Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(1):389-393

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Isolation of flavonoids from *Indigofera heterantha* seeds

Taj Ur Rehman[1,2*], Ghias Uddin[1] and Khanzadi Fatima Khattak[1] and Wajiha Liaqat[2]

[1]*Department of chemistry, Abdul Wali Khan University, Mardan, KPK, Mardan, Pakistan*
[2]*Institute of Chemical Sciences, University of Peshawar, Peshawar, K.P.K,* Pakistan

---

ABSTRACT

The seeds of Indigofera heterantha yielded a flavonoid characterized as quercetin 3-O-α-L-rhamnoside along with known 3,5,7-trihydroxy-6,4´-dimethoxyflavone and 3,5,4´-trihydroxy-6,7-dimethoxyflavone. The isolated compounds were characterized by using modern spectroscopic techniques such as UV, IR, NMR and mass.

Key words: *Indigofera heterantha* seeds, Leguminosae, flavonoids

---

INTRODUCTION

Indigofera heterantha Wall, commonly known as Himalayan Indigo is a deciduous shrub. It is widely distributed in tropical region of the world. In Pakistan, genus *Indigofera* is represented by about 24 species [1]. A number of phytochemicals such as lignins, triterpenes, steroids, alkaloids, flavonoids, acylphloroglucinols, saponins, tannins, quinines, rutin, cafffeic acid, gallic acid, myricetin, galangin and quercetin have been reported from the genus [2-4]. Some species of this genus contains nitro group containing chemical compounds including 3-nitropropionate and other toxic substances which act as suicide in-activators of succinate dehydrogenase [2]. The current phytochemical investigation on the seed constituents of *Indigofera heterantha*, led to the isolation of three flavonids. Out of these three compounds, one is new source and the remaining are known.

EXPERIMENTAL SECTION

General procedure

The ultraviolet (UV) spectral analysis was carried out in methanol by using Hitachi U-3200 spectrophotometer. Similarly, JASCO 302-A infrared spectrometer was used for carrying out infrared (IR) spectral analysis. The ^{1}H and ^{13}C NMR spectra were obtained at (400, 500, 100 and 125 MHz on Bruker AM-400 and AMX-500) nuclear magnetic resonance spectrometers using tetramethylsilane (TMS) as internal reference. The Heteronuclear 2D ^{1}H-^{13}C chemical shift experiments were done at 500 MHz. The NOE difference measurements were carried out on Bruker A M-600. The electron impact (EI) mass spectra were conceded *via* Finnegan MAT 311 and 312 spectrometers, joined with PDP 11/34 computer system. Both the high resolution mass measurements (HR-MS) and fast atomic bombardment (FAB) mass measurements were recorded on Joel JMS HX 110 mass spectrometer using glycerol or thioglycerol as the matrix and cesium iodide (CsI) as internal standard for exact mass measurements. Column chromatography was conceded on silica gel (Si 60, 70-230 mesh, Merck) and the vacuum liquid chromatography (VLC) was done on (Si 60, GF_{254}, E. Merck) silica gel. The Flash column chromatography was carried out *via* (Si 60, 230-400 mesh, E. Merck) silica gel as adsorbent using Eyela Flash Chromatograph model known as EF-10, by the process of purification. In addition to it, (20×20, 0.5 mm thick; E. Merck) precoated silica

gel GF_{254} preparative glass plates were used for thin layer chromatography. Purity of samples were tested and observed on the same precoated plates, visualized by spray reagents.

Plant material
The seeds of *Indigofera heterantha* plant were collected during the month of May, 2009 from Lower Dir in Northern areas of Pakistan. The taxonomic identification of the plant was carried out by Dr. Samin Jan, Associate Professor, Department of Botany, Islamia College University, Peshawar, Pakistan. The voucher specimen (SJ-36) was deposited in the herbarium of the University.

Extraction and isolation
Air shade dried powdered seeds (22 kg) were subjected to extraction (x 3) with 5% aqueous methanol for one week. The combined extract was concentrated under reduced pressure by a vacuum rotary evaporator, to get brownish residue F1 (2.29 kg), which was further fractionated by using chloroform and water to yield F2 (41 g) of chloroform and F3 (1.6 kg) of water fractions. The chloroform fraction was partitioned into *n*-hexane and methanol fractions, which afforded FX1A (3 g) and FX1B (36 g) respectively, using soxhlet extractor. Water fraction was also partitioned with ethyl acetate (EtOAc), as a result FX3A (1 kg) of ethyl acetate fraction was obtained, which was further fractionated using ether: petroleum ether (2:1) and water to get three fractions, FX3AC (400 g), FX3AB (160 g) and residue fraction FX3AA (360 g).

RESULTS AND DISCUSSION

Quercetin 3-O-α-L-rhamnoside
Compound 1 was obtained as yellow powder and the EI-MS showed the molecular ion $[M]^+$ peak at m/z 448, corresponding to the molecular formula $C_{21}H_{20}O_{11}$. The IR spectrum exhibited hydroxyl (3523 cm^{-1}), conjugated carbonyl (1658 cm^{-1}) and aromatic (1604 cm^{-1}) absorption bands and the UV spectrum showed maximum absorption at 267 nm indicating the presence of conjugated system. The spectral data (Table-1) of the compound indicated its flavonoid nature, in which 15 carbon atoms ascribed to the aglycone and 6 to the sugar moiety. The ^{1}H NMR spectrum displayed three aromatic proton signals at δ_H 7.30 (1H, d, J = 1.7 Hz, H-2´), 7.29 (1H, d, J = 8.5 Hz, H-5´) and 7.32 (1H, dd, J = 8.5, 1.6 Hz, H-6´) suggested the 3′,4′-disubstitution pattern of the ring B while two weakly coupled doublet signals were assigned to two protons on C-6 and C-8 in the A ring. An anomeric proton signal at δ_H 5.33 attached to carbon at δ_C 103.5 confirmed the presence of glucose moiety in the nucleus. Furthermore, HMBC from the ^{1}H signal at δ_H 5.33 to carbon at δ_C 136.2 suggested that the C-3 position of flavonol was glycosylated in compound 1. A proton signal at δ_H0.93 (3H, d, J = 6 Hz) and carbon signal at δ_C 17.6 indicated that the glucose had a terminal methyl group. Based on the spectral data the structure of compound 1 was identified as Quercetin 3-*O*-*α*-L-rhamnoside [5-6].

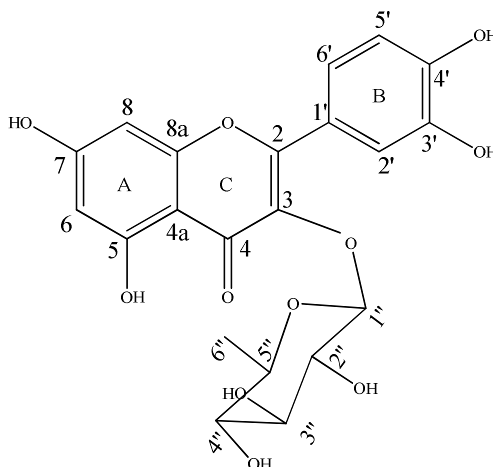

Table-1: ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of 1 in MeOD

C. No.	^{1}H NMR δ_H (*J* in Hz)	^{13}C NMR δ_C	Multiplicity
2	-	149.0	-C-
3	-	136.2	-C-
4	-	179.6	-C-
4a	-	105.7	-C-
5	-	158.5	-C-
6	6.94, (d, *J* = 1.4)	99.9	CH
7	-	166.2	-C-
8	6.35, (d, *J* = 1.2)	94.9	CH
8a	-	163.2	-C-
1´	-	122.9	-C-
2´	7.30, (d, *J* = 1.7)	122.8	CH
3´	-	116.3	-C-
4´	-	159.2	-C-
5´	7.29, (d, *J* = 8.5)	116.9	CH
6´	7.32, (dd, *J* = 8.5, 1.6)	122.9	CH
1″	5.33, (d, 7.7)	103.5	CH
2″	3.43, m	72.1	CH
3″	3.32, m	72.1	CH
4″	3.11, m	73.2	CH
5″	4.20, m	71.0	CH
6″	0.93, (d, *J* = 6)	17.6	CH_3

3, 5, 7-Trihydroxy-6, 4´-dimethoxyflavone

Compound **2** was purified as light yellow powder, and the EI-MS showed molecular ion $[M]^+$ peak at m/z 330 for $C_{17}H_{14}O_7$. The IR spectrum revealed absorption bands at 3313, 1642 and 1601 cm^{-1} for aromatic -OH, carbonyl group and aromatic double bonds, respectively. The UV spectrum showed maximum absorptions at 269 and 340 nm. The ^{1}H NMR spectral data (**Table-2**) for **2** showed 5,6,7-trisubstituted with two hydroxyl and one methoxy groups (ring A) while ring B is 4′-methoxylated. The spectral data were in accordance with those given in literature for 3,5,7-trihydroxy-6,4´-dimethoxyflavone [7-8].

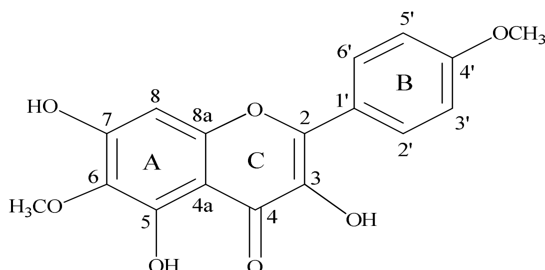

Table-2: ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of 2 in $CDCl_3$

C. No.	^{1}H NMR δ_H (*J* in Hz)	^{13}C NMR δ_C	Multiplicity
2	-	159.1	-C-
3-OH	12.50 br. s	134.1	-C-
4	-	176.9	-C-
4a	-	104.1	-C-
5-OH	12.50 br. s	152.3	-C-
6-OCH_3	-	132.9	-C-
6-OCH_3	3.90, s	55.8	CH_3
7-OH	12.50 br. s	151.2	-C-
8	6.67, s	93.5	CH
8a	-	148.3	-C-
1´	-	120.8	-C-
2´	8.14, (d, *J* = 8.7)	130.5	CH
3´	6.90, (d, *J* = 8.7)	114.7	-C-
4´-OCH_3	-	131.2	-C-
4´-OCH_3	3.70, s	55.1	CH_3
5´	6.90, (d, *J* = 8.7)	113.4	CH
6´	8.14, (d, *J* = 8.7)	130.5	CH

3,5,4´-Trihydroxy-6,7-dimethoxyflavone

Compound **3** was obtained as light yellow powder. The EI-MS displayed the molecular ion $[M]^+$ peak at m/z 330, corresponding to molecular formula $C_{17}H_{14}O_7$. The IR spectrum exhibited absorption bands at 3269 (OH), 1642 (C=O) and 1601 (aromatic C = C) cm^{-1}, while UV spectrum showed maximum absorptions at 263 and 368 nm. In the ^{1}H-NMR spectrum (**Table-3**), two aromatic signals at δ_H 8.23 (2H, d, J = 8.8 Hz, H-2´,6´) and 7.09 (2H, d, J = 8.8 Hz, H-3´,5´) indicated AA′BB′ spin system of the ring B, while an aromatic signal at δ_H 6.70 (1H, s) along with a low field singlet at δ_H 12.66 for the hydroxyl group indicated 5, 6, 7-substituted pattern of ring A.

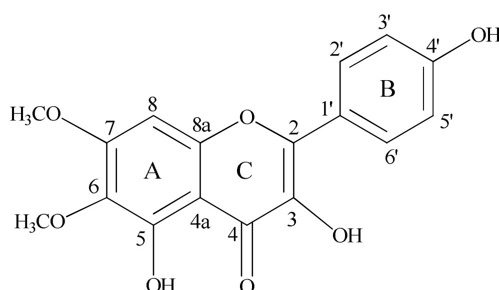

Table-3: ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of 3 in $CDCl_3$

C. No.	^{1}H NMR δ_H (J in Hz)	^{13}C NMR δ_C	Multiplicity
2	-	159.9	-C-
3-OH	12.66, br. s	133.5	-C-
4	-	176.7	-C-
4a	-	148.2	-C-
5-OH	12.66, br. s	152.3	-C-
6-OCH_3,	-	131.1	-C-
6-OCH_3	3.93, s	55.6	
7-OCH_3,	-	151.4	-C-
7-OCH_3	3.73, s	54.4	
8	6.70, s	94.8	CH
8a	-	102.1	-C-
1´	-	120.8	-C-
2´	8.23, (d, J = 8.8)	130.1	CH
3´	7.09, (d, J = 8.8)	114.4	CH
4´-OH	12.66, br. s	158.5	-C-
5´	7.09, (d, J = 8.8)	113.4	CH
6´	8.23, (d, J = 8.8)	130.1	CH

The spectral data (**Table-3**) were in accordance to the values given in literature for 3,5,4´-trihydroxy-6,7-dimethoxyflavone [9].

CONCLUSION

The isolated flavonoids were quercetin 3-*O*-*α*-L-rhamnoside along with known 3,5,7-trihydroxy-6,4´-dimethoxyflavone and 3,5,4´-trihydroxy-6,7-dimethoxyflavone. The structures were elucidated by UV, IR, MS and NMR spectral analysis. All compounds 1 to 3 displayed UV and IR absorption peaks, and ^{1}H and ^{13}C NMR signals (experimental) characteristic of a flavonoid nucleus. However to our knowledge, from the survey of literature Quercetin 3-*O*-*α*-L-rhamnosidewas previously unknown from *Indigofera heterantha* and hence its first isolation from this natural source.

Acknowledgements

The authors are thankful to the HEJ research institute of chemistry, university of Karachi, Pakistan, for providing lab facilities.

REFERENCES

[1] NE Ali; National Herbarium Agriculture Research "*Flora of West Pakistan", Council,* Rawalpindi., **1997**, (65), 100-106.

[2] AR Rehman; AR Malik; H Riaz; NA Ahmad; MI Choudhary. *Chem. Pharm. Bull.,* **2005**, (53), 63-68.

[3] TA Alston; I Mela; H Bright, *J. Nat. Proc. Acad. Sci. USA.,* **1977**, (74), 67-73.

[4] S Bakasso; AL Meda; CM Lamien; J Kiendrebeogo; A Millogo; OG Gouedraogo; *Biological Sciences,* **2008**, (11), 14-19.
[5] HM Yoon; JY Park; O MY; K Kim; J Han; WY Whang;. *Nat. Prod. Sci.,* **2005**, (11) 75-79.
[6] FP Zhao; S Dieter; B Alfred; R Subramaniam; N Goh; TF Chia; SN Tan; LS Chia, *Phytochemistry.,* **2003**, (62), 219-225.
[7] E Wollenweber; Z. *Naturforsch* **1971**, (26), 1188-1193.
[8] EI Wollenweber; WD Schober; G Clark; Yatskieych, *Phytochemistry.,* **1985**, (24), 2129-2135.
[9] L Quijano; F Malanco; Trios*Tetrahedron.* **2001**, (26), 308-314.