Kaempferol-3-O-α-L-glucosyl (1→2) rhamnoside from *Hymenophyllum crispatum*

[1]Rachana Mishra[*] and [2]D. L. Verma,
[1&2]Department of Chemistry, Kumaun University,
SSJ Campus, Almora-263601, (Uttarakhand) India.
[*]Email: 09411102476m@gmail.com
Phone Numbers: +91-9411102476[1], +91-5962-233849(R)[2]

Abstract: *Hymenophyllum crispatum*, Wall (family Hymenophyllaceae), is a Filicinae fern of leptosporangiate group. It is widely distributed in sub-alpine regions of Kumaun Himalaya. For the present chemical investigation, *H. crispatum* was collected from the forest vegetation near to the timber line of Pindari glacier of Kumaun, Uttarakhand (India). Various members of the genus *Hymenophyllum* have previously been screened for anti-microbial activities. *H. crispatum*, a species native to Himalaya has still not been investigated for various biological activities and active constituents. Present communication reveals the isolation of a flavonol-di-O-glycoside from the fern fronds of *H. crispatum*. About 1kg air dried and powdered sample of *H. crispatum* was extracted sequentially with 80% aq. MeOH and 50% aq. MeOH. Both the extracts were combined and concentrated under reduced pressure until only small H_2O layer (approx.50ml) remained. It was partitioned with CH_2Cl_2 and BuOH successively. The BuOH fraction was chromatographed on Whatman No. 3 PC using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as an eluent. After inspecting dried and developed chromatograms with UV light (360nm) a broad dark purple fluorescent band was observed. It was cut and eluted separately. The residue of the band was further chromatographed on Sephadex LH-20 column using 50% aq. MeOH as an eluent. Three flavonol-3-O-glycosides were isolated and identified. Out of these three glycosides, quercetin-3-O-ά-L-rhamnoside and quercetin-3-O-rutinoside were identified by CoPC with their standards and kaempferol-3-O-ά-L-glucosyl (1→2) rhamnoside was identified by color reactions, UV, ^{1}HNMR and hydrolytic methods. [Nature and Science. 2009;7(6):82-85]. (ISSN: 1545-0740).

Keywords: *Hymenophyllum crispatum,* flavonol-di-O-glycoside, active constituents.

Introduction

Hymenophyllum, genera of Filicinae fern of leptosporangiate group, is well known as a filmy ferns of family Hymenophyllaceae. It is a native to sub-alpine regions of Kumaun Himalaya and is widely distributed in moist shady places from 2000m-3800m. *H. crispatum* is a tribal folk medicinal plant of Kumaun Himalaya and various tribal inhabitants of the region use the plant extract for curing cough, bronchitis, asthma, wound healing and ulcers (Pande, 1992). Looking on the traditional medicinal significances of fern, the BuOH fraction of the fern was investigated chemically for flavonol-3-O-oligosacharide. The extracts derived from other medicinal plants have widely been investigated for various biological activities (Khetwal and Verma, 1983, 1984, 1986, 1990; Khetwal *et al.*, 1985, 1986; Mishra and Verma). Flavonoidal compounds, polyphenolic heterocyclic compounds, form a major family of natural products. Such compounds isolated from plants have widely been used for curing cancer, coronary dysfunction, inflammation, rheumatic arthritis, immune system decline, brain dysfunction and cataracts (Middleton *et al.*, 2000; Havsteen, 2002).

Material and methods

1. Authentification of fern species: *H. crispatum* Wall was collected from Kaphani and Sunderdhunga glaciers of Kumaun in the month of August. The Voucher specimen of fern was identified by Prof. P. C. Pande, Department of Botany, Kumaun University, SSJ campus, Almora (Uttarakhand) India, and deposited in the Department of Chemistry (Vouch. Sp. No.42).

2. Method of extraction: About 1kg air dried and powdered sample of *H. crispatum* was extracted sequentially with 80% aq. MeOH and 50% aq. MeOH. Both the extracts were combined and concentrated under reduced pressure in Rota-evaporator at 40^0C. The residue was fractionated between 50% CH_2Cl_2. The CH_2Cl_2 layer was separated and H_2O layer partitioned with BuOH. The BuOH Soluble was evaporated to dryness and residue was dissolved in MeOH and it was banded on Whatman No. 3 PC (10 sheets) using BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer) as an eluent. After three times repeated development

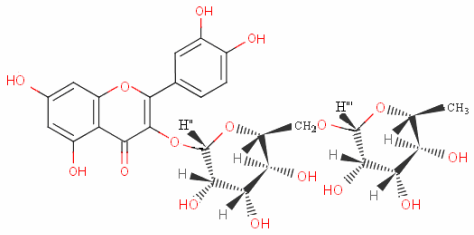
of PC with BAW, a dark purple fluorescent band was observed between two blue fluorescent bands on Pc with UV light. The broad dark purple fluorescent band was cut and eluted separately with 70% aq. EtOH. The aq. ethanolic elute was evaporated to dryness and residue was chromatographed on sephadex LH-20 CC using 20% aq. MeOH as an eluent. On eluting CC with 20% aq MeOH, three purple fluorescent bands were observed on PC with UV light of which the middle band was eluted separately from CC by monitoring with UV light. The aq. methanolic elute was evaporated to dryness. The residue was dissolved in 80% aq. MeOH and it was chromatographed on whatman No.1 PC using 30% HOAc as an eluent. Two purple fluorescent bands were observed on PC with UV light and were eluted with 70% aq. MeOH separately by monitoring PC with UV light.

Results and discussion

The slower moving compound was identified as quercetin-3-O-rutinoside (A) by CoPC with its standard using three solvent systems, BAW (n-BuOH-AcOH-H_2O, 4:1:5, V/V, upper layer), 30% HOAc and BEW (n-BuOH-EtOH-H_2O, 4:1:2.2, upper layer). It was finally confirmed by ^{1}HNMR in DMSO-d_6, 500 MHz (table [1]):

Table [1]: ^{1}HNMR of compound (A) in DMSO-d_6, 500MHz

Shift (δ)	Multiplicity	Identification
6.20	1H, d, J=2.0Hz	H-6
6.40	1H, d, J=2.0Hz	H-8
6.89	1H, d, J=8.5Hz	H-5'
7.20	1H, dd, J=8.5 and 2.0Hz	H-6'
7.35	1H, d, J=2.0Hz	H-2'
5.28	1H, d, J=7.5Hz	Anomeric proton glucose
4.38	1H, d, J=1.0Hz	rhamnosyl anomeric proton
3.0-4.20	10H, m	For remaining sugar protons of gluco.&rham.



(A) Quercetin-3-O-rutinoside

The faster moving compound (B) gave an amorphous grey powder, mp 210^{0}C. The molecular formula of compound was deduced as $C_{27}H_{29}O_{15}$ from FABMS (-ve). The compound gave positive tests with $FeCl_3$, Mg+HCl and α-naphthol, indicating a polyphenolic heterocyclic glycoside (Harborne, 1967). It appeared as a dark purple fluorescent on PC with UV light and changed to yellow-green with NH_3 vapours, indicating presence of hydroxyls at C-4' and C-5 (Mabry *et al.*, 1970).

When cellulose TLC of the compound was sprayed with methanolic solution of NA reagent, the purple fluorescence of compound turned to yellow, indicating absence of ortho-di-hydroxyl group in B-ring and presence of 4'-OH group (Homberg and Geiger, 1960; Geiger and Homberg, 1963). The purple fluorescence of compound changed into fluorescent yellow-green with $AlCl_3$ and $ZrOCl_2$, indicating presence of 5-OH group in the A-ring (Feigl, 1960). Thus, on the basis of color reactions, the flavone has free hydroxyls at C-4' and C-5.

Acid hydrolysis of the compound with 2N-HCl at 100^{0}C for an hour, gave an aglycone, kaempferol and glucose and rhamnose.

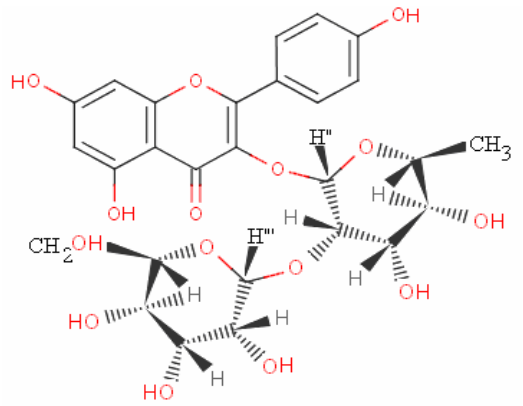
The aglycone was identified by ^{1}HNMR (DMSO -d_6, 400Hz): ^{1}HNMR showed two meta coupled doublets at δ 6.20 (1H, d, J=2.0Hz) and δ 6.33 (1H, d, J=2.0Hz) assignable to H-6 and H-8 of A-ring. Two symmetrical doublets, each with J=9.0Hz, appeared at δ 6.94 and δ 8.02 representing H-3', 5' and H-2', 6' of B-ring. A broad singlet at δ 12.5 indicated presence of free OH at C-5. Thus, the aglycone was identified as kaempferol. The acid hydrolysed sugars, glucose and rhamnose were identified by respecttive CoPC with their standards. UV spectra of compound (B) in MeOH at (λ_{max}, nm) at 268, 366 and shifts obtained with diagnostic reagents indicated the compound is a kaempferol-3, 7-di-O-glycoside.

The compound (B) was hydrolysed with the emulsin derived from almonds, gave a compound, kaempferol-3-O-α-L-rhamnoside (CoPC). The compound (B) gave dark purple fluorescence on PC with UV light while its acid hydrolysed aglycone gave dull yellow fluorescence on PC, indicating release of sugar moiety from C-3. Further the compound (B) was identified by ^{1}HNMR spectra in DMSO-d_6, 400MHz (Table [2]):

Table [2]: ^{1}HNMR of compound (B) in DMSO-d_6, 400MHz

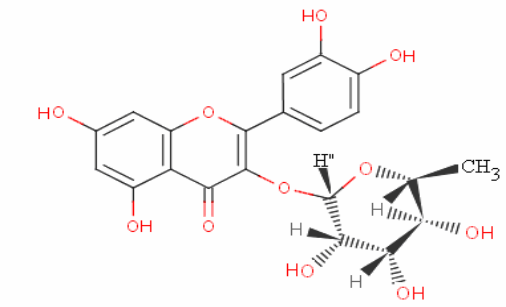
Shift (δ)	Multiplicity	Identification
6.22	1H, d, J=2.0Hz	H-6
6.43	1H, d, J=2.0Hz	H-8
6.86	2H, d, J=9.0Hz	H-3', H-5'
8.05	2H, d, J=9.0Hz	H-2', H-6'
5.56	1H, d, J=7.5Hz	H-1"rham.
4.19	1H, d, J=7.5Hz	H-1"' gluco.
4.10	1H, d, J=2.0Hz	H-2"rham
3.0-3.56	10H, m	For remaining sugar protons of gluco. & rham.
12.50	1H, s	5-OH

The downfield shift of H-2''of rhamnose indicated the terminal glucose and is attached with C-2''of rhamnose. Further, the compound (B) was identified as kaempferol-3-O-α-L-glucosyl (1→2) rhamnoside by CoPC with its authentic isolated from the leaves of *Ginko biloba* (Markham and Geiger, 1992).



(B) Kaempferol-3-O-α-L-glucosyl (1→2) rhamnoside

The slower moving component on PC, a dark purple fluorescent band derived from the fractionation of BuOH soluble on PC (Whatman No. 3) with BAW (4:1:5, V/V, upper layer) solvent gave a single compound (C). It was purified on Sephadex LH-20 column using H_2O-MeOH (60: 40) and identified as quercetin-3-O-α-L-rhamnoside by CoPC with its authentic.



(C) Quercetin-3-O-α-L-rhamnoside

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Correspondence to: [1]Mrs. Rachana Mishra, Department of Chemistry, Kumaun University, S. S. J. Campus, Almora – 263601, Uttarakhand (India).
[2]Dr. D. L. Verma, Associate Professor, Department of Chemistry, Kumaun University, S. S. J. Campus, Almora – 263601, Uttarakhand (India).

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