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Extraction and identification of flavonoid in *Phlomis Olivieri* benth

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Abstract: *Phlomis olivieri* Benth is an important medicinal plant belonging to family Labiatae. Flavonoid 6,7-dimethoxy-2-phenyl-2,3-dihydro-4*H*-chromen-4-one was isolated from *in vivo* leaf of the species. The dried samples were separately soxhlet extracted in n-hexane. The structure of the extracts was determined by means of ^{1}H NMR, ^{13}C NMR, FT-IR, and UV techniques.

Keywords: Extraction, Isolation, Identification, Flovonoid, Phlomis *Olivieri*.

Introduction

Phenolic compounds (phenolic acids, flavonoids, and flavonoid polymers) are secondary metabolites, ubiquitous in the plant kingdom, that have been shown to impact human health (1-3). The U.S. public consumes as much as 250 mg of flavonoids per person per day (3) in a wide variety of forms (fruits, vegetables, nuts, drinks, spices, herbal and botanical supplements, and vitamin and mineral supplements). Accurate assessment of the relationship between ingestion of phenolic compounds and human health requires a food composition database to support clinical and epidemiological studies (4, 5).The large number of phenolic compounds, their structural diversity, the numerous dietary sources, the large variation in concentration, and the diversity of analytical methods presenta considerable challenge to developing a comprehensive database. Consequently, a systematic analytical approach is needed for the identification and quantification of flavonoids and other phenolic compounds in the U.S. food supply. Use of a standard screening method for phenolic identification will allow each analysis to contribute to a growing database rather than being just another isolated experiment.

The flavonoids comprise a large class of natural polyphenolic compounds (Table **1**), which are known to occur frequently in fruits and vegetables that are regularly consumed by humans.

Table 1. Subclasses of flavonoids

Class	Flavonoids
Flavonols	Quercetin, kaempferol, myricetin,isorhamnetin
Flavones	Luteolin, apigenin
Flavanones	Hesperetin, naringenin, eriodictyol, pentahydroxyflavanone
Flavans	Catechin, gallocatechin, epicatechin, epigallocatechin, dihydrokaempferol, dihydroquercetin, dihydromyricetin
Isoflavones	Daidzein, genistein, glycitein
Anthocyanidins	Cyanidin, delphinidin, malvidin, pelargonidin
Chalcones	Chacone, tetrahydroxychalcone

The genus *Phlomis* (Labiatae) comprises 17 species, which have been widely distributed in Azarbaijan, Fars, Giulan, Hamedan, Isfahan, Kurdestan, and Mazandaran provinces of Iran [6,7]. A lot of *Phlomis* species are used in herbal medicine, e.g. for treatment of respiratory tract diseases or externally for treatment

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of wounds [8]. Phlomis olivieri is a perennial plant, endemic in Iran [7–9]. Chemical composition of some *Phlomis* species has shown flavonoids, iridoids, phenyl-ethanoid glycosides, diterpeneglycosyl esters and nortriterpenes [8, 10–15].

At present, there are no scientific reports on the extraction of flavonoids from the leaves of *P. olivieri*. In this study, the optimal conditions to isolate a flavonoid derivative (**1**) of *P. olivieri* leave were used.

Results and discussion

The compound was isolated from the n-hexane extract. These results also suggested that the compound is a flavonoid derivative with a free hydroxyl group at C-5. In its electrospray mass spectrum, the molecular ion peak of the compound at m/z 300 [M+H] + corresponded to the molecular formula $C_{17}H_{16}O_5$. The UV spectrum exhibited absorption maxima at 272 and 334, suggesting that the compound belongs to the flavones family, unsubstituted at the 3-position. The IR spectra of the compound showed absorption bands for hydroxyl group(3488 cm–1) and methoxy group (1039 cm^{-1}). The ^{1}H NMR spectrum of the compound exhibited a signal at δ12.0 (1H, s), attributed to a chelated hydroxyl group. Further, two signals observed at δ 3.79 (3H, s) and δ 3.90 (3H, s) were due to a methoxy groups. The mass spectrum of the compound showed important mass peaks at m/z 300 [M+H]+, 285 [M –CH_3]+, 272 [M –CO]+, 270 [M –2XCH_3]+ and 247 [M –CO–CH3]+. The MS fragmentation pattern clearly indicated that two methoxy and one hydroxyl groups were attached to the ring-A. The UV spectrum of the compound, in the presence of aluminum chloride, remained unchanged upon the addition of hydrochloric acid, which confirmed the presence of a hydroxyl functional group at C-5. In view of these spectral data, the compound was identified as 6,7-dimethoxy-2-phenyl-2,3-dihydro-4*H*-chromen-4-one (Figure **1**).

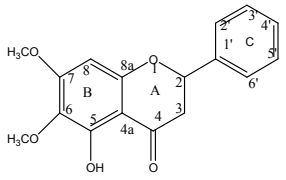

Fig. 1: 6,7-dimethoxy-2-phenyl-2,3-dihydro-4*H*-chromen-4-one

This structure was further confirmed by^{13}C NMR spectralstudies. The ^{13}C NMR spectrum of the compound showed a total of 15 signals for 17 carbons. A signal was observed at δ 198 and was allocated to C-4. Signals observed at δ 56.2 and 61 were ascribed to 2 methoxy groups at C-6 and C-7. All these spectral data were in good concurrence with those reported in the literature (16, 17).

Table 2. ^{1}H NMR (in $CDCl_3$, 400 MHZ) and ^{13} C NMR (in $CDCl_3$, 400MHZ)

Position	δ_H,ppm (J in Hz)	c(ppm)
1	--	-
2	5.48	80
3	2.8, 3.2	45
4	-	198
4a	-	104
5	-	159
6	-	131
7	-	161
8	6.12	91
8a	-	155
1'	-	138
2',6',4'	7.38	127.1
3',5'	7.49	128.9

Experimental

Materials and methods:

*General***:**

The industrial and analytical reagent grade solvent was used for extraction and column chromatography. Silica gel 60 and G-60 70-230 mesh ASTM (merck774) were used for column chromatography. Aluminium and support silica gel 60F_{254} were used for Thin Layer Chromatography and preparative TLC, respectively. The silica and plates were activated at 100^0 for one hour stored in a dessicator until needed. TLC spots were visualized under ultra-violet light (254 nm and 365nm) followed by spraying with the required spotting regent.

The ^{1}H NMR and ^{13} C NMR spectra were recorded in chloroform-D on a JOEL JNM-FX400. Chemical shift were report in ppm and the coupling constants were given in Hz.

The mass spectra were measured on a JMS 700 spectrometer using NBA as the matrix for FAB analysis. The Automass Thermofinnigan was used for HR $ESI^+$and ESI^- analysis. The EIMS spectra were obtained on Shimadzu GC-MS QP2000A spectrometer 70 eV.

The infrared spectra were obtained with chloroform as a solvent on a Perkin Elmer spectrum 2000-FT IR spectrometer. The UV spectra were measured on a UV visible recording spectrophotometer, Model Shimadzu UV-160A with ethanol as a solvent.

Plant Materials:

The plant was collected from region of Gadook of Mazandaran province in north of Iran and the species was identified, confirmed by a Botanist in department of Biology in Qaemshahr Azad University.

Extraction and Isolation Plant Material:

The extraction of the plants material was carried out by cold percolation or exhaustive extraction using the Soxhlet extractor. The milled dried leaves (450 g) of the plants were first defatted with hexane for 5 days at room temperature. The n-hexane extract was then dried on the rotary evaporator. The crude n-hexane plant was subjected to column chromatography over silica gel 60 as stationary phase. The solvent system used for chromatography was n-hexane with increasing portion od dichloromethane (gradient elution system). The ratio of the solvent between n-hexane and dichloromethane were (100:0, 99:1, 98:2, 96:4, 94:6, 90:10, 85:15, 80:20, and 50:50) and finally 100% Dichloromethane. Fractions were collected every 100 ml and each fraction was tested with aluminium TLC plate. In this regard, when the solvent evaporated under reduced pressure, a Colorless needle of compound **1** was obtained (2.3 g, 0.51%).

Colorless needles from n-hexane-CH_2Cl_2, mp 97^0C. EIMS m/z (rel. int.) 300 [M]+ (100). ^{1}H NMR ($CDCl_3$, 400 MH_Z): 5.48(1H, dd , J =12.1 and 3.3 Hz, H-2), 2.89 (1H, dd, J=17.2 and 3.4 Hz, H^A-3), 3.2 (1H, dd ,J=17.3 and 12.1 Hz, H^B3), 6.12 (1H, s, H-8), 7.38-7.49 (5H, m, H2'/3'/4'/5'/6'), 3.90 (3H, s, 6-OCH_3), 3.79 (3H, s,7-OCH_3), 12.0 (1H, s. 5-OH). ^{13}C NMR ($CDCl_3$, 400 MH_Z): 80 (C-2), 45 (C-3), 198 (C-4), 104 (C-4a), 159 (C-5), 131 (C-6),161 (C-7), 91(C-8), 155 (C-8a), 127 (C2', 4',6'), 128.9 (C-3',5'), 138 (C-1') , 56.2 (7-OCH_3), 61 (6-OCH_3) .

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