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Original Article

Two Flavones from *Salvia leriaefolia*

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Abstract

The methanolic extract of the aerial parts of *Salvia leriaefolia* (labiatae) afforded 5-hydroxy-4′**,** 6, 7-trimethoxy flavone (Salvigenin) and (5,4′-dihydroxy-7-methoxy flavone (Genkwanin). The structures of the isolated compounds were elucidated using 1 and 2 D-NMR, IR, UV and MS.

Keywords: Flavone; Salvigenin; Genkwanin; *Salvia leriaefolia.*

Introduction

Salvia is an important genus of the labiatae family consisting of over 900 species and is widely distributed in various regions of the world, namely, the Mediterranean area, South Africa, Central and South America, and South East Asia. These plants are perennial, rarely biennial, or annual, with attractive flowers in various colors (1). Over 58 species of this genus have been found in Iran, seventeen of which are endemic (2). These plants have been used in folk medicine all around the world for culinary purposes (3) as well as for their antibacterial, antioxidant, antidiabetic, antitumor and antituberculotic activities (1, 4). Previous chemical investigations on different species of *Salvia* have shown the presence of flavonoids, diterpenoids, triterpenoids, sesterterpenes, and essential oils (1, 3, 5) exhibiting antitumor, antimicrobial, cytotoxic and anti-inflammatory activities (1, 6). Flavonoids are widely distributed in species of *Salvia*, being mostly present as flavones, flavonols and their glycosides (3). To the best of our knowledge, except for a report on the isolation of a labdan diterpene from *Salvia leriaefolia* (7), no flavonoid has been reported of this species. In this article isolation of two flavones from this plant is described and their spectroscopic data have been discussed.

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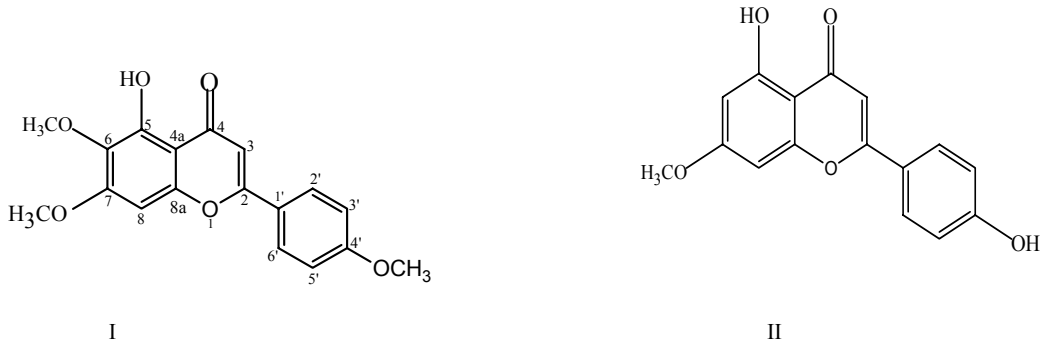
Experimental

The FT-IR spectra were recorded on a vector 22 instrument. The ^{1}H-NMR was recorded on a Bruker AM400 and AMX 500 NMR (Avance) instruments using the UNIX data system at 400 and 500 MHz, respectively. The ^{13}C-NMR spectrum was recorded at 100 and 125 MHz, respectively, using $CDCl_3$ and CD_3OD as solvents. ^{1}H-^{13}C HMBC and HMQC spectra were



I II

Figure 1. Structures of Salvigenin (I), Genkwanin (II).

recorded as mentioned above. EI-MS spectra were recorded on a Finnigan MAT 312. Fab mass measurements were performed on Jeol JMS HX 110 mass spectrometer using glycerol as the matrix. HR-EIMS were carried out on Jeol JMS 600 mass spectrometer. Column chromatography was carried out on silica gel (M&N), 70-230 and 230-400 meshes. Compounds on the TLC was employed to detect compounds at 254 and 366 nm using ceric sulfate as spraying reagent.

Plant material

The aerial parts of *Salvia leriaefolia* were collected from Sabzewar, Province of Khorassan, in the north-east of Iran, at an altitude of 1400 m, in July 2006. A voucher specimen was deposited at the Herbarium of the Department of Botany, Shahid Beheshti University, Tehran, Iran.

Extraction and isolation

The fresh aerial parts of *Salvia leriaefolia* were dried in the shade for 3 weeks. The powdered materials (3 Kg) were extracted three times with methanol by maceration at room temperature. The solvent was evaporated under vacuum to give 300 g of extract. The obtained dry extract was suspended in H_2O followed by extraction with hexane, $CHCl_3$ and n-butanol for three times consecutively. The $CHCl_3$ fraction was chromatographed on silica gel column (70-230 mesh) and eluted with varying portions of n-hexane, EtOAc and methanol to obtain fractions A-O. Two fractions (I, II) were selected from eluted fraction of n-hexane/EtOAc (7:3) (fraction F). Fraction IF was re-chromatographed on a silica gel column using 30% $CHCl_3$/n-Hexane as eluent to obtain three fractions (IF_a-IF_c). Fraction IF_b gave 15 mg of a yellow powder after evaporating the solvent. The compound had a melting point of 185°C and showed the following spectral characteristics:

Salvigenin (5-hydroxy-4´, 6, 7-trimethoxy flavone) (IF_b):

UV max (EtOH): 274, 330 nm; IR (KBr)

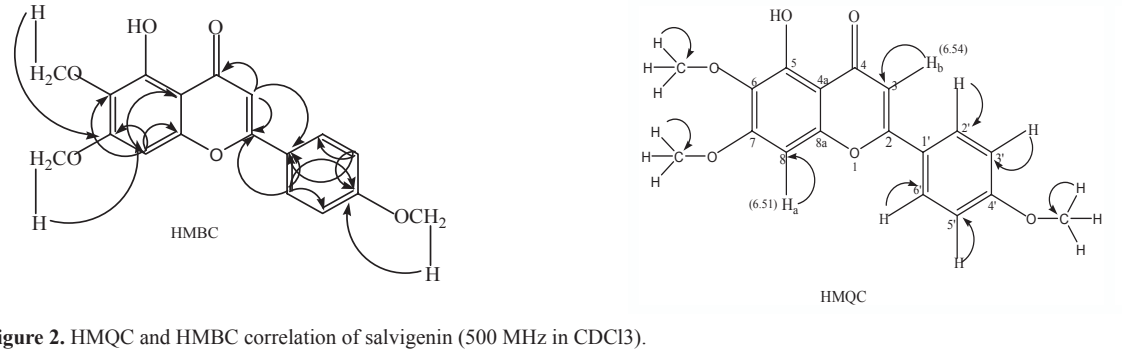


Figure 2. HMQC and HMBC correlation of salvigenin (500 MHz in CDCl3).

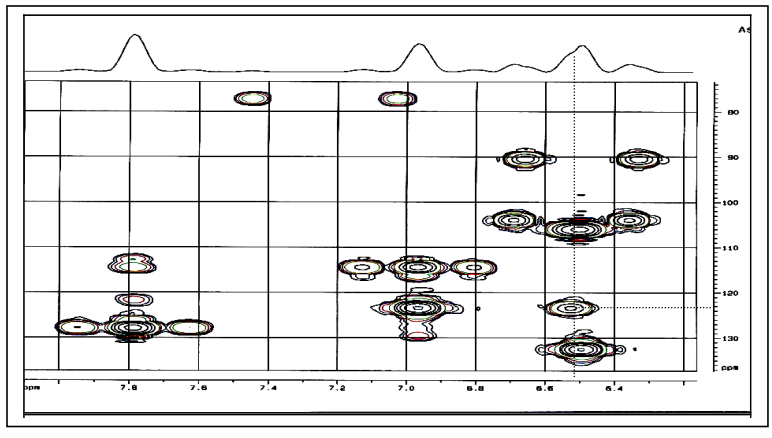


Figure 3. HMBC of salvigenin; correlation between H-3 and C-1′.

max: 1635 (C=O), 1600, 830 cm^{-1}; EI-MS: m/z = 328 $[M]^+$; ^{1}H- NMR (400 MHz, $CDCl_3$), δ ppm: 3.88, 3.9, 3.94 (s,OMe), 6.51 (s, 1H, H-8), 6.54 (s, 1H, H-3), 6.97 (d, 2H, J = 8.8 Hz, H-3′,5′), 7.79 (d, 2H, J = 8.8 Hz, H-2′,6′), 12.74 (s, OH); ^{13}C-NMR (100 MHz, $CDCl_3$), δ ppm: 55.47, 56.24, 60.77 (OMe), 90.5 (C-8a), 104(C-3), 106.4 (C-5), 114.44 (C-3′,5′), 123.43 (C-1′), 127.9 (C-2′,6′), 132.54 (C-7), 152.98 (C-6), 153.13 (C-4a), 158.65 (C-8), 162.55 (C-4′), 163.91 (C-2), 182.58 (C-4) (Table 1). Fraction IIF was purified by preparative TLC with 1% MeOH/Hexan as eluent to obtain three fractions (IIF_a-IIF_c). Fraction IIF_c gave 10 mg of a yellow powder after evaporating the solvent. The compound had a melting point of 285 °C and showed the following spectral characteristics:

Genkwanin (5, 4′-dihydroxy-7-methoxy flavone) (IIF_c):

UV max (MeOH): 267, 300, 333 nm ; IR (KBr) :3270, 1660, 1600, 1340, 1200, 1180, 830 cm^{-1}; EI-MS : m/z = 284 $[M]^+$; ^{1}H- NMR (500 MHz, CD_3OD), δ ppm: 3.88 (s,OMe), 6.31 (d, *J* = 2 Hz, H-6), 6.53 (s, 1H, H-3), 6.63 (d, *J* = 2 Hz, H-8), 6.78 (d, *J* = 8.8, H-3′,5′), 7.78 (d, *J* = 8.8 Hz, H-2′,6′); ^{13}C-NMR (125 MHz, CD_3OD), δ ppm: 55.8 (OMe), 93.3 (C-8), 99.1 (C-6), 103.4 (C-3), 105.9 (C-4a), 116.3 (C-3′,5′), 118.8 (C-

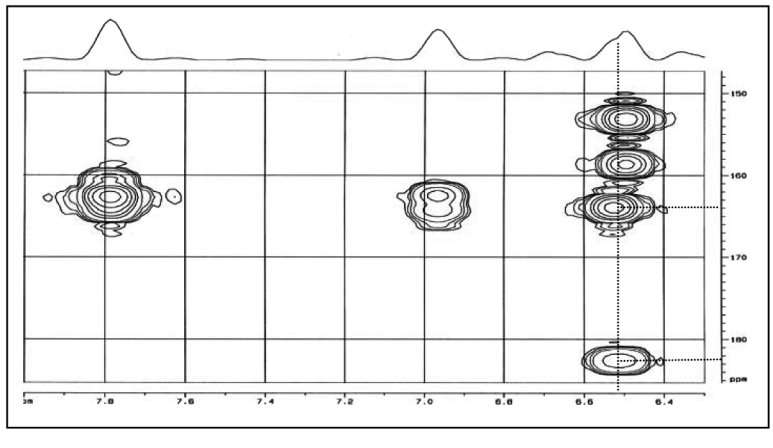


Figure 4. HMBC of salvigenin; correlation between H-3 and C-2, C-4.

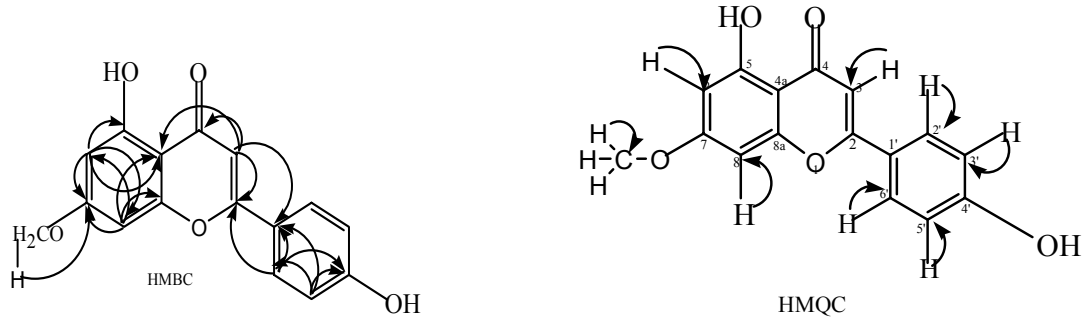


Figure 5. HMQC and HMBC correlation of genkwanin (500 MHz in CD3OD).

1′), 129.2 (C-2′,6′), 157.7 (C-5), 159.2 (C-8a), 161.8 (C-4′), 167.2 (C-7), 167.8 (C-2), 183.8 (C-4) (Table2).

Results and Discussion

The compound obtained from fraction IFb was identified as salvigenin and the one obtained from fraction IIFc was identified as genkwanin (Figure 1) by interpretation of their MS, NMR and IR spectra as well as by comparison of their spectral data with those reported in the literatures (8-10). EI-MS and FAB MS $[M+1]^+$ spectra of salvigenin confirmed the molecular weight at m/z 328(fragments at m/z 313,299,285,181,153). HR EI-MS showed the $[M]^+$ at m/z 328.0956 in agreement with the molecular formula $C_{18}H_{16}O_6$ (calcd. 328.0947) and indicated eleven degrees of unsaturation. The UV absorption maxima at 274 and 330 nm suggested the presence of a flavonoid moiety (11). The IR spectrum showed *v* max at 1635 (for C=O) and 830 cm^{-1} (for p-substituted phenyl ring). The ^{1}H-NMR spectrum proved it to be a flavone (δ 6.54, 1H, s, H-3) (12). Three single peaks at δ=3.94, 3.9, 3.88, showed the position of methoxyl groups at C4′, C6, C7, respectively. A single peak at δ=6.51 assignable to H-8 and a pair of doublets at δ=7.79 and 6.97 (each 2H, *J*=8.8 Hz) assignable to the protons located at 2′,6′and 3′, 5′which was confirmed by 2D COSY. The single peak at δ=12.74, showed the position of hydroxyl group at C5. The ^{1}H-^{13}C HMBC spectrum confirmed the placement of the methoxyl groups at positions

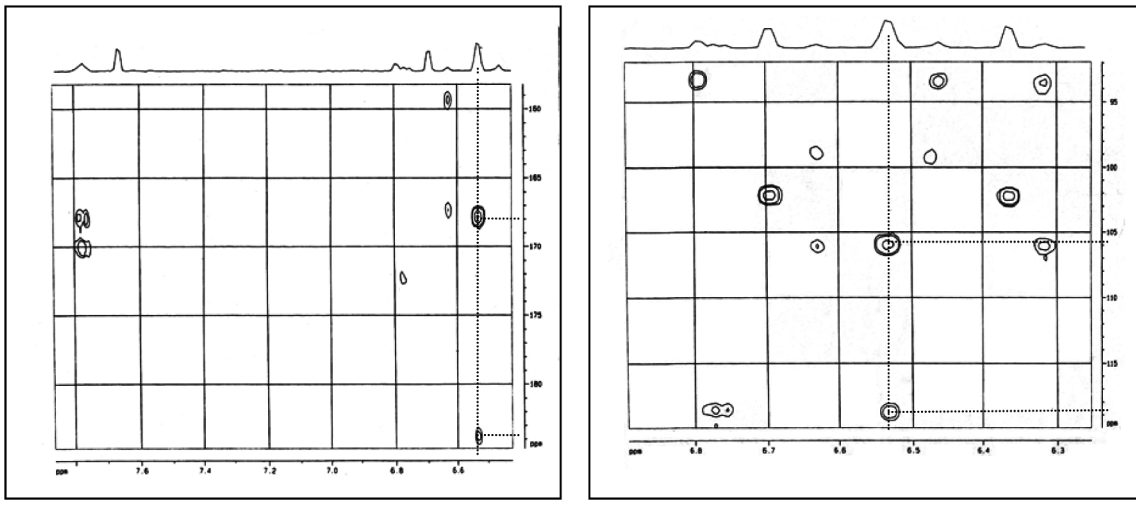


Figure 6. HMBC of genkwanin; correlations between H-3 and C-1′, C-2, C-4, C-4a.

Table 1. ^{1}H&^{13}C-NMR data of Salvigenin (I).

Pair proton or carbon	^{1}H-NMR	^{13}C-NMR
2		163.91C
3	6.54 s	104C-H
4		182.58C
6		152.98C
7		132.54C
8	6.51 s	158.65C-H
8a		90.5C
4a		153.13C
1′		123.43C
2′	7.79 (d,8.8)	127.9C-H
3′	6.97 (d, 8.8)	114.44C-H
4′		162.55C
5′	6.97 (d, 8.8)	114.44C-H
6′	7.79 (d, 8.8)	127.9C-H
5 (OH)	12.74 s	106.4C
OMe	3.94 s	55.47
OMe	3.9 s	56.24
OMe	3.88 s	60.77

Table 2. ^{1}H & ^{13}C-NMR data of Genkwanin (II).

Pair proton or carbon	^{1}H-NMR	^{13}C-NMR
2		167.8C
3	6.53 s	103.4C-H
4		183.8C
5		157.7C
6	6.31(d,2)	99.1C-H
7		167.2C
8	6.63 (d,2)	93.3C-H
8a		159.2C
4a		105.9C
1′		118.8C
2′	7.78 (d,8.8)	129.2C-H
3′	6.78 (d,8.8)	116.3C-H
4′		161.8C
5′	6.78 (d,8.8)	116.3C-H
6′	7.78 (d,8.8)	129.2C-H
OMe	3.88 s	55.8

6, 7 and 4′ (Figure 2). Also H-3 (δ=6.54) showed correlation with C4, C2, C1′ in HMBC, confirming the position of H-3 (Figures 3 and 4). The ^{13}C-NMR spectra showed the presence of twelve aromatic carbons, which comprise of 5 methines[δ=158.65, 127.9 (2CH), 114.4(2CH)] and 7 quaternary carbons (Table 1). All these data confirming the structure of IF_b to be 5-hydroxy-4′, 6, 7 trimethoxyflavone (salvigenin). The EI-MS and FAB MS $[M+1]^+$ spectra of genkwanin (II) confirmed the molecular weight at m/z 284. The UV absorption maxima at 267, 300, 333 nm (MeOH). HR EI-MS showed the $[M]^+$ at m/z 284.0925 in agreement with the

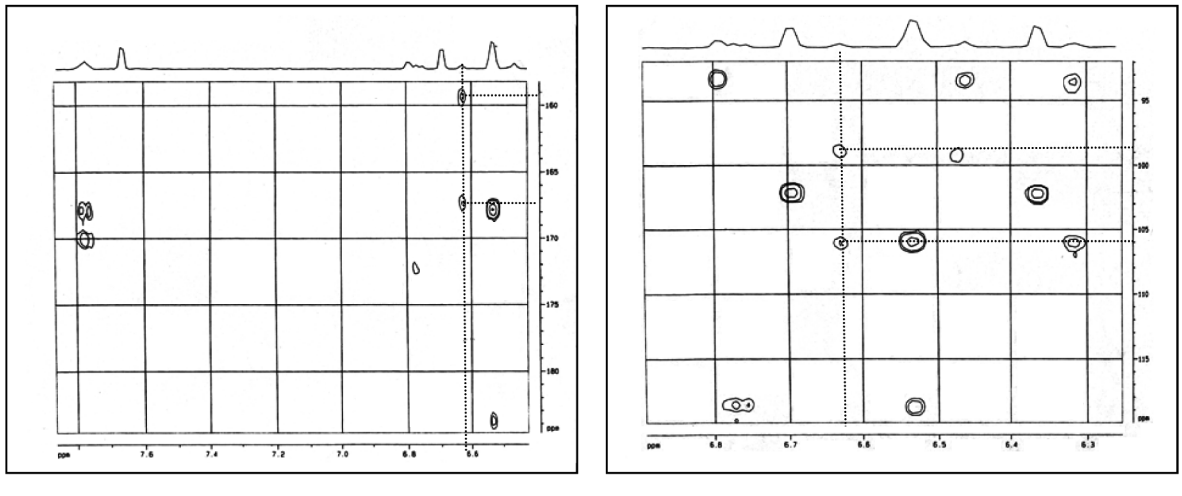


Figure 7. HMBC of genkwanin; correlations between H-8 and C-6, C-7, C-8a, C-4a.

molecular formula $C_{16}H_{12}O_5$. Flavone structure of genkwanin was revealed by comparison of ^{1}H-NMR with salvigenin and the position of H-3 at δ = 6.53. The ^{1}H-NMR spectrum exhibited one methoxy group (δ= 3.88, 3H, s), one set of meta coupled aromatic protons δ= 6.31(1H, d, *J* = 2.04 Hz) and δ= 6.63(1H, d, *J* = 2.04 Hz), two sets of ortho coupled aromatic hydrogens, δ= 7.78(2H, d , *J* = 8.8 Hz) and δ= 6.78 (2H, d, *J*= 8.8 Hz), and a non-coupled olefinic hydrogen at δ= 6.53 (1H, s) (Table2). Supporting evidence for the structure of genkwanin was provided by HMBC spectral data, which confirmed the position of methoxyl group at C-7 (Figure 3).Also H-3 showed cross peaks with C-1′, C-2, C-4, C-4a and H-8 showed HMBC correlation with C-8a, C-7, C-4a, C-6, confirming the position of these hydrogens (Figure 6, 7). The ^{13}C-NMR spectra showed the presence of twelve aromatic carbons, which comprise of 6 methines [δ= 93.3, 99.1, 129.2 (2CH), 116.3 (2CH)] and 6 quaternary carbons (Table 2).

Some biological activities of salvigenin and genkwanin have been reported in the literature. In a study by Cottiglia,etal. genkwanin showed antimicrobial and antifungal activities (13). Kamatou, et al. have screened extracts of seventeen *salvia* species used in traditional medicine in South Africa for their ability to inhibit the *in vitro* growth/proliferation of *Plasmodium falciparum* (FCR-3 strain) and the cytotoxic effects on three human cancer cells (breast adenocarcinoma, colon adenocarcinoma and glioblastoma). *Salvia radula* has displayed the most favorable activity. Two compounds have been subsequently isolated from the active fraction of *S.radula* and identified as betulafolientriol oxide and salvigenin. The IC_{50} value of 24.60 mg/mL has been reported for salvigenin against *Plasmodium falciparum* (14).

Brozic et al. have determined the inhibitory activity of some flavonoids on 17ß-hydroxy steroid dehydrogenase (17ß-HSD) type1 which converts estrone to estradiol. In this study genkwanin has demonstrated more than 80% inhibition of 17ß-HSD type1 activity at 6mM. 17ß-HSD type 1 represents an important target for the development of drugs for treatment of estrogen-dependent diseases (15).

Isolation and purification of other fractions of this plant is being carried out. Some of these fractions contain terpenoids or flavonoids that may be novel compounds.

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