

Extraction and Determining of Chemical Structure of Flavonoids in *Tanacetum parthenium* (L.) Schultz. Bip. from Iran

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Abstract

Flavonoids, the most important of secondary metabolism products, occur in the plants. They have medicine and biological effects such as: Purifies blood, strengthens immune system, monitors cholesterol level, regulates blood pressure, suppresses acid secretion, prevents thrombus, suppresses cytophy, antibacterial, prevents cancer, promotes metabolism. In this study, the flavonoids of methanolic extract from *Chrysanthemum parthenium* (syn. *Tanacetum parthenium*) (Compositae Family) were separated and purified by column chromatography and TLC methods. Flavonol, Kaempferol, Fisetin and Naringenin are four flavonoid compounds were extracted, separated and detected by spectroscopy methods (¹H-NMR, ¹³C-NMR, Mass and IR).

Keywords: Flavonoids, Methanolic extract, *Chrysanthemum parthenium*, Compositae, Flavono, Kaempferol, Fisetin, Naringenin.

Introduction

The genus *Tanacetum*, (Family: Compositae or Asteraceae) with approximately 200 species, is distributed over Europe and west Asia. Twenty six different species has been reported from the genus of *Tanacetum* grow in various region of Iran .⁽¹⁾

Feverfew, the common name of *Tanacetum parthenium* (L.) Schultz. Bip. is a folk medicine used in Europe and Iran as alleviative the symptoms of migraine, arthritis and psoriasis, and to inhibit blood platelet aggregation⁽²⁻⁴⁾. Also it had been used as an insect repellent and had antibacterial, antioxidant and antifungal activity .^(5,6)

Parthenolide, flavonoides and a number of related sesquiterpene lactones and considered to be responsible for these activities. Investigations on different species of *Tanacetum* have shown the presence of essential oils⁽⁷⁻¹⁴⁾, sesquiterpene lactones,⁽¹⁵⁾ and flavonoids .⁽¹⁶⁾ *T. parthenium* has high camphor (44.0%) and trans-chrysanthenyl acetate (23.0%) content, *T. vulgare* contains lyratyl acetate, thujone and germacrene-D.⁽¹⁷⁾

The essential oil of *T. fruticosum* from Iran have been investigated by GC , GC/MS and NMR spectroscopy and some new farnesyl derivatives have been identified by their NMR spectra⁽⁸⁾. The flavonoids have been reported to have multiple biological effects including antibacterial , anti – inflammatory , antiallergic , antiviral , and also can be utilized in

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preventative and treatment protocols for cardiovascular disease, cancer, asthma, periodontal disease, liver disease, cataracts and macular degeneration.⁽¹⁸⁾

We report here the isolation and the structure elucidation of three flavone: 3-hydroxyflavone (Flavonol **1**); 3, 4', 5, 7-tetrahydroxyflavone (Kaempferol **2**); 3, 3', 4', 7-tetrahydroxyflavone (Fisetin **3**); and a flavanone, 4', 5, 7-trihydroxyflavanone (Naringenin **4**) from the MeOH aerial parts extract of *Tanacetum parthenium*.

Materials and Methods

Plant materials

The aerial parts of *Tanacetum parthenium* were collected in August 2005 from Khalkhal-Asalem road in northwest of Iran at an altitude of 2300m. A voucher specimen has been deposited at the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Methods:

Mass spectra were recorded on an AEI MS-50 spectrometer. IR spectrums were recorded on a Shimadzu IR-470 spectrometer. ¹H and ¹³C-NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker AM 300 instrument and TMS was used as internal standard. The ¹H and ¹³C-NMR spectrums were obtained in a mixture of CDCl₃ and CD₃OD as solvents.

Experimental:

Dried and finely powdered *Tanacetum parthenium* aerial parts (600g) were extracted with methanol in a Soxhlet apparatus during 3 days. The crude residue (70g) obtained after filtration and evaporation of the solvent and washing with petroleum ether, was dissolved in water then extracted successively with AcOEt and BuOH, yielding 4g and 12g fractions, respectively.

The ethyl acetate extract (4g) was resolved by silica gel column chromatography (70-230 mesh, Merck, petroleum ether, AcOEt, methanol gradients) so that 19 main fractions were collected. Fraction 1 was in turn chromatographed over silica gel with CHCl₃ to provide 7 subfractions. Subfraction 4 (80 mg) was rechromatographed on silica gel into 35 fractions (35×15 mL) using as eluents a 9.5:0.5 CHCl₃: MeOH mixture. The combined fractions 17 to 29 (23 mg) were further purified on a silica gel column to give compound **1** (17 mg).

From the 19 main fractions indicated above, fraction 5 was divided into 6 subfractions on a silica gel column using CHCl₃ as eluant. The most polar one was further purified on a silica gel column using CHCl₃: MeOH gradients to provide compound **2** (6 mg).

Fraction 9 (600 mg) was divided into six subfractions on a silica gel column using water: methanol as eluant. The subfraction with medium polarity (15 mg) was further resolved on a silica gel column using a gradient of CHCl₃: MeOH as eluant to afford 3 subfractions. The third one (7 mg) was compound **3**. The subfraction 5 (25 mg) of main fraction 9 was further purified on a silica gel column to give compound **4** (8mg).

Results and Discussion

Compound 1

Compound **1** (Flavonol) was obtained in the form of an amorphous solid. The molecular formula, C₁₅H₁₀O₃ was obtained on the basis of the [MH⁺] ion at m/z 239 and from the ¹³C-NMR analysis. The IR absorptions displayed a hydroxyl at (3227 cm⁻¹) and ketone (1628 cm⁻¹) groups. An examination of its NMR data (Table I) suggested that compound **1** was a flavonol. Thus, its ¹H-NMR spectrum revealed characteristic resonances of aromatic protons such as H-2',6' (δ 8.25, m, 2H), H-5 (δ 8.15, d, J=8.2Hz, 1H), H-7 (δ 7.82, m, 1H), H-8 (δ 7.78, d, J=8.2Hz, 1H), H-3', 5' (δ 7.59, m, 2H), H-4' (δ 7.53, m, 1H), and H-6 (δ 7.50, m, 1H). A typical hydroxyl signal at δ 9.68 ppm was also observed.

The ^{13}C -NMR spectrum showed 15 signals corresponding to nine $-\text{CH}-$ and six quaternary carbons (Table I). ^{13}C -NMR spectrum indicated the presence of a carbonyl carbon, which showed signal at $\delta 172.99(\text{C}-4)$.

Compound 2

The structure elucidation of compound **2** is as follow; compound **2** (Kaempferol) showed IR bonds for hydroxyl and carbonyl groups at 3418 , and 1660 cm^{-1} , respectively.

Its molecular formula was $\text{C}_{15}\text{H}_{10}\text{O}_6$ obtained from the $[\text{M}^+]$ ion at m/z 286 and ^{13}C -NMR analysis. The investigation of ^1H and ^{13}C -NMR spectrum data suggested that compound **2** also is a flavonoid (Table I). Its ^1H -NMR spectrum showed the typical signals of aromatic at δ 8.06(d, $J=8.4\text{Hz}$, H-2', 6'), δ 6.95(d, $J=8.4\text{Hz}$, H-3', 5'), δ 6.46(d, $J=1.9\text{Hz}$, H-8), and δ 6.2(d, $J=1.9\text{Hz}$, H-6). The ^{13}C -NMR spectrum showed 15 signals corresponding to six $-\text{CH}-$ and nine quaternary carbons (Table I).

Compound 3

Compound **3**, was obtained in the form of a pale yellow amorphous solid, also is a flavonoid (Fisetin). The EI-MS of **3** exhibited $[\text{M}^+]$ ion at m/z 286.2 for $\text{C}_{15}\text{H}_{10}\text{O}_6$ which is in accord with a flavonoid containing four hydroxyl groups. This compound showed IR bonds for hydroxyl and carbonyl groups at 3397 , and 1604 cm^{-1} , respectively. The signals at δ 6.5-8.5 ppm in ^1H -NMR and δ 100-180 ppm; In ^{13}C -NMR spectra (Table I), were suggestive of a flavone type skeleton. Compound **3** ^1H -NMR spectrum showed signals at δ 7.96(d, $J=8.2\text{Hz}$, H-5), δ 7.73(d, $J=3\text{Hz}$, H-8), δ 7.58(dd, $J=8.2, 3\text{Hz}$, H-6), δ 6.95(d, $J=1.9\text{Hz}$, H-2'), δ 6.94(d, $J=8.2\text{Hz}$, H-5') and δ 6.93(dd, $J=8.2, 1.9\text{Hz}$, H-6'). The ^{13}C -NMR spectrum showed 15 signals of carbons (see Table I).

Compound 4

The structure of this compound after assignments of the ^1H and ^{13}C -NMR signals and investigation of MS and IR spectrum was established as **4** and its name is naringenin. The molecular formula, $\text{C}_{15}\text{H}_{12}\text{O}_5$, was obtained of the $[\text{M}^+]$ ion at m/z 272 in EI-MS-50. The IR spectrum showed 2 bonds of $-\text{OH}$ and CO groups (3307 and 1641 cm^{-1} , respectively). In the ^1H -NMR spectrum, the doublet was observed at δ 7.32(d, 2H, H-2', 6') and δ 6.81(d, 2H, H-3', 5'). Its other signals of ^1H -NMR spectrum showed at δ 5.9(d, $J=1.9\text{Hz}$, H-6, 8), δ 5.43(t, 1H, H-2), δ 3.25(dd, 1H, H_a-3) and δ 2.69(d, 1H, H_b-3). (Fig.1)

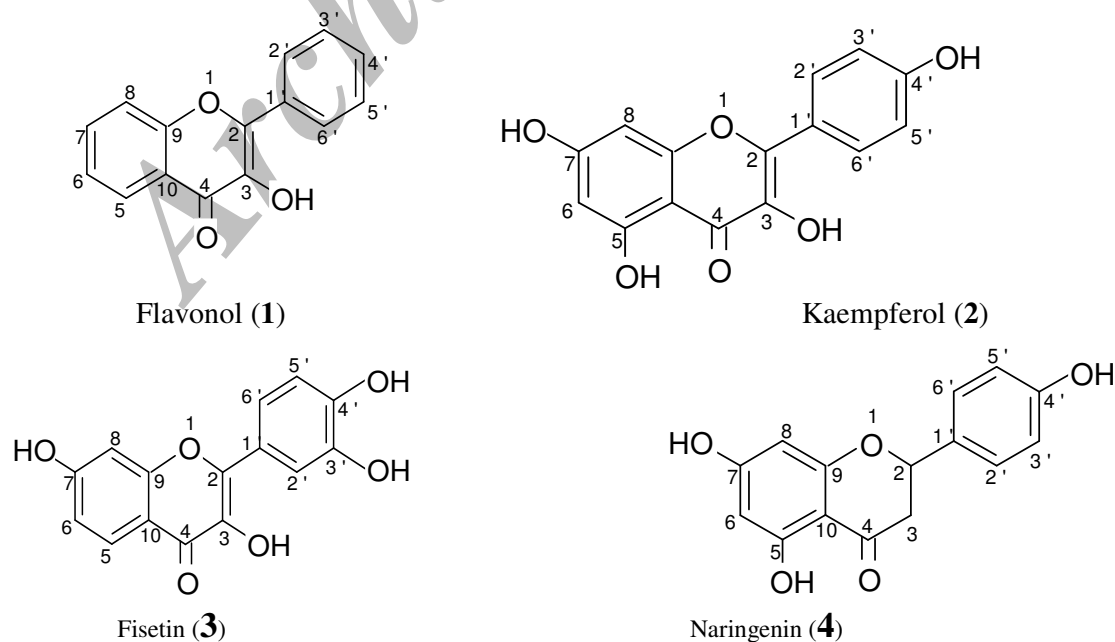


Fig. (1): Chemical structure of compound **1**, **2**, **3** and **4**.

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