

Isolation and Characterization of Methylated Flavones from *Artemisia kermanensis*

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 febrile 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 febrile 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, cirsiin, eupatin, mearnsetin, chryso-splenol 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'-trimethoxyflavone, **2** has been isolated for the first time, in large amounts.

Materials and Methods

General experimental procedures

¹H and ¹³C NMR spectra were recorded by Bruker 400 (¹H at 400 MHz and ¹³C at 100 MHz) spectrometers using the solvent signal for calibration (CDCl₃: δ^H 7.26, δ^C 77.0; CD₃OD: δ^H 3.31, δ^C 49.0). The multiplicities of ¹³C 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 (SiO₂) plates with hexane/acetone (7:3) as a mobile phase and cerium sulfate in 2 N sulfuric acid (H₂SO₄) 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 plant 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

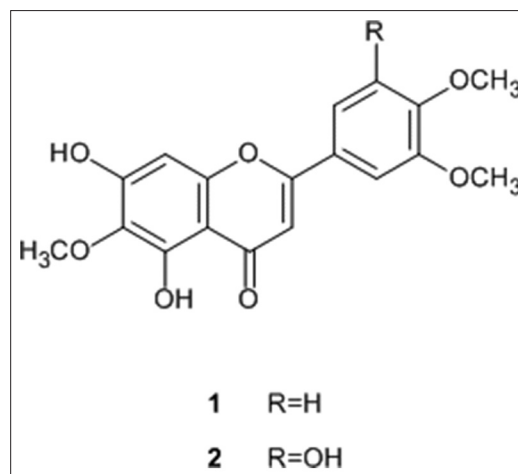


Figure 1: Chemical structures of compounds 1 and 2

at m/z 343.175 $[M-H]^-$ and m/z 345.237 $[M + H]^+$ at ESIMS (positive ions), which following ¹³C NMR data suggested the molecular formula C₁₈H₁₆O₇ 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 sp² 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.

¹³C 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 C₁₈H₁₆O₈, determined by ESIMS analysis and ¹³C NMR data. ¹H NMR spectrum of 2

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

Position	1		2	
	δ H, mult., int., J in Hz	δ C, mult.	δ H, mult., int., J in Hz	δ C, 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 CDCl₃ for compound 1 and in CD₃OD 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. ¹³C 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.

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