Original Article

Isolation and Characterization of Methylated Flavones from *Artemisia kermanensis*

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Abstract

Background: *Artemisia kermanensis* Podl. is a green aromatic perennial shrub that belongs to the family *Asteraceae* and it grows widely in central deserts and south-eastern mountains of Iran such as Taftan Mountain in Sistan and Baluchestan Province. *Artemisia* species have been used traditionally as a remedy for various feverous diseases, including malaria, treatment of colds, infections, parasites, inflammations of the liver, as well as dyspepsia, diabetes, hypertension, and so many other conditions. **Materials and Methods:** Air-dried *A. kermanensis* extraction from all parts of the plant was done using different organic solvents. The methanolic extract was selected for isolation of flavonoids, using thin-layer chromatography. The chemical structures of the isolated compounds were determined based on analysis of mass and nuclear magnetic resonance spectra. **Results:** Two flavone aglycones were isolated and identified for the first time from this plant's methanolic extract, including 5,7-dihydroxy-3',4',6-trimethoxyflavone (eupatilin) and 5,7,3'-Trihydroxy-6,4',5'-trimethoxyflavone. **Conclusions:** Eupatilin is known for its anticancer, antioxidant, and anti-inflammatory activities. In future researches on *A. kermanensis*, as a rich source of these flavone compounds, it is wise to investigate for the proven eupatilin's biological activities that have been mentioned.

Keywords: *Asteraceae, eupatilin, flavonoids, flavones*

Introduction

Artemisia kermanensis Podl. is a green aromatic perennial plant that belongs to the family *Asteraceae*, and it grows widely in central deserts and south-eastern mountains of Iran such as Taftan mountain in Sistan and Baluchestan Province. So far, *A. kermanensis* has been reported for its antimicrobial and antioxidant effects.[1] In general, *Artemisia* species has been used traditionally as a remedy for various feverous diseases, including malaria, treatment of colds, infections, parasites, inflammations of the liver, dyspepsia, diabetes, hypertension, and other conditions,[2-5] and in modern medicine, Grech-Baran and Pietrosiuk[6] reported the synthesis of two drugs, artemisinin and arglabin, first isolated from *Artemisia* species[7] using for the treatment of malaria and multiple tumor cell lines, respectively.[8]

A variety of secondary metabolites has been discovered in *Artemisia* genus so far, such as terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols, and acetylenes.[9,10] The flavonoids include apigenin, luteolin, chrysoeriol, kaempferol, rhamnocitrin, quercetin, tamarixetin, mikanin, casticin, cirsineol, eupatin, mearnsetin, chrysosplenol E and flavonoid glycosides include kaempferol-3-O-glucoside and isorhamnetin 3-glucoside.[11]

In this study, the known natural pharmacologically active flavone, 5,7-dihydroxy-3',4',6-trimethoxyflavone (eupatilin), **1** and 5,7,3'-Trihydroxy-6,4',5'-trimethoxyflavanone, **2** has been isolated for the first time, in large amounts.

Materials and Methods

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded by Bruker 400 (^{1}H at 400 MHz and ^{13}C at 100 MHz) spectrometers using the solvent signal for calibration ($CDCl_3$: δ^{H} 7.26, δ_C 77.0; CD_3OD: δ_H 3.31, δ_C 49.0). The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. ESIMS spectrometer was done on LCQ mass spectrometer using MeOH as the solvent. Medium-pressure

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liquid chromatography (MPLC) was performed on a Büchi 861 apparatus using glass columns (26 mm × 460 mm i.d.) and millipore silica gel (15–40 µm) as the stationary phase. Thin-layer chromatography (TLC) was performed on silicon dioxide (SiO_2) plates with hexane/acetone (7:3) as a mobile phase and cerium sulfate in 2 N sulfuric acid (H_2SO_4) as a reagent for visualizing the spots.

Plant material

A. kermanensis was authenticated and collected by Mohammad Amir Heidari in the September of 2018 from Taftan Mountain in south-east of Iran. A voucher specimen (4001) was deposited in the Herbarium of Pharmacy Faculty of Medical Sciences University of Isfahan. The collected plan was air dried away from the direct sunlight.

Extraction and isolation

5.0 kg of all parts of *A. kermanensis* was air-dried and extracted several times by maceration method. The solvent combination was two parts of dichloromethane, one part acetone. The extract was filtered and then evaporated under reduced pressure to get crude extract. The crude extract was coated on reverse silica gel and treated with methanol: water (7:3) to remove chlorophyll from the extract. The methanolic extract was filtered and the solvent evaporated and a waxy extract was left.

The methanolic crude extract (288.2 g) was pulverated by normal silica gel and was subjected to fractionation using MPLC. The pulverated extract was loaded on a previously packed MPLC silica gel column, fractionated using a gradient solvent system from n-hexane 100% to EtOAc 100%. The fractions were collected in 250 cc volumes, riched to 185 fractions. They were analyzed by TLC and combined into 23 fractions that were concentrated and kept for later use. Before the combination, the fractions were given a few days in room temperature in case of possible crystallization. Fortunately, considerable amounts of yellow crystals were observed and collected from the middle polarity zone tubes. TLC analysis showed three compounds that were isolated one after another. They were isolated from 19 tubes, and the first crystal was pure. The second compound isolated by preparative TLC and extra fractionation by column chromatography to the isolation of the third compound was failed [Figure 1].

Results

Two methylated flavones [Figure 1] were isolated from the methanol soluble part of the acetone/DCM extract of *A. kermanensis* by silica gel MPLC and preparative TLC methods. Their structure elucidation was obtained by extensive spectroscopic analysis, including NMR and ESIMS experiments.

Compound 1 was obtained as yellow crystals. The ESIMS (negative ions) of 1 showed a molecular ion peak

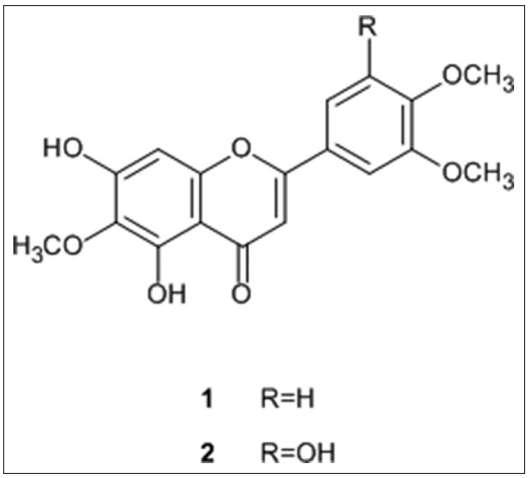

Figure 1: Chemical structures of compounds 1 and 2

at m/z 343.175 $[M-H]^-$ and m/z 345.237 $[M + H]^+$ at ESIMS (positive ions), which following ^{13}C NMR data suggested the molecular formula $C_{18}H_{16}O_7$ and it was assigned to 1 [Table 1]. Its structural assignment was obtained by extensive use of NMR spectroscopy and in comparison, with literature data.[12] Preliminary inspection of the ^1H NMR spectrum of 1 [Table 1] suggested the presence of a flavone skeleton methylated with three methoxy groups (3H singlets at δ3.95, 3.96, 4.02). The ^1H NMR spectrum showed signals attributed to two hydroxyl groups 1H signals at 6.53 (bs) and 13.05 (s). Other signals in the ^1H NMR spectrum at δ6.59 (s, H-3) indicated the presence of typical flavone aglycone type of flavonoids.[13] The proton spectrum also showed that four aromatic protons at 6.56 (s, H-8), 6.96 (d, J = 8.4 Hz, H-5'), 7.31 (d, J = 2 Hz, H-2'), 7.50 (dd, J = 8.4-2 Hz, H-6') were ascribable to protons on sp^2 carbon on A and B ring of highly oxygenated flavones. Two of them appeared as doublets (J = 2 Hz) at δ 7.31 and 7.50, suggesting meta coupling, a doublet (J = 8.4) at 6.96 corresponding to ortho coupling and one as a singlet at δ 6.56.

^{13}C NMR and DEPT experiments showed that the skeleton is composed of 15 carbons: five methines and ten quaternary carbons between δ 93.6 and 164.3, a carbonyl carbon at δ 183.1. Signals at δ 56.3, 56.3, and 61.1 indicate the presence of three methoxyl groups.

This evidence pointed to flavone skeleton for 1 with oxygenated carbons at positions 5, 6, 7, 3', and 4' as a dihydroxy-trimethoxyflavone. Comparison of NMR data with analog compounds reported in the literature pointed to the 5,7-Dihydroxy-3',4',6-trimethoxyflavone (eupatilin) structure.[14,15]

Analysis of NMR and MS spectral data of the second yellow crystals obtained from 12 fractions of *A. kermanensis* by preparative TLC method showed the flavone aglycone skeleton. The molecular formula of $C_{18}H_{16}0_8$, determined by ESIMS analysis and ^{13}C NMR data. ^1H NMR spectrum of 2

Table 1: ^{1}H and ^{13}C nuclear magnetic resonance data of compounds 1 and 2 at 400 and 100 MHz

Position	1		2	
	δH, mult., int., *J* in Hz	δC, mult.	δH, mult., int., *J* in Hz	δC, mult.
2	-	164.3	-	164.8
3	6.59	104.2	6.54	103.0
4	-	183.1	-	183.5
5	-	153.3	-	155.2
6	-	130.5	-	134.9
7	-	155.2	-	155.8
8	6.56	93.6	6.40	97.2
9	-	152.5	-	152.4
10	-	105.9	-	104.7
1’	-	123.9	-	128.3
2’	7.31 d (2)	108.8	7.10 d (2)	108.8
3’	-	149.5	-	153.7
4’	-	152.2	-	141.1
5’	6.96 d (8.4)	111.3	-	155.6
6’	7.50 dd (8.4-2)	120.3	7.06 d (2)	108.8
OCH3	3.95	56.3	3.94	64.5
OCH3	3.96	56.3	3.87	61.3
OCH3	4.02	61.1	3.86	56.8

Spectra were run in $CDCl_3$ for compound 1 and in CD_3OD for compound 2

exhibited a typical flavone skeleton with the signals of three distinct methyl groups (3H singlets at 3.94, 3.87, and 3.86). Aromatic protons showed two singlets and two doublets at δ 6.40 (s, H-8), 6.54 (s, H-3), 7.06 (d, J = 2 Hz, H-6’), and 7.10 (d, J = 2 Hz, H-2’). The characteristic small germinal values (2 Hz) indicated the presence of meta-aromatic protons. ^{13}C NMR data were closely related to compound 1 and agreed with the molecular formula, thus showing 18 carbon signals. The carbon flavone skeleton, including five methines and ten quaternary carbons, was appeared between δ 97.3 and 164.8 and carbonyl carbon at δ 183.5. Methoxyl group carbons signals were exhibited at δ 56.8, 61.3, and 64.5. A comparative NMR and MS analysis of compound 2 with compound 1 indicated compound 2 to be the derivative of compound 1 with oxygenated carbons at positions 5, 6, 7, 3’,4’, and 5’.

Compound 2 was found identical in all the characteristics, including NMR and MS data, with 5,7,3’-Trihydroxy-6,4’,5’-trimethoxyflavone previously isolated from *Artemisia ludoviciana*[12] and *Artemisia frigida*.[13]

Discussion

Artemisia is a diversified genus encompassing about 500 species in the temperate regions of Europe, Asia, and North America. It has been reported as a rich source of flavonoids, including eupatilin.[16,17] In general, flavonoids of *Artemisia* species have been demonstrated to have cytotoxic, antioxidant, antimalarial, antihemolytic, and estrogenic activities.[7,18-20] As we discussed, there are two pharmacologically active flavone compounds, eupatilin, and its hydroxylated form, in *A. kermanensis* with their wide range of biological activity. Eupatilin is known for its anticancer,[17,18,21-25] anti-inflammatory,[26,27] anti-oxidant,[28] neuroprotective,[29] and antiallergic[30] activities. It is speculated that eupatilin could be subjected to structural optimization to synthesize derivative analogs to reinforce its efficacy, minimize toxicity, and optimize absorption profiles, ultimately leading to potent drug candidates.

Conclusions

In future researches on *A. kermanensis*, as a source of these flavone compounds, it is wise to investigate for the proven eupatilin’s biological activities that have been mentioned earlier.

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Conflicts of interest

There are no conflicts of interest.

References

1. Kazemi M, Dakhili M, Dadkhah A, Yasrebifar Z, Larijani K. J Med Plant Res 2011;5:18:4481-86.
2. Patil G, Dass S, Chandra RJ. *Artemisia afra* and modern diseases. J Pharmacogenomics Pharmacoproteomics 2011;2:1-22.
3. Suresh J, Reddy V, Rajan D, Ihsanullah M, Khan MJ. Antimicrobial activity of *Artemisia* abrotanum and *Artemisia* pallens. J Pharmacogn Phytochem Res 2010;3:18-21.
4. Abad MJ, Bedoya LM, Apaza L, Bermejo P. The Artemisia L. genus: A review of bioactive essential oils. J Mol 2012;17:2542-66.
5. Mighri H, Hajlaoui H, Akrout A, Najjaa H, Neffati M. Antimicrobial and antioxidant activities of *Artemisia* herba-alba essential oil cultivated in Tunisian arid zone. J C R Chim 2010;13:380-6.
6. Grech-Baran M, Pietrosiuk A. Artemisia species *in vitro* cultures for production of biologically active secondary metabolites. BioTechnologia 2012;93:371-80.
7. Ferreira JF, Luthria DL, Sasaki T, Heyerick A. Flavonoids from *Artemisia* annua L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules 2010;15:3135-70.
8. Lone SH, Bhat KA, Khuroo MA. Arglabin: From isolation to antitumor evaluation. Chem Biol Interact 2015;240:180-98.
9. Koul B, Taak P, Kumar A. The *Artemisia* genus: A review on traditional uses, phytochemical constituents, pharmacological

properties and germplasm conservation. J Glycomics Lipidomics 2017;7 (1):142.
10. Brown GD, Liang GY, Sy LK. Terpenoids from the seeds of *Artemisia* annua. Phytochemistry 2003;64:303-23.
11. Lee JY, Chang EJ, Kim HJ, Park JH, Choi SW. Antioxidative flavonoids from leaves of carthamus tinctorius. Arch Pharm Res 2002;25:313.
12. Kim AR, Ko HJ, Chowdhury MA, Chang YS, Woo ER. Chemical constituents on the aerial parts of *Artemisia* selengensis and their IL-6 inhibitory activity. Arch Pharm Res 2015;38:1059-65.
13. Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids. New York etc., Springer. 1970.
14. Liu YL, Mabry T. Flavonoids from *Artemisia* ludoviciana var. ludoviciana. Phytochemistry 1982;21:209-14.
15. Liu YL, Mabry T. Two methylated flavones from *Artemisia* frigida. Phytochemistry 1981;20:309-11.
16. Bora KS, Sharma A. The genus *Artemisia*: A comprehensive review. Pharm Biol 2011;49:101-9.
17. Son JE, Lee E, Seo SG, Lee J, Kim JE, Kim J, *et al.* Eupatilin, a major flavonoid of *Artemisia*, attenuates aortic smooth muscle cell proliferation and migration by inhibiting PI3K, MKK3/6, and MKK4 activities. Planta Med 2013;79:1009-16.
18. Yuan H, Lu X, Ma Q, Li D, Xu G, Piao G. Flavonoids from *Artemisia sacrorum* Ledeb. and their cytotoxic activities against human cancer cell lines. Exp Ther Med 2016;12:1873-8.
19. Naqinezhad A, Nabavi SM, Nabavi SF, Ebrahimzadeh MA. Antioxidant and antihemolytic activities of flavonoid rich fractions of *Artemisia* tschernieviana Besser. Eur Rev Med Pharmacol Sci 2012;16 Suppl 3:88-94.
20. Lee SJ, Chung HY, Maier CG, Wood AR, Dixon RA, Mabry TJ. Estrogenic flavonoids from *Artemisia* vulgaris L. J Agric Food Chem 1998;46:3325-9.
21. Kim JS, Lee SG, Min K, Kwon TK, Kim HJ, Nam JO. Eupatilin inhibits adipogenesis through suppression of PPARγ activity in 3T3-L1 cells. Biomed Pharmacother 2018;103:135-9.
22. Lee JY, Bae H, Yang C, Park S, Youn BS, Kim HS, *et al.* Eupatilin promotes cell death by calcium influx through ER-mitochondria axis with SERPINB11 inhibition in epithelial ovarian cancer. Cancers (Basel) 2020;12 (6):1459.
23. Zhong WF, Wang XH, Pan B, Li F, Kuang L, Su ZX. Eupatilin induces human renal cancer cell apoptosis via ROS-mediated MAPK and PI3K/AKT signaling pathways. Oncol Lett 2016;12:2894-9.
24. Wang X, Zhu Y, Zhu L, Chen X, Xu Y, Zhao Y, *et al*. Eupatilin inhibits the proliferation of human esophageal cancer TE1 cells by targeting the Akt-GSK3 β and MAPK/ERK signaling cascades. Oncol Rep 2018;39:2942-50.
25. Li YY, Wu H, Dong YG, Lin BO, Xu G, Ma YB. Application of eupatilin in the treatment of osteosarcoma. Oncol Lett 2015;10:2505-10.
26. Kim J, Kim Y, Yi H, Jung H, Rim YA, Park N, *et al.* Eupatilin ameliorates collagen induced arthritis. J Korean Med Sci 2015;30:233-9.
27. Liu H, Hao J, Wu C, Liu G, Wang X, Yu J, *et al.* Eupatilin Alleviates lipopolysaccharide-induced acute lung injury by inhibiting inflammation and oxidative stress. Med Sci Monit 2019;25:8289-96.
28. Du L, Chen J, Xing YQ. Eupatilin prevents H_2O_2-induced oxidative stress and apoptosis in human retinal pigment epithelial cells. Biomed Pharmacother 2017;85:136-40.
29. Nageen B, Sarfraz I, Rasul A, Hussain G, Rukhsar F, Irshad S, *et al.* Eupatilin: A natural pharmacologically active flavone compound with its wide range applications. J Asian Nat Prod Res 2020;22:1-6.
30. Song EH, Chung KS, Kang YM, Lee JH, Lee M, An HJ. Eupatilin suppresses the allergic inflammatory response *in vitro* and *in vivo*. Phytomedicine 2018;42:1-8.