duangrat, 2003. Screening of some rubiaceous plants for cytotoxic activity against *Cervix carcinoma* (KB-3-1) cell line. Thai J. Pharm. Sci., 27: 167-172.