

Antiproliferative evaluation of terpenoids and terpenoid coumarins from *Ferulago macrocarpa* (Fenzl) Boiss. fruits

Seyed-Ebrahim Sajjadi¹, Maryam Jamali², Yalda Shokoohinia^{3,4}, Gisy Abd⁵, Behzad Shahbazi³, Ali Fattahi⁶

¹Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, ²Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, ³Novel Drug Delivery Research Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, ⁴Department of Pharmacognosy and Biotechnology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, ⁵Department of Organic Chemistry, Faculty of Chemistry, Razi University, Kermanshah 67149, ⁶Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Submitted: 03-01-2015

Revised: 09-02-2015

Published: 21-10-2015

ABSTRACT

Background: *Ferulago macrocarpa* is a plant used as flavoring agent and protectant in the food industry and as a folk medicinal plant in Iran with no available information on its chemical identity. *Ferulago* spp. showed to contain biologically terpenoids and coumarins. **Objective:** The objective was to isolate and characterize terpenoids and coumarins from the acetone extract of *F. macrocarpa* fruits and to evaluate their antiproliferative effects on several cell lines. **Materials and Methods:** A series of normal and reverse phase gravity and high-performance liquid chromatography analyses were used to purify constituents. Compounds 1–5 and 7 were evaluated for their cytotoxic effects on MCF-7, HT-29 and H-1299 cell lines. **Results:** Six compounds including bornyl acetate (1), 1,10-di-*epi*-cubenol (2), stigmaterol (3) and three coumarins grandivittin (4), prantschimgin (5) and 4"-hydroxygrandivittin (7) along with mixtures of feruloyl derivatives (6a-6c) have been purified. Their structures were established by spectroscopic methods including nuclear magnetic resonance and MS analyses. Compound 2 showed moderate cytotoxicity effect with IC₅₀ values of 5.0 and 6.7 μ M on MCF-7 and HT-29, respectively. **Conclusion:** 1,10-di-*epi*-Cubenol could be considered as a potential proliferation inhibitor of MCF-7 and HT-29 cell lines.

Key words: 1,10-di-*epi*-Cubenol, Bornyl acetate, Grandivittin, HT-29, MCF-7, Prantschimgin

Access this article online

Website:

www.phcogres.com

DOI: 10.4103/0974-8490.158437

Quick Response Code:



INTRODUCTION

Natural products have been a prominent source of pharmacologically active molecules in medicines for thousands of years with advantages of effectiveness and low occurrence of side effects. A multitude of medicinal herbs have anticancer properties that are mediated through different mechanisms including altered carcinogen metabolism, induction of DNA repair systems, immune activation and suppression of cell cycle progression/induction of apoptosis.^[1] A study at 2007 has indicated that among 155 Food and Drug

Administration-approved small molecule anticancer drugs, 47% were either natural products or their analogues.^[2]

The genus *Ferulago* is represented by 40 species in the world. Among them, eight species exist in Iran with three endemics.^[3] The plants of the genus *Ferulago* have been employed in traditional Asian medicine against ulcers, snake bite, as well as headache and diseases of the spleen.^[4,5] *Ferulago* spp. showed different pharmacological effects such as antimicrobial,^[6,7] cytotoxic, immunomodulatory,^[8] antioxidant,^[9] acetylcholine esterase inhibitory,^[10] *Corpus cavernosum* relaxant,^[11] antilipidemic^[12] and fibrinolytic.^[13]

Ferulago macrocarpa (Fenzl) Boiss, known as Chavil (Chavir)-e-Roshanbal, is a perennial glabrous 40-100 cm high plant with cylindrical dichotomously branched stem. Leaves are shortly petiolate, pinnatisect, terminal segment, linear-oblong, acute; upper leaves reduced in short sheaths. Flowers are yellowish, synflorescence corymbose-

Address for correspondence:

Dr. Yalda Shokoohinia, Novel Drug Delivery Research Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran. Department of Pharmacognosy and Biotechnology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.
E-mail: yshokoohinia@kums.ac.ir; yshokoohinia@gmail.com

paniculiform. Fruits have a flat mericarp narrowly winged, more or less denticulate.^[3] *F. macrocarpa* is a commonly used Iranian folk medicine and food additive^[14,15] especially in western Iran (Kermanshah, Ilam, Kurdistan) as a flavoring agent in animal oil and ghee.^[16]

The plant genus *Ferulago* is widely distributed in Western Asia. Previous phytochemical and pharmacological studies on this genus mainly focus on coumarins^[16-20] due to their pharmacological effects.

Several pharmacological properties such as sedative, tonic, digestive and aphrodisiac besides treatment of intestinal worms, hemorrhoids and spleen diseases have been reported from *Ferulago* spp.^[9,19] However, there is some negotiations on traditional uses of these plants because of the similarity of local names of different species of *Ferula*, *Prangos* and *Ferulago* as “Jashir” in Persian and Turkish languages.^[3,21] Moreover, these two genera show strong similarity in chemotaxonomic pattern.^[22] However, since several *Ferula* spp. constituents^[23] along with coumarins.^[24,25] and terpenoids^[23] of Apiaceae family, exert cytotoxic effects, in the current study, antiproliferative effects of 1-5 and 7 have been studied on MCF-7, HT-29 and H-1299 cell lines. Furthermore, this is the first report on phytochemical analysis of *F. macrocarpa* and the very foremost biological evaluation of pure 1,10-di-*epi*-cubenol.

MATERIALS AND METHODS

General

Nuclear magnetic resonance (NMR) spectra were measured on a Bruker® (400 MHz) spectrometer. Chemical shifts were referenced to the residual solvent signal (CDCl₃: δ_H 7.26, δ_C 77.0). MS spectra were performed on an Agilent 5975C Network mass selective spectrometer. Open column chromatographies were performed using silica gel (70-230 mesh); separations were monitored by thin layer chromatography (TLC) on Merck 60 F254 (0.25 mm) plates and were visualized by ultraviolet (UV) inspection and/or staining with 0.2% cerium sulfate/4.2% sodium molybdate and heating; high-performance liquid chromatography (HPLC) were achieved on a waters apparatus equipped with a pump module 600 and a dual wavelength UV detector. HPLC apparatus was used to purify final products. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was bought from Sigma Aldrich (St. Louis, MO, USA). Cell culture medium, penicillin-streptomycin, and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). All the solvents used for extraction and purification were purchased from Merck (Germany).

Plant material

Ferulago macrocarpa (Fenzl) Boiss. fruits were collected from Saleh-Abad, Ilam, Western Iran, in May 2010 at an altitude of ca. 800 m above sea level, and the plant identity was confirmed by the Ilam Agricultural and Natural Resource Research Center, Iran.

Extraction and Isolation of coumarins

The air dried fruits of *F. macrocarpa* (500 g) were exhaustively macerated with acetone at room temperature. After the removal, the solvent *in vacuo*, the residue (87.5 g) was dissolved in methanol, kept in – 20°C and underwent chill filtration to get rid of long chain triglycerides. Defatted extract (75 g) was purified by vacuum liquid chromatography using mixtures of heptane (H) and EtOAc (E) (10:0-0:10) to afford 7 fractions (A–G). Fraction A was fats. Fraction B (1.5 g) was purified through open column chromatography on silica using 5-2% EtOAc in toluene to get pure 13 mg of compound 1 [Figure 1]. Other subfractions of B were mixed and purified using normal phase HPLC (hexane: EtOAc, 80:20-90:10) resulted in compound 2. Fraction C was mixture of B and D. Fraction D was subjected to several normal phase column chromatography and HPLC analyses, were led to isolation of a plant sterol, compound 3 (200 mg) and a pure coumarin, compound 4 (H: EtOAc, 50:50, 9 mg). Fraction E (H: E, 8:2) rendered a mass of impure crystals in which recrystallization resulted in a pure compound 5. Mother liquor of 1E was mixed with 1F and fractionated on normal phase open column and HPLC analyses to get mixtures of compounds 6a-6c (10 mg). Fraction G along with the most polar subfractions of other fractions were subjected to reversed-phase solid phase extraction using mixtures of MeOH and water, and finally furnished with HPLC analysis using RP18 column and 60-100% MeOH in H₂O solvent system to get compound 7 (5 mg).

Compound 1: Bornyl acetate - ¹H NMR (400 MHz, CD₃OD): δ_H 0.73 (s, CH₃-8), 0.80 (s, CH₃-9), 0.83 (s, CH₃-10), 2 (s, CH₃-2'), 1.11-1.26 (m, 2CH₂-3, 4 and H-5), 1.68 (s, 1H-6), 1.86 (s, 1H-6), 4.80 (m, *J* = 9.6, 2.4, H-1).

Compound 2: 1,10-di-*epi*-cubenol - ¹H NMR (400 MHz, CD₃OD): δ_H 0.77 (d, *J* = 6.8, CH₃-12) 0.82 (d, *J* = 6.8, CH₃-13), 0.94 (d, *J* = 6.8, CH₃-15), 1.05 (m, CH₂-4), 1.25 (m, CH₂-7), 1.30 (m, CH₂-8), 1.22 (m, H-9), 1.45 (m, H-6), 1.62 (s, CH₃-14), 1.75 (m, H-11), 1.90 (m, CH₂-3), 2.03 (m, H-10), 5.30 (m, H-1).

Compound 3: Stigmasterol.(+) EIMS *m/z* 412 (M) +, 397 (M-CH₃) +, 394 (MH₂O) +. FT-IR (KBr): ν_{max} = 3320, 2946, 2854, 1648, 1600, 1450, 1220, 890. ¹H NMR (400 MHz, CD₃OD): δ_H 5.30 (H-6), 5.12 (H-23), 4.91 (H-22), 3.71 (H-3).

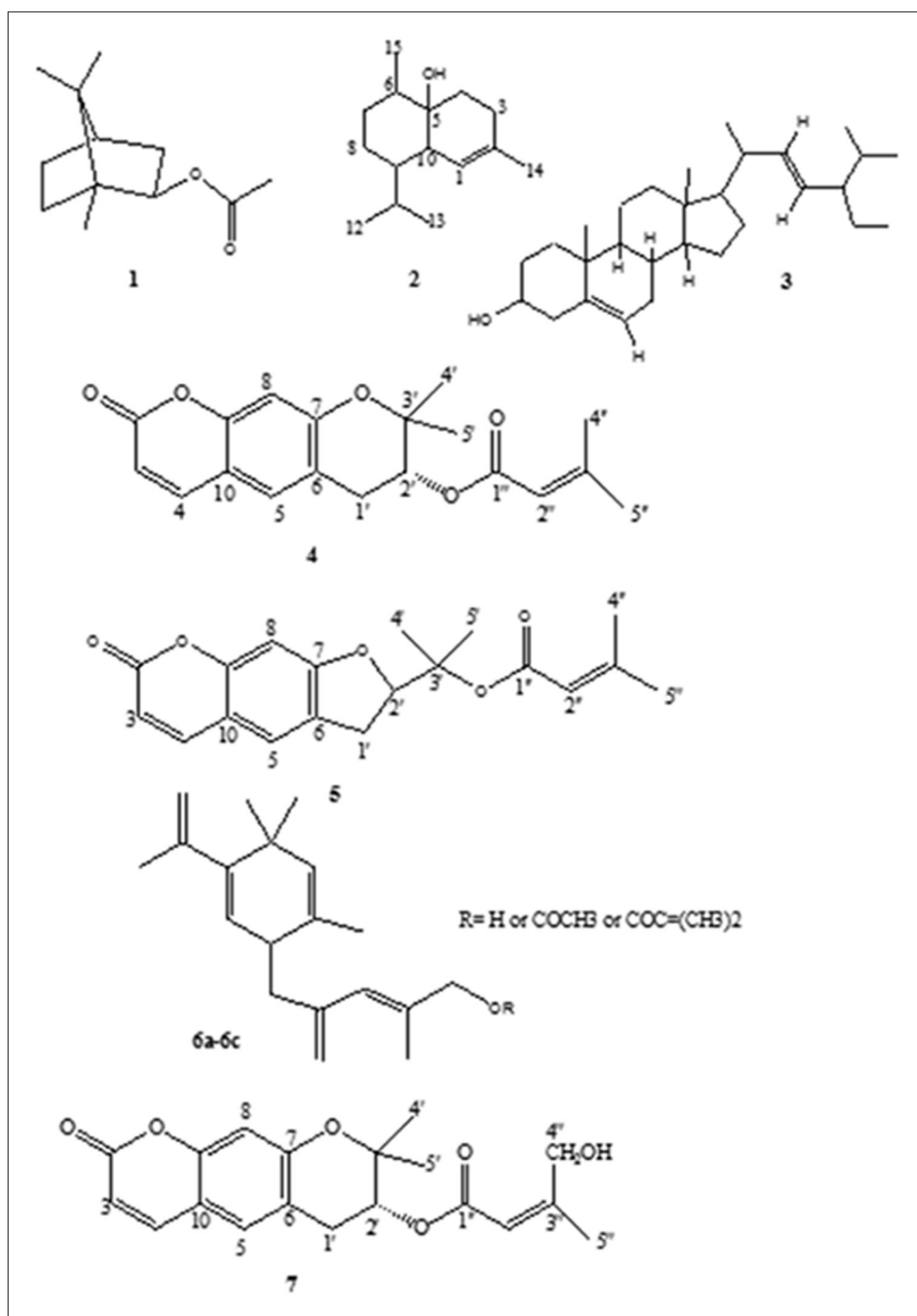


Figure 1: Chemical structure of terpenoids and terpenoid coumarins of *Ferulago macrocarpa*

Compound 4: Grandivittin - ^1H NMR (400 MHz, CD_3OD): Table 1.

Compound 5: Prantschimgin - ^1H NMR (400 MHz, CD_3OD): Table 1.; ^{13}C NMR (100 MHz, CD_3OD): δ C 20.66 (C-4''), 21.12 (C-5'), 22.29 (C-4'), 27.40 (C-5''), 29.57 (C-1'), 81.28 (C-3'), 88.87 (C-2'), 97.91 (C-8), 112.19 (C-3), 112.24 (C-10), 116.90 (C-5), 123.23 (C-6), 124.57 (C-2''), 143.72 (C-4), 155.74 (C-9), 156.47 (C-3''), 161.46 (C-7), 163.36 (C-2), and 165.85 (C-1').

Compound 7: Hydroxy grandivittin - ^1H NMR (400 MHz, CD_3OD): Table 1.

Cell culture

HT-29 (human colorectal adenocarcinoma), H-1299 (human nonsmall cell lung carcinoma) and MCF-7 (mammary adenocarcinoma) cell lines were obtained from Pasteur Institute (Tehran, Iran) and incubated at 37°C in a humidified atmosphere (90%) containing 5% CO_2 . Cells were cultured in RPMI-1640

with 10% (v/v) FBS, 100 U/mL penicillin and 100 µg/mL streptomycin. The medium was changed 2-3 days and sub-cultured when the cell population density reached to 70-80% confluence.

Assessment of cell proliferation

The cytotoxic effects of components were determined against MCF-7, HT-29 and H-1299 cell lines by a colorimetric assay using MTT and compared with the untreated control. Cells were plated onto 96-well plates at a concentration of 5.0×10^4 cells/mL and in a volume of 180 µL. After 24 h, cells were treated with various concentrations of components. At 48 h, the medium was removed and replaced by 100 µL of 0.5 mg/mL of MTT in growth medium and then the plates transferred to a 37°C incubator for 3-4 h. Supernatants were removed, and the reduced MTT dye was solubilized with dimethyl sulfoxide (100 µL/well). Absorbance was determined on an ELISA plate reader (Biotek, H1M, USA) with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain sample signal (OD570-OD630).

RESULTS AND DISCUSSION

By a combination of gravity column chromatography and HPLC purifications, several constituents of *F. macrocarpa* fruits have been identified as two monoterpenoids, bornyl acetate (1) and 1,10-di-*epi*-cubenol (2), and three coumarins, grandivittin (4), prantschimgin (5) and hydroxygrandivittin (7), along with mixtures of ferulol derivatives (6).^[22]

The coumarins were visualized as blue fluorescent spots on TLC plate while the nonfluorescent nonabsorbent spot was stigmasterol.

The mass (m/z 328) and ^{13}C -NMR spectra of 5 are in agreement with the molecular formula $\text{C}_{19}\text{H}_{21}\text{O}_5$. The presence of in the ^1H NMR spectra of the 4, 5, and 7 compounds, separately, in the regions δ 6.80, 6.70, 6.92 (H-8 of the 4, 5, and 7 products) and δ 7.16, 7.23, 6.57 (H-5 of the 4, 5, and 7 products), respectively, suggested substitution in position C-6 and C-7 of the coumarin nucleus for all three substances and most probably a linear fused ring system.^[26] Besides, the ^1H -NMR spectrum shows one AB system at two regions, δ 6.20, 6.20, 6.00 (d, H-3 of 4, 5, 7) and δ 7.59, 7.60, 7.35 (d, H-4 of 4, 5, 7) which are characteristic of the dihydrocoumarin skeleton. Methyls of C-4',-5' at δ 1.5 and one methylene at 3.24 (2H, m, $J = 1.2$, H-1') have completed the dihydro feature. The remaining signals are in agreement with the presence of a senecioid group^[19] including two methyls 1.86 (3H, d, $J = 1.6$ Hz, H-4''), 2.11 (3H, d, $J = 1.2$ Hz, H-5'') and one allylic hydrogen 5.50 (1H, s, H-2'') in 5, and corresponding data δ H 1.37 (3H, s, H-5'), 1.39 (3H, s, H-4'), 5.67 (1H, m, $J = 1.2$, H-2'') for 4.

The ^1H NMR of 4''-hydroxy grandivittin displayed similar peaks except for 4'' substitutes. Disappearing one allylic methyl and appearing one oxygenated methylene at δ 3.90 indicated the existence of the hydroxyl group on CH_2 -4'' and introducing the compound 7 as 4''-hydroxylated derivative of grandivittin. According to current data, it could be elucidated as 4''-hydroxy grandivittin, tentatively.

Isolated compounds were evaluated for potential antitumoral cytotoxicity against human MCF-7, HT-29 and H-1299 cell lines under MTT method. The results demonstrated that the cell proliferation was inhibited in the order of 1,10-di-*epi*-cubenol > bornyl acetate \geq prantschimgin \geq hydroxygrandivittin \geq grandivittin in MCF-7 cell line. This order for HT-29 was 1,10-di-*epi*-cubenol > prantschimgin > grandivittin > bornyl acetate \geq hydroxygrandivittin [Table 2]. As shown in Figure 2 (A and B), exposure to 1,10-di-*epi*-cubenol for 48 h resulted in a concentration dependent decrease in cell viability, with approximate IC_{50} value of 6.7 µM and 5 µM, in HT-29 and MCF-7 cells, respectively.

The Apioideae subfamily has been most intensely investigated and is characterized by the widespread occurrence of furo- and pyranocoumarins.^[26-28]

Most of the previously isolated compounds from *Ferulago* spp. were different coumarins such as dihydrofuranocoumarins,^[29]

Table 1: ^1H NMR data of compounds 4, 5, 7 in CDCl_3

| Number | 4 (m, J) | 5 (m, J) | 7 (m, J) |
|--------|---|---------------|--|
| 1'' | - | - | - |
| 2 | - | - | - |
| 7 | - | - | - |
| 3'' | - | - | - |
| 9 | - | - | - |
| 4 | 7.59 (d, 9.6) | 7.6 (d, 9.2) | 7.35 (d, 9.2) |
| 2'' | 5.67 (m, 1.2) | 5.50 (s) | 5.76 (m, 1.6) |
| 6 | - | - | - |
| 5 | 7.16 (s) | 7.23 (s) | 6.57 (s) |
| 10 | - | - | - |
| 3 | 6.20 (dd, 2.8, 9.6) | 6.20 (d, 9.6) | 6.00 (d, 9.2) |
| 8 | 6.80 (s) | 6.7 (s) | 6.92 (s) |
| 2' | 5.10 (t, 5.2) | 5.14 (t, 8.8) | 4.87 (m, 4.8) |
| 3' | - | - | - |
| 1' | 3.18 (dd, 0.8, 4.8) 2.87 (dd, 17.6, 4.8) | 3.24 (m, 1.2) | 3.00 (ddd, 1.2, 4.8, 17) 2.64 (dd, 4.8, 19.2) |
| 5'' | 2.15 (d, 1.2) | 2.11 (d, 1.2) | 1.83 (t, 0.4) |
| 4' | 1.39 (s) | 1.55 (s) | 1.13 (s) |
| 5' | 1.37 (s) | 1.60 (s) | 1.15 (s) |
| 4'' | 1.88 (d, 1.2) | 1.86 (d, 1.6) | 3.9 (d, 1.2) 5.07 |

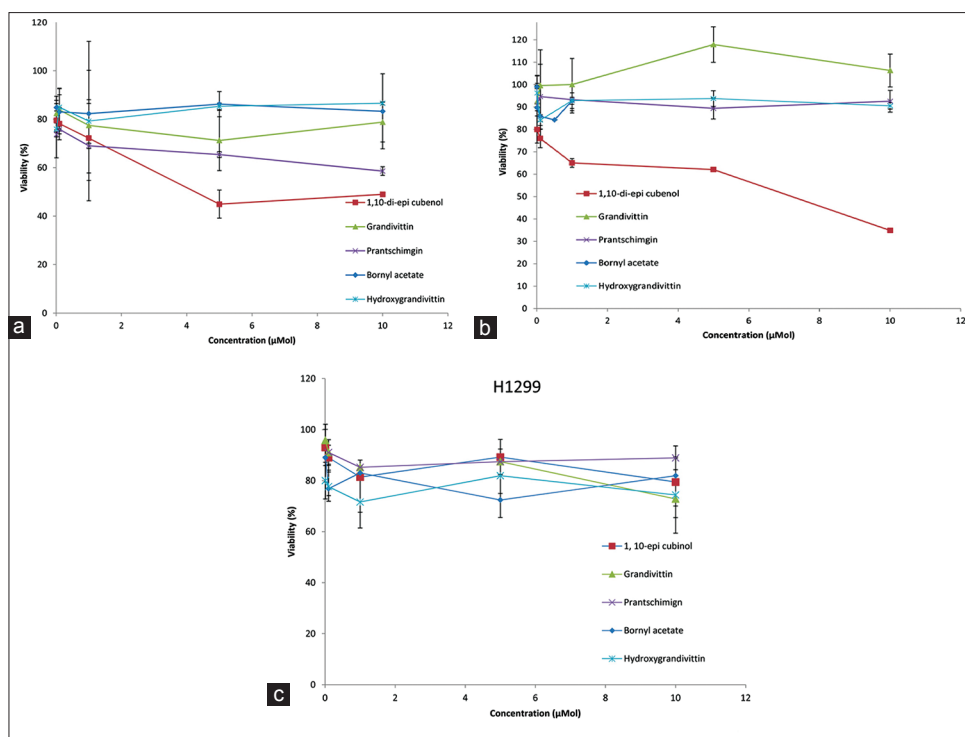


Figure 2: Cytotoxic activities of isolated compounds against (a) HT-29, (b) MCF-7 and (c) H-1299 cell lines. Values are mean \pm standard deviation of three experiments. Significance was calculated by ANOVA ($P < 0.05$)

Table 2: Antiproliferative effects of terpenoids and coumarins isolated from *Ferulago macrocarpa* against human cell lines

| | HTs-29 | | MCF-7 | | H-1299 | |
|-----------------------------|-----------------------|----------|-----------------------|---------|-----------------------|---------|
| | IC ₅₀ (μM) | MAE* (%) | IC ₅₀ (μM) | MAE (%) | IC ₅₀ (μM) | MAE (%) |
| 1,10-di- <i>epi</i> Cubenol | 6.7 | 55.07 | 5 | 65.12 | >10 | 20.52 |
| Prantschimgin | >10 | 41.42 | >10 | <10 | >10 | <10 |
| Grandivittin | >10 | 28.79 | >10 | <10 | >10 | <10 |
| Hydroxygrandivittin | >10 | 14.7 | >10 | 15.73 | >10 | <10 |
| Bornyl acetate | >10 | 13.42 | >10 | <10 | >10 | <10 |

*MAE=Maximum antiproliferative effect

and dihydropyranocoumarins,^[9] besides some other compounds such as flavonoids,^[30] phenylpropanoids, sesquiterpene aryl esters, sesquiterpenoids^[31] and polyacetylenes.^[22]

Lee *et al.* showed that seneciolylic acid moiety in dihydrocoumarins was more important in exhibiting the antitumor effects than the angeloylic acid moiety.^[32]

Among the isolated compounds, 1,10-di-*epi*-cubenol is the only compound that possesses a medium cytotoxic property. Previous studies showed that cubenol or essential oil containing cubenol and 1-*epi*-cubenol possess cytotoxicity effects on several cell lines such as M14, Hela, Hep-G2 and Bel-7402, which indicate that these groups of compound can be potentially cytotoxic.^[33-36] However, until now no data have been recorded regarding cytotoxicity

of 1,10 di-*epi*-cubenol. Although effects of prantschimgin on HT-29 cell line were dose-dependent, this compound did not reach to IC₅₀ value in the concentration range used in this study. Moreover, prantschimgin was not toxic against MCF-7 and H-1299, and no significant reducing on cell viability was observed in these cells. Ben Salem *et al.* had also shown that prantschimgin did not show strong cytotoxicity against the human colorectal cancer cell lines HCT-116 and HT-29, which is in a good agreement with our results.^[37] The rest of compounds were almost nontoxic and showed dose-independent effects in both of MCF-7 and HT-29 cell lines.

All chemical compounds extracted in this study shown no cytotoxicity against H-1299 cells which lack the expression of p53 protein. The p53 tumor suppressor proteins play an essential role in starting apoptosis by sensing

different intrinsic and extrinsic stresses. Deficiency in p53 gene leads to cell resistance and chemotherapeutic failure of cancer treatment. The lack of p53 expression in H-1299 may account for its higher resistance to extracted chemicals comparing to the other cell lines used in this study.^[38]

CONCLUSION

1, 10-di-*epi*-Cubenol (1) and prantschimgin (5) in comparison to other tested compounds exerted more cytotoxic effects on MCF-7 and HT-29. None of the compounds shows the cytotoxicity on H-1299 cells. Although 1 and 5 are more toxic than others, only compound 1 has shown a moderate cytotoxicity effect on MCF-7 and HT-29 cancerous cell lines with IC₅₀ values of 5 and 6.7 μ M, respectively.

REFERENCES

1. Taghizadeh Rabe SZ, Mahmoudi M, Ahi A, Emami SA. Antiproliferative effects of extracts from Iranian *Artemisia* species on cancer cell lines. *Pharm Biol* 2011;49:962-9.
2. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod* 2012;75:311-35.
3. Mozaffarian V. A Dictionary of Iranian Plant Names. Tehran: Farhang Moaser Publications; 1996.
4. Baser KH, Demirci B, Demirci F, Hashimoto T, Asakawa Y, Noma Y. Ferulagone: A new monoterpene ester from *Ferulago thirkeana* essential oil. *Planta Med* 2002;68:564-7.
5. Demetzos C, Perdetzoglou D, Gazouli M, Tan K, Economakis C. Chemical analysis and antimicrobial studies on three species of *Ferulago* from Greece. *Planta Med* 2000;66:560-3.
6. Abedini A, Roumy V, Mahieux S, Gohari A, Farimani MM, Rivière C, et al. Antimicrobial activity of selected Iranian medicinal plants against a broad spectrum of pathogenic and drug multiresistant micro-organisms. *Lett Appl Microbiol* 2014;59:412-21.
7. Darderafshi M, Bahrami G, Sadeghi E, Khanahmadi M, Mohammadi M, Mohammadi R. The effect of *Ferulago angulata* essential oil on *Staphylococcus aureus* during the manufacture and preservation of Iranian white cheese. *Iran J Nutr Sci Food Technol* 2014;8:13-20.
8. Amirghofran Z, Bahmani M, Azadmehr A, Javidnia K. Anticancer effects of various Iranian native medicinal plants on human tumor cell lines. *Neoplasma* 2006;53:428-33.
9. Basile A, Sorbo S, Spadaro V, Bruno M, Maggio A, Faraone N, et al. Antimicrobial and antioxidant activities of coumarins from the roots of *Ferulago campestris* (Apiaceae). *Molecules* 2009;14:939-52.
10. Dall'Acqua S, Maggi F, Minesso P, Salvagno M, Papa F, Vittori S, et al. Identification of non-alkaloid acetylcholinesterase inhibitors from *Ferulago campestris* (Besser) Grecescu (Apiaceae). *Fitoterapia* 2010;81:1208-12.
11. Ozturk B, Gur S, Coskun M, Kosan M, Erdurak CS, Hafez G, et al. A new relaxant on human *Corpus cavernosum*: *Ferulago syriaca* root extract. *Afr J Pharm Pharmacol* 2012;6:2652-6.
12. Rafeia-Kopaei M, Shahinfard N, Rouhi-Boroujeni H, Gharipour M, Darvishzadeh-Boroujeni P. Effects of *Ferulago angulata* Extract on Serum Lipids and Lipid Peroxidation. *Evid Based Complement Alternat Med* 2014;2014:680856.
13. Ugur M, Gürkan E, Koeksal E, Tuzlaci E. Cytotoxicity assay and fibrinolytic evaluation of *Heracleum sphondylium* and *Ferulago thirkeana*. *Fitoterapia* 1998;69:378-9.
14. Ghasemi Pirbalouti A, Momeni M, Bahmani M. Ethnobotanical study of medicinal plants used by Kurd tribe in Dehloran and Abadan Districts, Ilam Province, Iran. *Afr J Tradit Complement Altern Med* 2012;10:368-85.
15. Sajjadi SE, Shokoohinia Y, Jamali M. Chemical composition of essential oil of *Ferulago macrocarpa* (Fenzl) Boiss. *Fruits. Res Pharm Sci* 2012;7:197-200.
16. Masumi M. Wild growing plants in kermanshah. Kermanshah: Kosar publications; 2001.
17. Andrianova VB, Sklyar YE, Pimenov MG. Coumarins of the roots of *Ferulago turkomanica*. *Chem Nat Comp* 1975;11:532.
18. de Pascual T, Jimenez B, Corrales B, Grande M. Coumarins from *Ferulago granatensis* group: Isovaleryl marmesin. *An Quim* 1979;75:175-6.
19. Jiménez B, Grande MC, Anaya J, Torres P, Grande M. Coumarins from *Ferulago capillaris* and *F. brachyloba*. *Phytochemistry* 2000;53:1025-31.
20. Kilic CS, Okada Y, Coskun M, Okuyama T. New furanocoumarins isolated from the roots of *Ferulago isaurica* pesmen growing in Turkey. *Heterocycles* 2006;69:481-6.
21. Akalin E, Demirci B, Can Başer KH. A chemotaxonomic study on the genus *Ferulago*, sect. Humiles (Umbelliferae). *Biodiversity: Springer*; 2002. p. 309-13.
22. Ruberto G, Cannizzo S, Amico V, Bizzini M, Piattelli M. Chemical constituents of *Ferulago nodosa*. *J Nat Prod* 1994;57:1731-3.
23. Iranshahy M, Iranshahi M. Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assa-foetida* oleo-gum-resin) – A review. *J Ethnopharmacol* 2011;134:1-10.
24. Shokoohinia Y, Hosseinzadeh L, Moieni-Arya M, Mostafaei A, Mohammadi-Motlagh HR. Osthole attenuates doxorubicin-induced apoptosis in PC12 cells through inhibition of mitochondrial dysfunction and ROS production. *Biomed Res Int* 2014;2014:156848.
25. Shokoohinia Y, Sajjadi SE, Gholamzadeh S, Fattahi A, Behbahani M. Antiviral and cytotoxic evaluation of coumarins from *Prangos ferulacea*. *Pharm Biol* 2014;52:1543-9.
26. Erdelmeier CA, Sticher O. Coumarin Derivatives from *Eryngium campestre* L. *Planta Med* 1985;51:407-9.
27. Sajjadi SE, Shokoohinia Y, Hemmati S. Isolation and identification of furanocoumarins and a phenylpropanoid from the acetone extract and identification of volatile constituents from the essential oil of *Peucedanum pastinacifolium*. *Chem Nat Compd* 2012;48:1-4.
28. Sajjadi S, Zeinvand H, Shokoohinia Y. Isolation and identification of osthol from the fruits and essential oil composition of the leaves of *Prangos asperula* Boiss. *Res Pharm Sci* 2009;4:19-23.
29. Kilic CE, Coskun M. Felamedin and prantschimgin content of chloroform fractions of *Ferulago isaurica* and *F. syriaca* growing in Turkey. *Chem Nat Compd* 2006;42:351-2.
30. Doganca S, Ulubelen A, Tuzlaci E. 1-Acetylhydroquinone 4-galactoside from *Ferulago aucheri*. *Phytochemistry* 1991;30:2803-5.
31. Miski M, Moubasher HA, Mabry TJ. Sesquiterpene aryl esters from *Ferulago antiochia*. *Phytochemistry* 1990;29:881-6.
32. Lee S, Lee YS, Jung SH, Shin KH, Kim BK, Kang SS. Anti-tumor activities of decursinol angelate and decursin from *Angelica gigas*. *Arch Pharm Res* 2003;26:727-30.
33. Yu JQ, Liao ZX, Cai XQ, Lei JC, Zou GL. Composition, antimicrobial activity and cytotoxicity of essential oils from *Aristolochia mollissima*. *Environ Toxicol Pharmacol* 2007;23:162-7.

34. Yang Y, Yue Y, Runwei Y, Guolin Z. Cytotoxic, apoptotic and antioxidant activity of the essential oil of *Amomum tsao-ko*. *Bioresour Technol* 2010;101:4205-11.
35. Shakeri A, Khakdan F, Soheili V, Sahebkar A, Rassam G, Asili J. Chemical composition, antibacterial activity, and cytotoxicity of essential oil from *Nepeta ucrainica* L. spp. *Kopetdagensis*. *Ind Crops Prod* 2014;58:315-21.
36. Halabi MF, Sheikh BY. Anti-proliferative effect and phytochemical analysis of *Cymbopogon citratus* extract. *Biomed Res Int* 2014;2014:906239.
37. Ben Salem S, Jabrane A, Harzallah-Skhiri F, Ben Jannet H. New bioactive dihydrofuranocoumarins from the roots of the Tunisian *Ferula lutea* (Poir.) Maire. *Bioorg Med Chem Lett* 2013;23:4248-52.
38. Shokoohinia Y, Hosseinzadeh L, Alipour M, Mostafaie A, Mohammadi-Motlagh HR. Comparative Evaluation of Cytotoxic and Apoptogenic Effects of Several Coumarins on Human Cancer Cell Lines: Osthole Induces Apoptosis in p53-Deficient H1299 Cells. *Adv Pharmacol Sci* 2014;2014:847574.

Cite this article as: Sajjadi SE, Jamali M, Shokoohinia Y, Abdi G, Shahbazi B, Fattahi A. Antiproliferative evaluation of terpenoids and terpenoid coumarins from *Ferulago macrocarpa* (Fenzl) Boiss. fruits. *Phcog Res* 2015;7:322-8.

Source of Support: Ministry of Health, Kermanshah University of Medical Sciences, Iran., **Conflict of Interest:** None declared.