

Isolation and characterization of steroids, phthalide and essential oil of the fruits of *Kelussia odoratissima* Mozaff., an endemic mountain celery

S.E. Sajjadi¹, Y. Shokoohinia^{2,*} and P. Mehramiri¹

¹Department of Pharmacognosy and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

²Department of Pharmacognosy and Biotechnology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, I.R. Iran.

Abstract

Kelussia odoratissima Mozaff. is an Iranian endangered endemic edible plant with enormous use in the middle region of Iran as food and spice especially yogurt seasoning, and as medicinal herb for anti-inflammatory and cardiovascular purposes. Although antioxidant, anti-inflammatory and antilipidemic effects of this plant have so far been studied, but chemical analyses of the non volatile constituents of the plant have not yet been reported. Therefore, identification of chemical constituents of different parts of plant was investigated in the present study. In this direction, two steroids including stigmasterol and β -sitosterol and one phthalide namely 3-butyliden-4,5-dihydrophthalide were isolated from the plant fruits. In addition, the essential oil composition of the fruits was studied. Thirty eight compounds were identified, of which the major components were found to be Z-ligustilide (29.2%), germacrene-B (15.9%) and germacrene-D (15.5%). According to the previous bioassays of the isolated compounds such as anti-inflammatory and antilipidemic effects of phthalides and steroids, a chemical-biological relation can be postulated.

Keywords: Apiaceae; Stigmasterol; β -Sitosterol; Z-Ligustilide; 3-Butyliden-4,5-dihydrophthalide; Germacrene

INTRODUCTION

Apiaceae have aroused interest in food science because of its common used plants such as celery, carrot, fennel, caraway and dill. Coumarins, polyacetylenes, flavonoids, sesquiterpenes and phthalides are among the important chemical constituents of this family along with biologically active essential oil (1-3).

Kelussia is a monotypic endemic genus to Apiaceae, and *K. odoratissima* is wild growing in high altitude of western Zagros mountains, Iran (4). In some studies, its previous name *Amirkabiria odoratissima* has been used. As an endangered plant, it has recently drawn more attention for its regular cultivation. Being called “karafs koochi” (mountain celery) or “keloss” in the region and bearing a unique flavorful smell, *K. odoratissima* is vastly used as spice especially in yogurt. Pickles of small

leaves and shoots are common in Chaharmahal Bakhtiari province in south west of Iran. It is believed in folk medicine to be effective as anti-inflammatory and sedative, and to treat hypertension (interview with local people).

The antioxidant activity of the methanolic extract of the plant has been evaluated using β -carotene bleaching assay, reducing power, thiocyanate, accelerated oxidation of sunflower oil, and DPPH radical-scavenging and it was effective in some assays (5,6). In spite of its *in vitro* and *in vivo* efficiencies in lipid profile (7) the results could not be confirmed in the clinical trial, except for an increase in HDL (8). Furthermore, providing rabbits' food with aerial parts of the plant, suggested bearing beneficial effects to prevent development of fatty streak (9). Besides, gastric acid secretion has been reduced meaningfully in rats fed with *K. odoratissima*, but pepsin secretion was unaffected (10).

*Corresponding author: Y. Shokoohinia, this paper is extracted from the Pharm.D thesis No. 385505
Tel. 0098 831 4276488, Fax. 0098 831 4276493
Email: yshokoohinia@kums.ac.ir

Polyphenolic extract and essential oil were tested for fibrinolytic effects, and the latter was effective on thrombolytic agent (11). Essential oil showed a pronounced antibacterial effect as well (12). In folkloric medicine the use of this plant is proposed in inflammatory conditions which were confirmed in vitro by carrageenan test (13).

In spite of consumption of keloss as food additive and flavor and several pharmacological effects reported earlier, chemical identification of non volatile constituents of the plant except for clarification of fatty acid pattern and total phenolic content (6) have not yet been reported.

MATERIALS AND METHODS

General instrument

Medium pressure liquid chromatography (MPLC) was performed on a Büchi apparatus equipped with two pump modules 601/605, a pump manager (C-615) and a fraction collector C-60 using silica gel (70-230 mesh) columns. IR spectrum was recorded on a Rayleigh WQF-510 FTIR instrument.

¹HNMR was recorded on a Bruker (500 MHz) instrument, using CDCl₃ as the solvent and TMS as the internal standard. EI-MS spectrum was recorded on Hewlett Packard 5972A mass spectrometer. Compounds on the TLC (Silicagel 60 GF₂₅₄ precoated plates, Merck) were detected at 254 and 365 nm followed by KOH/ethanol, vaniline- sulfuric acid or cerium sulfate molibdate as spraying reagents (2). All reagents and solvents were purchased from Merck (Germany). GC/MS analysis was carried out on a Hewlett-Packard 6890 gas chromatograph fitted with a fused silica HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The oven temperature was programmed from 60 to 280°C at 4°C/min increment. Helium was used as the carrier gas at a flow rate of 2 ml/min with inlet pressure set at 17.7 psi and injection volume of 0.1 μl. The gas chromatograph was coupled to a Hewlett-Packard 5972A mass selective detector. The EI-MS operating parameters were as follows: ionization voltage, 70 eV; ion

source temperature, 200°C. Identification of components of the volatile oil was based on retention indices relative to *n*-alkanes and computer matching with the Wiley 275.L library, as well as comparison of the fragmentation patterns of the mass spectra with those reported in the literature (14,15).

Plant material

The fruits of *K. odoratissima* were collected in July 2008 from Zardkooh mountain which is located in Charmahal Bakhtiari province (south western of Iran), at an altitude of *ca.* 2400 m above sea level. The plant identity was confirmed by the Botany Department of Isfahan University and a voucher specimen of the plant (No. 2022) was deposited in the herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

Extraction and isolation of non-volatile compound

Ground fruits (300 g) were extracted with 600 ml of *n*-hexane for 6 h using Soxhlet apparatus which left 74 g crude extract (24.6%). The extract was then solubilized in methanol, kept in -20°C overnight and filtered which resulted in 30 g filtrate. The filtrate was purified using medium pressure liquid chromatography (MPLC) on a normal silica 60 A (15-40 μ) column eluted with heptane-ethylacetate (10:0 to 0:10) to get 14 fractions as A1-N1. The fractions were pooled according to the TLC profile using chromogenic reagents including vaniline-sulfuric acid and cerium sulfate molibdate. Fraction G1 compiled of a mass of crystals was recrystallized repeatedly in hexane to get crystals of compound **I** (127 mg) and compound **II** (100 mg). Fractions H1-L1 were added together (2 g) and purified using second normal silica MPLC eluted with heptane:ethyl acetate (8:2-6:4) which resulted in fractions A2-R2. The fractions were pooled according to the TLC profile using chromogenic reagent of vaniline-sulfuric acid and KOH 10% in ethanol and considering UV absorbance pattern at 366 nm. Fraction E2 collected as a mass of crystals were re-crystallized to obtain compound **III** (50 mg).

Isolation of essential oil

Plant material was hydrodistilled in a Clevenger-type apparatus for 3 h according to the method recommended in the British Pharmacopoeia (16). The volatile oil was dried over anhydrous sodium sulfate and stored in sealed vial and stored at 4°C until analysis. The yield of oil was calculated based on the dried weight of the plant material.

Compound I

Stigmasterol; colorless needle like crystals, ^1H NMR (CDCl₃, 500 MHz, J in Hz): Table 1; ^{13}C NMR (CDCl₃, 125 MHz) Table 1; (+) EI-MS m/z 412 $[\text{M}]^+$, 397 $[\text{M}-\text{CH}_3]^+$, 394 $[\text{M}-\text{H}_2\text{O}]^+$. FT-IR (KBr): ν_{max} = 3320, 2946, 2854, 1648, 1600, 1450, 1220, 890.

Compound II

β -sitosterol; colorless needle like crystals; m.p. 139°C; ^1H NMR (CDCl₃, 500 MHz, J in Hz): Table 1; (+) EI-MS m/z 414 $[\text{M}]^+$, 396, 381. FT-IR (KBr): ν_{max} = 3319, 2946, 2854, 1640, 1060, 870.

Compound III

Ligustilide; yellow crystals, ^1H NMR (CDCl₃, 500 MHz, J in Hz): Table 1; ^{13}C NMR (CDCl₃, 125 MHz) Table 1; (+) EI-MS m/z 190 $[\text{M}]^+$, 161 $[\text{M}-\text{Et}]^+$, 134 $[\text{M}-\text{side chain}]^+$. FT-IR (KBr): ν_{max} = 3030, 1755, 1455, 1170, 978.

RESULTS

Using a combination of winterization, medium pressure chromatography and recrystallization, three compounds were obtained in pure state from hexane extract of *K. odoratissima*.

According to the ^1H NMR spectrum of compound I, the multiplet signal at δ 3.71 was assigned to the resonance of methine proton at C-3 which is a common figure in 3-OH bearing triterpenoids and steroids. Additional data such as methyls (δ_{H} 0.9-1.2, δ_{C} 19-20, m/z 397 $[\text{M}-\text{CH}_3]^+$), OH strong stretch bond at 3471 cm^{-1} in IR spectrum and $[\text{M}-\text{H}_2\text{O}]^+$ ion fragment at m/z 394 confirmed the initial

Table 1. Selected ^1H NMR and ^{13}C NMR data of isolated compounds.

Compound I			Compound II		Compound III	
C/H	δ_{C}	δ_{H}	C/H	δ_{H}	H	δ_{H}
					4	2.60, 2H, t, $J=13.5$
					5	2.45, 2H, m
3	72.2	3.71	3	3.71	6	5.92, 1H, m
5	141.1	----			7	6.20, 1H, dt, $J=9.5, 1.5$
6	122.1	5.30	6	5.36	8	5.20, 1H, t, $J=7.9$
22	138.7	4.91			9	2.38, 2H, dt, $J=7.9$
23	129.7	5.12			10	1.20, 2H, m
					11	0.92, 3H, t, $J=7.5$

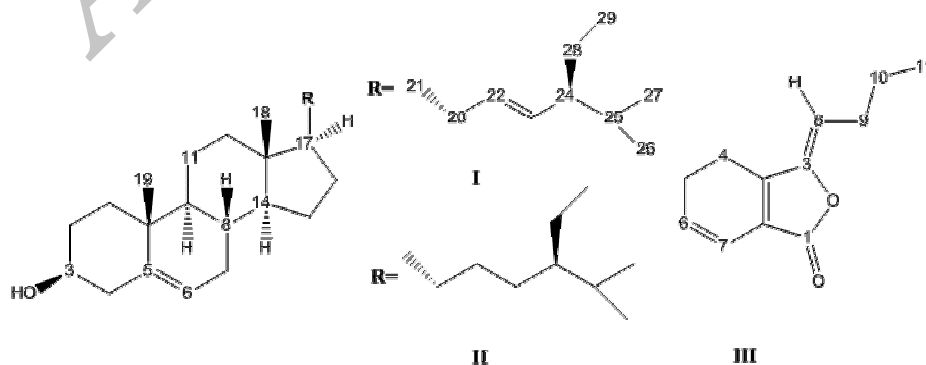


Fig. 1. Structures of isolated pure compounds. I. stigmasterol; II. β -sitosterol; III. ligustilide (3-butylden-4,5-dihydrophthalide)

proposal which is in accordance with stigmasterol. There are two unsaturation bonds represented by one broad singlet (δ_H 5.30, δ_C 122.1) and two doubled doublets (δ_H 5.12, δ_C 138.7 and δ_H 4.91, δ_C 129.7), well in consistent with H-6, H-22 and H-23 of stigmasterol (Fig. 1), respectively (17).

The characteristic feature of its mass fragmentation, except the molecular ion at 412 is the presence of a fragment ion peak at m/z 271 due to the loss of the side chain of stigmasterol (Fig. 1) followed by the loss of two hydrogen atoms (Table 1).

RESULTS

Using a combination of winterization, medium pressure chromatography and recrystallization, three compounds were obtained in pure state from hexane extract of *K. odoratissima*.

According to the ^1H NMR spectrum of compound **I**, the multiplet signal at δ 3.71 was assigned to the resonance of methine proton at C-3 which is a common figure in 3-OH bearing triterpenoids and steroids. Additional data such as methyls (δ_H 0.9-1.2, δ_C 19-20, m/z 397 $[\text{M}-\text{CH}_3]^+$), OH strong stretch bond at 3471 cm^{-1} in IR spectrum and $[\text{M}-\text{H}_2\text{O}]^+$ ion fragment at m/z 394 confirmed the initial proposal which is in accordance with stigmasterol. There are two unsaturation bonds represented by one broad singlet (δ_H 5.30, δ_C 122.1) and two doubled doublets (δ_H 5.12, δ_C 138.7 and δ_H 4.91, δ_C 129.7), well in consistent with H-6, H-22 and H-23 of stigmasterol (Fig. 1), respectively (17). The characteristic feature of its mass fragmentation, except the molecular ion at 412 is the presence of a fragment ion peak at m/z 271 due to the loss of the side chain of stigmasterol (Fig. 1) followed by the loss of two hydrogen atoms (Table 1).

Compound **II**, was assigned as β -sitosterol (Fig. 1) according to Rayleigh IR library comparing the finger print region with confirmation from mass data, with molecular ion at m/z 414 and m/z 396 for $[\text{M}-\text{H}_2\text{O}]^+$. Further IR characteristics were stretch absorbing features for OH (3471 cm^{-1}), C=C (1671 cm^{-1}) and unsaturated CH (after 3000 cm^{-1}).

EI-mass analysis defined the molecular ion peak of compound **III** at m/z 190. It showed to contain an α - β unsaturated lactone because of stretching absorption at 1750 cm^{-1} in IR spectrum, and two further unsaturated bonds as below: H-6 and H-7 were attached to unsaturated carbons with peaks at δ 5.98 (dt, $J = 4, 9.5\text{ Hz}$) and 6.20 (dt, $J = 2, 9.5\text{ Hz}$), respectively. H-8 was coupled with two protons of C-9 and appeared as a triplet at δ_H 5.2 ppm (Table 1).

The absorption sharp band at 1059 cm^{-1} , typical of C-O bond and multiple weak sharp peaks at $1300\text{-}1600\text{ cm}^{-1}$, characteristics of stretching absorption of C-C(=O)-O of α - β unsaturated esters (15) supported the elucidation.

The remaining unsaturation degree goes well with an extra cycle which is a cyclohexane bearing H-4 and H-5 as multiplets at δ 2.4-2.6, to represent the molecule as a phthalide called ligustilide (3-butylden-4,5-dihydrophthalide) (Fig. 1) (18). The ion fragment at m/z 161, due to the loss of an ethyl group and a characteristic feature of fragmentation with the presence of a fragment ion peak at m/z 134 due to the loss of the side chain of the compound, supported the identification.

DISCUSSION

To the best of our knowledge, the only previous investigation on the non-volatile constituents of *K. odoratissima* fruits concerned identification of fatty acid pattern and total phenolic contents (6). Here, we reported the isolation and identification of two sterols and a phthalide from fruits of *K. odoratissima*.

In order to explain the health promoting effects of various fruits and vegetables, focus has primarily been on vitamins, minerals, fibers and antioxidants, but still remains to be clear which components are responsible for these beneficial effects of food plants. A possible explanation could be that plants contain other bioactive compounds, i.e. compounds having a direct or indirect effect on living tissue *in vitro* and/or *in vivo*, which provide benefits to the health, even though they are not essential nutrients (1). Although application of "keloss" as a yogurt and pickle

Table 2. Percentage composition of the oil of *Kelussia odoratissima* fruits analyzed by GC/MS.

No.	Compound	%	RI
1	α -pinene	0.6	935
2	camphene	t	949
3	sabinene	t	972
4	β -pinene	t	977
5	myrcene	0.1	987
6	α -phellandrene	0.3	1001
7	δ -3-carene	0.1	1007
8	β - phellandrene	9.2	1028
9	<i>cis</i> - β -ocimene	3.8	1038
10	<i>trans</i> - β -ocimene	t	1047
11	γ -terpinene	t	1059
12	α -terpinolene	t	1085
13	allo-ocimene	0.1	1126
14	Unknown	1.0	1155
15	citronellol	0.1	1225
16	bornyl acetate	t	1283
17	lavandulyl acetate	0.1	1286
18	citronellyl acetate	0.2	1351
19	α -copaene	1.1	1373
20	β -elemene	2.3	1388
21	β -caryophyllene	0.4	1415
22	linalyl butyrate	0.4	1420
23	γ -elemene	3.4	1430
24	α -guaiene	0.5	1436
25	α -humulene	0.3	1450
26	germacrene-D	15.5	1477
27	β -selinene	0.7	1483
28	δ -selinene	1.3	1492
29	α -muurolene	0.7	1496
30	<i>trans</i> - β -guaiene	1.2	1497
31	germacrene-A	1.5	1500
32	δ -cadinene	5.8	1521
33	Unknown	1.1	1525
34	cadinal,4-diene	0.9	1529
35	α -cadinene	0.9	1536
36	germacrene-B	15.9	1554
37	germacrene-D-4-ol	t	1574
38	<i>cis</i> -3-butylidene phthalide	1.3	1667
39	3-butylidene-4,5-dihydrophthalide	29.2	1734
40	3N-butyl phthalide	t	1815

T = trace (<0.05%)

RI : Retention indices determined on HP-5MS capillary column

RT : Retention time (min)

% : Calculated from TIC data

seasoning is mainly because of its pleasant smell, but the regional beliefs about anti-flatulence and stomach tonic effect of the plant which is now attributed to its high amount of

phthalides in addition to evidences of being effective on *Helicobacter pylori* (19) and inflammation play an important role (20). Besides, effects of phytosterols on blood

cholesterol (21) and inflammation (22), and decreasing effect of phthalides on platelet aggregation would propose a clue to the folk belief regarding its use in cardiovascular diseases. Z-ligustilide, the pure isolated phthalide has shown antifungal effects (23).

The percent of essential oil was found to be 0.8%. The chemical composition of the *K. odoratissima* volatile oil is presented in Table 2. The compositions are listed in order of their elution on the DB-5 column. Thirty eight compounds were identified, of which the major components were found to be Z-ligustilide (29.2%), germacrene-B (15.9%) and germacrene-D (15.5%). Omidbaigi and coworkers (24) reported higher amount of ligustilide in essential oil of the aerial parts of *K. odoratissima* once compared to our findings. While considerable amount of ligustilide and piperitone epoxide were reported as other constituents in the aerial parts of *K. odoratissima*, here we found germacrene B and D in about 15% of the total essential oil. The plant sample used in our study and the one used by Omidbaigi (24) were both collected from Zagros mountain chain in Iran, but from different places and heights. The essential oil of *K. odoratissima* has shown fibrinolytic (11) and antibacterial effects (12). Overall, essential oil of Apiaceous plants has shown various biological and nutritional efficiencies (25,26).

Since phytosterols are effective on blood cholesterol (21) and inflammatory processes (22) in human, and phthalides could decrease platelet aggregation (27-28) and were effective on *Helicobacter pylori* (19) and inflammation (20), a rational relation between the major plant constituents and its proposed pharmacological effects may be postulated. Since it is an edible plant, further biological analyses on these and other isolated compounds are proposed.

The major components of the essential oil have been previously reported from Apiaceous plants. Ligustilide have been identified in edible and medicinal plants like celery (*Apium graveolens*), *Angelica sinensis* and lovage (*Levisticum officinale*) (29). Germacrene B has been found in *Ferula spp* (30) and Germacrene D in *Prangos asperula*

(31). The essential oil of the fruits of *K. odoratissima* consisted of thirteen monoterpene hydrocarbons (15.2%), five oxygenated monoterpenes (0.8%), sixteen sesquiterpene hydrocarbons (53.5%) and one oxygenated sesquiterpene (trace amount). Three phthalide compounds were also consisted 30.5% of the oil. As it could be concluded, the oil of *K. odoratissima* fruit was characterized by a high content of sesquiterpene hydrocarbons and phthalides, respectively.

CONCLUSION

Kelussia odoratissima is a good source of pharmacologically active phthalides and steroids. Previous cardioprotective and antihyperlipidemic effects of the plant could be attributed to these constituents.

ACKNOWLEDGMENT

This work was supported by a grant of deputy of research, Isfahan University of Medical Sciences. We gratefully acknowledge Isfahan University of Medical Sciences, School of Pharmacy and Isfahan Pharmaceutical Sciences Research Center for providing us with instruments and materials. Assistance of Mrs. Negin Mahdinejad is gratefully acknowledged.

REFERENCES

1. Christensen LP, Brandt K. Bioactive polyacetylenes in food plants of the Apiaceae family: occurrence, bioactivity and analysis. J Pharm Biomed Anal. 2006;41:683-693.
2. Harborne JB. Flavonoid and phenylpropanoid patterns in the Umbelliferae. Bot J Linn Soc. 1971;64:438.
3. Sajjadi SE, 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.
4. Mozaffarian V. Two new genera of Iranian Umbellifera. Bot Zhurn. (Moscow & Leningrad). 2003;88:88-94.
5. Ahmadi F, Kadivar M, Shahedi M. Antioxidant activity of *Kelussia odoratissima* Mozaff. in model and food systems. Food Chem. 2007;105:57-64.
6. Saeedi KA, Omidbaigi R. Evaluation of content and composition of fatty acids, total phenolic and essential oil content of *Kelussia odoratissima*

- Mozaff. Seed. Iran J Med Arom Plants Res. 2009;25:113-119.
7. Shahrani M, Pilehvarian AA, Khayri S, Asghari A, Farokhi A, Parvin N, *et al.* Effects of *Kelussia odoratissima* Mozaff. (KOM) extract on blood lipid in balb/c mice. Shahrekord Univ Med Sci J. Suppl. 2009;4:50-56.
 8. Rafieian M, Shahrani M, Pilehvarian AA, Khayri S, Rabii RA, Momeni A, *et al.* Effect of *Kelussia odoratissima* Mozaff. (KOM) on hyperlipidemia: a clinical trial study. Shahrekord Univ Med Sci J. Suppl. 2009;4:70-76.
 9. Asgari S, Naderi G, Dashti G, Paknahad Z. Effect of *Amirkabiria odoratissima* Mozaffarian on the development and progression of fatty streaks in hypercholesterolemic rabbits. Phytother Res. 2004;18:370-372.
 10. Shahrani M, Rafieian M, Pilehvarian A, Shirzadeh H, Hashemzadeh M, Yusofi H, *et al.* The effect of *Amirkabiria odoratissima* extract on gastric acid and pepsin secretion level in rat, Shahrekord Univ Med Sci J. 2007;8:88-95.
 11. Asgari S, Naderi G, Jafarian-Dehkordi A, Askary N, Behagh AR. Fibrinolytic activity of *Amirkabiria odoratissima* Mozaffarian. J Med Plants. 2005;13:50-59.
 12. Ghasemi-Pirbalouti A, Malekpoor F, Enteshari S, Yousefi M, Branch I, Momtaz H, *et al.* Antibacterial Activity of Some Folklore Medicinal Plants Used by Bakhtiari Tribal in Southwest Iran. Int J Biol. 2010;2:55-63.
 13. Hajhashemi V, Ghannadi AR, Soltani L. Analgesic and anti-inflammatory effects of *Amirkabiria odoratissima*. J Res Med Sci. 2003;7:121-125.
 14. Adams RP. Identification of essential oil components by gas chromatography/mass spectroscopy. Illinois: Allured Publication Corporation. 1995.
 15. Swigar AA, Silverstein RM. Monoterpenes, infrared, mass, ¹H-NMR, ¹³C-NMR spectra and Kovats indices. Wisconsin: Aldrich Chemical Company Incorporation. 1981.
 16. British Pharmacopoeia Commission. British pharmacopoeia. Vol 2: London: HMSO. 1998. p. 137-138.
 17. Forgo P, Kövér KE. Gradient enhanced selective experiments in the ¹HNMR chemical shift assignment of the skeleton and side-chain resonances of stigmasterol, a phytosterol derivative. Steroids. 2004;69:43-50.
 18. Zschocke S, Klaiber I, Bauer R, Vogler B. HPLC-coupled spectroscopic techniques (UV, MS, NMR) for the structure elucidation of phthalides in *Ligusticum chuanxiong*. Mol Diversity. 2005;9:33-39.
 19. Dekker KA. CJ 12,954 and its congeners, new anti *Helicobacter pylori* compounds produced by *Phanerochaete velutina*: fermentation, isolation, structural elucidation, and biological activities. J Antibiot. 1997;50:833-839.
 20. Zheng G, Kenney PM, Zhang J, Lam LKT. Chemoprevention of benzo [a] pyrene-induced forestomach cancer in mice by natural phthalides from celery seed oil. Nutr Cancer. 1993;19:77-86.
 21. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clinic Proc. 2003;78:965-978.
 22. Boukes GJ, Van de Venter M, Oosthuizen V. Quantitative and qualitative analysis of sterols/sterolins and hypoxoside contents of three *Hypoxis* (African potato) spp. Afr J Biotech. 2010;7:1624-1629.
 23. Meepagala, KM, Sturtz G, Wedge DE, Schrader KK, Duke SO. Phytotoxic and antifungal compounds from two Apiaceae species, *Lomatium californicum* and *Ligusticum hultenii*, rich sources of Z-ligustilide and apiol, respectively. J Chem Ecol. 2005;31:1567-1578.
 24. Omidbaigi R, Sefidkon F, Saeedi K. Essential oil content and composition of *Kelussia odoratissima* Mozaff. as an Iranian endemic plant. J Essent Oil Bear Plants. 2008;11:594-597.
 25. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a review. Food Chem Tox. 2008;46:446-475.
 26. Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review, Int. J Food Microbiol. 2004;94:223-253.
 27. Cao YX, Zhang W, He JY, He LC, Xu CB. Ligustilide induces vasodilatation via inhibiting voltage dependent calcium channel and receptor-mediated Ca²⁺ influx and release. Vascu Pharmacol. 2006;45:171-176.
 28. Zhang L, Du JR, Wang J, Yu DK, Chen YS, He Y, *et al.* Z-ligustilide extracted from *Radix Angelica sinensis* decreased platelet aggregation induced by ADP *ex vivo* and arterio-venous shunt thrombosis *in vivo* in rats. Yakugaku Zasshi. 2009;129:855-859.
 29. Zhong J, Pollastro F, Prenen J, Zhu Z, Appendino G, Nilius B. Ligustilide: A novel TRPA1 modulator. Eur J Physiol. 2011;462:841-849.
 30. Sahebkar A, Iranshahi M. Biological activities of essential oils from the genus *Ferula* (Apiaceae). Asian Biomedicine. 2010;4:835-847.
 31. Sajjadi SE, Mehregan I. Chemical composition of the essential oil of *Prangos asperula* Boiss. *Subsp.* *Haussknechtii* (Boiss) Herrnst. Et Heyn Fruits. Daru. 2003;11:79-81.