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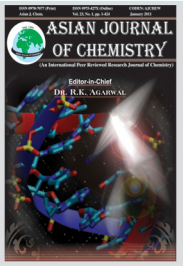

NOTE

Characterization of Flavonoid Aglycones in Aerial Parts of *Hypericum oblongifolium* L.

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The genus *Hypericum* is known worldwide for its traditional and modern uses. Several species of this genus have been used in folk medicine. Extracts from a number of species of the genus were found to possess antifungal, antibacterial, antimalarial, antioxidant and antidepressant activities. The main constituents of these extracts are reported to be phloroglucinols and flavonoids. *Hypericum oblongifolium* L. is an important representative of the genus in the northern areas of Pakistan used in traditional herbal medicine. The present paper describes a simple, fast, specific and precise method coupling high-performance liquid chromatography (HPLC) with UV detection for the separation and identification of six flavonoid aglycons in the extracts of aerial parts of *H. oblongifolium*. Quercetin, myricetin, rhamnetin, isorhanmetin, kaempferol and luteolin were used as standards. The compounds were separated on a prepacked analytical C_{18} column (250 mm × 4.6 mm, 5 µm particle size) using acetonitrile/water 1:1 acidified with 1 % acetic acid as mobile phase. The flow rate was kept constant at 1.0 mL/min at 25 °C and the peaks were detected at 254 nm. The flavonoid content was expressed as quercetin equivalent and the quantification was by peak area measurement. The results showed that the most abundant flavonoid aglycones were luteolin (0.368 mg/g dry wt.) and myricetin (0.322 mg/g dry wt.) while isorhamnetin was not detected in this species.

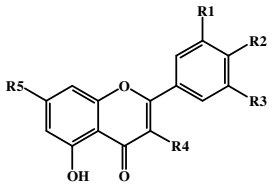
Key Words: *Hypericum oblongifolium*, Phloroglucinols, Flavonoids, Antibacterial, HPLC.

Flavonoids are one of the largest groups of known natural products. They are important secondary metabolites with widespread occurrence in plant kingdom. Over 6000 flavonoids have been identified to date, widely distributed in the leaves, seeds, bark and flowers of plants[1]. In plants, flavonoids have important roles in providing flower pigmentation to attract pollinators, in defending plants against pathogens, as signal molecules in plant-microbe interactions and in protecting plants from UV radiation[2,3]. In the recent years a number of studies have been carried out regarding the isolation and identification of flavonoids in plant material using different methods mainly chromatographic and spectroscopic. High performance liquid chromatography has proved to be the most convenient method which enables separation and identification of flavonoids using various detection systems[4-6]. As for the quantitative analysis, much data have been published in the last few years confirming the suitability of this technique for simultaneous determination of flavonoid compounds in various plant materials which gives an insight into the distribution of flavonoids in the studied material[7,8].

Hypericum oblongifolium is an evergreen shrub belonging to the family Hypericaceae. The plant commonly grows on Khasia Hill at an altitude of 5000-6000 m in China and in the Himalya hills[9]. A number of compounds have been detected from the plant such as flavonoids, xanthones and triterpenes[10,11]. Several plants of the genus *Hypericum* are well known for their medicinal properties and there are a number of reports on the use of these plants to treat infections in local system of medicine[12,13]. The crude methanol extracts of many species of *Hypericum* have been analyzed for antimicrobial activity against several microorganisms (bacteria and fungi). The prominent compounds in these extracts were tannins, flavonoids and phenolic acids[14]. Keeping this in view methanol extract of *H. oblongifolium* was evaluated for qualitative and quantitative analysis of flavonoid content by reverse-phase HPLC. Structures of the aglycons studied are depicted in Fig. 1.

All solvents used were purchased from Merck, Germany and were of analytical grade. Chemical standards for quercetin, luteolin, myricetin, rhamnetin, isorhamnetin, kaempferol and apigenin were purchased from Sigma-Aldrich. Acetonitrile, methanol and water used for HPLC analysis were HPLC grade purchased from Merck, Germany.

H. oblongifolium flowers and leaves were collected in the area between Murree and Abbotabad in July of 2008. The plants were identified by Dr. Mir Ajab Ali Khan, Professor



Flavonoids	R1	R2	R3	R4	R5
Quercetin	OH	H	H	OH	OH
Myricetin	OH	OH	OH	OH	OH
Isorhamnetin	OMe	OH	H	OH	OH
Rhamnetin	OH	OH	H	OH	OMe
Kaempferol	H	OH	H	OH	OH
Luteolin	H	OH	OH	H	OH

Fig. 1. Chemical structures of selected flavonoids

Department of Biological Sciences, Quaid-e-Azam University, Islamabad and the specimen deposited in the Prem Madan Herbarium of Lahore College for Women University, Lahore (Specimen Voucher No. PM# 0132).

Extraction procedure: The plant material (1 Kg) was air-dried at room temperature. The dried material (400 g) was grinded into small pieces 2-6 mm by using a crushing machine. The powdered plant material was extracted with 80 % methanol at room temperature for 15 days with occasional stirring. The solvent was evaporated under reduced pressure in a rotary evaporator to give a gummy residue (52 g).

HPLC analysis

Sample preparation: A weighed amount of the crude extract was dissolved in HPLC grade methanol to give a concentration of 100 μg mL^{-1}. All solutions were filtered through a 0.45 μm filter before HPLC analysis.

Standards: Stock solutions of the standard flavonoids were prepared in HPLC grade methanol at a concentration of 100 μg mL^{-1} and stored in a refrigerator at -20 °C until use. All solutions were filtered through a 0.45 μm filter before HPLC analysis.

The HPLC system (Waters) equipped with a pump (1500 series), a UV detector (2487), a column oven and a reversed-phase, prepacked C_{18} column (250 mm × 4.6 mm, 5 μm particle size) was used for qualitative and quantitative analysis of flavonoids. The mobile phase used was acetonitrile/water 1:1 acidified with 1 % acetic acid. The flow rate was kept constant at 1.0 mL/min at 25 °C and throughout the experiment all injection volumes were 10 μL. The peaks were detected at 254 nm and were initially identified by direct comparison of the retention times with those of standards. Standard solution was then added to the sample and peaks were identified by the observed increase in their intensity. This procedure was performed separately for each standard. The flavonoid content was expressed as quercetin equivalent and the quantification was by peak area measurement of the HPLC chromatograms from the 3 replicate samples.

The result of qualitative and quantitative analysis of flavonoids in the crude methanol extracts of *H. oblongifolium* species is summarized in Table-1. Only limited data is available on the phytochemical analysis of the plant studied. This study is the first report on the flavonoid content of the species using HPLC as the chromatographic technique and shows its valuable chemical composition justifying its use in traditional medicine. The method developed for HPLC analysis provided a quick analysis of the crude methanol extract in minimum run time (7 min). This is important especially in order to optimize equipment use and reduce solvent consumption. The compounds were identified by comparing the chromatogram for the crude methanol extract with the chromatogram of the six reference standard compounds obtained under similar experimental conditions.

TABLE-1
CONTENTS OF FLAVONOIDS (mg/g dry weight) IN CRUDE METHANOL EXTRACTS OF *Hypericum oblongifolium*s

Flavonoid	Retention time (min)	Flavonoid content
Quercetin	2.059	0.192
Myricetin	1.787	0.322
Isorhamnetin	2.740	Not detected
Rhamnetin	3.750	0.312
Kaempferol	2.639	0.126
Luteolin	1.966	0.368

Flavonoids, present in plants, generally occur as flavonoid aglycons and flavonoid glycosides. The method most commonly employed for phytochemical analysis, involves the hydrolysis of the crude extracts prior to investigation for their flavonoid contents to get maximum aglycones available. However, this does not give the information regarding the aglycons naturally present in the plant material studied. In the present study the methanol extract of *H. oblongifolium* was analyzed for its flavonoid aglycons without hydrolysis to determine the aglycons naturally occurring in the plant. The major flavonoids were myricetin (0.322 mg/g dry wt.), rhamnetin (0.312 mg/g dry wt.) and luteolin (0.368 mg/g dry wt.) while isorhamnetin was not detected in the extract.

ACKNOWLEDGEMENTS

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