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5-O-Glycosylated Flavonols from *Cheilanthes grisea*

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Abstract: *Cheilanthes grisea* Blanford, a rare fern of Kumaun hills, is a member of psinopteridacea family of leptosporangiate group of ferns. The fern fronds of *Cheilanthes grisea* (Voucher species No. 21) was collected from Pithoragarh district of Kumaun hills, Uttarakhand (India) altitude ranging 2800-3000m. About 500gm air dried fern fronds were extracted sequentially with 80%MeOH and 60%MeOH by cold percolation methods for six days. The two extracts were combined and concentrated under reduced pressure until only H_2O layer remained. It was partitioned with CH_2Cl_2:H_2O (1:1). The CH_2Cl_2 soluble was evaporated to dryness and residue was chromatographed on cellulose CC using 50%HOAc as an eluent. A blue fluorescent band observed between two dark purple fluorescent bands on CC with UV light. It was eluted and collected separately by monitoring CC with UV light. The eluent of blue fluorescent band was concentrated and chromatographed on Whatman No. 3 PC using 30%HOAc as an eluent. Three blue fluorescent bands observed on PC, representing Frac-1, Frac-2 and Frac-3 at Rf 45, 52 and 56 were eluted separately and gave three flavonoidal compound [1], [2] and [3] respectively. On the basis of chromatographic behaviour, hydrolytic method (acid, enzymatic and HI), H_2O_2 oxidation, UV, MS and ^{1}HNMR studies, compounds [1], [2] and [3] were identified as quercetin-3-OCH_3-5-O-β-D glucoside, kaempferol-3-OCH_3-5-O-β-D-glucoside and quercetin-3, 4'-dimethyl ether-5-O-β-D glucoside respectively. [New York Science Journal. 2009;2(5):93-95]. (ISSN: 1554-0200).

Keywords: *Cheilanthes grisea*, rare species, Kumaun Himalaya

Introduction

Cheilanthes Swartz, a group of leptosporangiate ferns of family psinopteridaceae, distributed widely in temperate and humid regions of Indian Himalayas. Nine species of *Cheilanthes* have been reported from the hills of central Himalayas (Pande, 1990). Various species of *Cheilanthes* have widely been recommended as medicines of traditional uses (Chopra *et al.,* 1958).

Therefore, *Cheilanthes* species have been screened for various biological activities (Banerjee and Sen, 1980). *C. grisea* Blanford is a rare species of Kumaun Himalaya. Literature survey revealed that the species of fern has neither been investigated for biological activities nor for active constituents. Present communication reveals the isolation and identification of flavonoidal compounds from *Cheilanthes grisea.* 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).

Material and method

Cheilanthes grisea Blanford, family psinopteridacea, was collected from the hills of Pithoragarh district (Uttarakhand), altitude ranging, 2800m to 3000m. The authentification of species was made by Prof. P. C. Pande, Department of Botany, Kumaun University, SSJ Campus, Almora, Uttarakhand (India). Its voucher species No. 21 has been deposited in the Chemistry Department of Kumaun University SSJ Campus, Almora, Uttarakhand (India).

Extraction and isolation of flavonoidal compounds

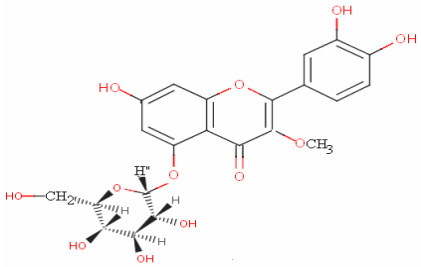
About 500gm of fern fronds of *C. grisea* was extracted sequentially with 80%MeOH and then 60%MeOH by cold percolation methods for six days. Two extracts were combined and reduced pressure until H_2O layer remained. It was partitioned with CH_2Cl_2: H_2O (1:1). The CH_2Cl_2 fraction was chromatographed on cellulose CC using 50%HOAc as an eluent. A broad blue fluorescent band was observed on CC under UV light. It was eluted separately. The eluent was dried under reduced pressure and residue was further chromatographed on Whatman No. 3 PC using 30%HOAc. Three blue fluorescent bands were observed on PC at Rf, 45, 52 and 56, representing Frac-I, II and III. Each fraction was cut and eluted separately gave

compounds [1], [2] and [3] respectively. The eluent of each fraction was re-chromatographed in BAW and finally purified on Sephadex LH-20 CC.

Results and discussion

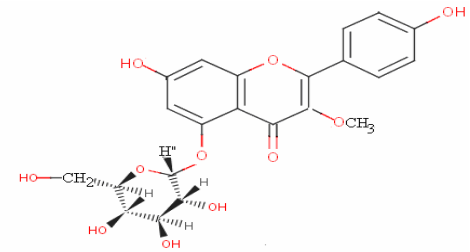
A blue fluorescent band derived from 30%HOAc fractionation of CH_2Cl_2 soluble on cellulose CC, afforded three compounds [1], [2] and [3]. They were isolated by the RPPC followed by their final purification on Sephadex LH-20 CC. The compound [1] a blue fluorescent on PC, gave a molecular ion at m/z 447 $[M-H]^-$ in FABMS (-ve) calculated for $C_{22}H_{22}O_{12}$ and other prominent ion observed at m/z 315 $[M\text{-glucosyl}]^-$, represent an aglycone. The blue fluorescent spot turned to orange after spraying with methanolic solution of Naturstoffreagenz A (NA) reagent, indicating the presence of ortho-di-hydroxyl group in the B-ring. UV spectra of compound [1] in MeOH ($\lambda_{max,}$ nm) gave two absorption bands at 255 (band II) and 353 (band I) and shifts obtained with diagnostic shift reagents, NaOMe (267, 401); NaOAc (268, 379); NaOAc+H_3BO_3 (260, 372); $AlCl_3$ (245, 378) and $AlCl_3$+HCl (251, 352), indicating the presence of free hydroxyls at C-3', C-4' and C-7 (Markham, 1982). The positive $AlCl_3$ shift which returned to neutral on addition of HCl indicated that the 5-hydroxyl was substituted (Mabry *et al.*, 1970).

Acid hydrolysis of [1] gave an aglycone, [1(a)] and glucose (CoPC). The aglycone was identified as quercetin-3-OCH_3 by MS studies: Mass spectra indicated the values m/z at 316$[M]^+$ 100%, 315(71%), 301$[M\text{-}CH_3]^+$, 298$[M\text{-}H_2O]^+$, 287$[M\text{-}HCO]^+$, 285$[M\text{-}OMe]^+$, 273$[M\text{-}CoMe]^+$, 153$[A+H]^+$ 137$[B_2]^+$. Hydrolysis of compound [1] with HI in presence of $NaHSO_3$, gave quercetin (CoPC). Finally, the compound [1] was identified as quercetin-3-OCH_3-5-O-β-D-glucoside by ^{1}HNMR studies in DMSO-d_6, 400 MHz: ^{1}HNMR showed five signals in aromatic region at δ 6.40 (1H, d, J=2.0Hz), δ 6.70 (1H, d, J=2.0Hz), δ 6.90 (1H, d, J=8.5Hz), δ 7.50 (1H, dd, J=8.5 and 2.0Hz) and δ 7.69 (1H, d, J=2.0Hz) attributed to H-6, H-8, H-5', H-6' and H-2' respectively of quercetin. The anomeric proton appeared as doublet at δ 4.78 (J=7.2Hz) and remaining protons of sugar appeared as multiplet at δ 3.0-4.20. A singlet appeared at δ 3.86 (3H, s), assignable to OCH_3 group attached at C-3.



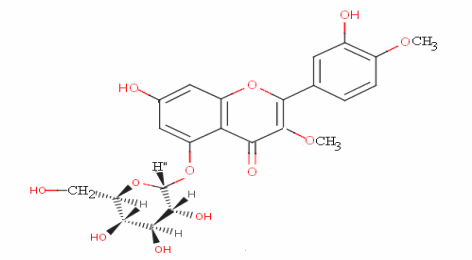
Quercetin-3-OCH_3-5-O-β-D-glucoside

The compound [2], a blue fluorescent on PC under UV light, gave a molecular ion m/z at 461$[M-H]^-$ in FABMS (-ve) and other prominent ion observed at 299$[M\text{-glucosyl}]^-$. On the basis of color reactions, compound [2] has free hydroxyls at C-4' and C-7 (Markham, 1982). The positions of free hydroxyls have also been supported by UV spectra in MeOH ($\lambda_{max,}$ nm): MeOH (257sh, 341); NaOMe (267sh, 319, 395); NaOAc (269, 311,376); NaOAc+H_3BO_3 (257sh, 343); $AlCl_3$ (256sh, 340) and $AlCl_3$+HCl (257sh, 341), indicating a flavone with disustituted at C-3 and C-5 (Mabry *et al.*, 1970). Acid hydrolysis of compound [2], gave kaempferol-3-O-CH_3 (CoPC) and glucose (CoPC). In ^{1}HNMR the sugar region of the compound [2] was found similar to the corresponding sugar region of compound [1]. Thus, the compound [2] was identified as kaempferol-3-O-CH_3- 5-O-β-D-glucoside.



Kaempferol-3-OCH_3-5-O-β-D-glucoside

FABMS (-ve) of compound [3] gave a molecular ion m/z at 491 $[M-H]^-$ and prominent ion observed at 329 $[m/z\ 491\text{-glucosyl}]^-$, represent an aglycone. Acid hydrolysis of compound [3], gave quercetin-3, 4'-dimethyl ether and identified by ^{1}HNMR studies in DMSO-d_6, 400 MHz: ^{1}HNMR showed four signals in aromatic region at δ 6.18 (1H, d, J=2.0Hz), δ 6.38 (1H, d, J=2.0Hz), δ 7.08 (1H, d, J=9.0Hz) and 7.50-7.60 (2H, m for H-2' and H-6'). Two singlet at δ 3.87 (3H, s) and 3.96 (3H, s) represent OCH_3 groups attached and C-5 position. Thus, aglycone was identified as quercetin-3, 4'-di-OCH_3 (CoPC). ^{1}HNMR studies in DMSO-d_6, 400MHz of compound [3] gave δ 6.63(1H, d, J=2.0Hz), δ 6.75 (1H, d, J=2.0Hz), δ 7.09 (1H, d, J=9.0Hz) and δ 7.50 to 7.61 (2H, m) represent H-6, H-8, H-5', H-2' and H-6' respectively. The anomeric proton singlet appeared at δ 4.80 (1H, d, J=7.5Hz). Thus, the compound [3] was identified as quercetin-3, 4'- dimethyl ether-5-O-β-D- glucoside.



Quercetin-3, 4'- dimethyl ether-5-O-β-D- glucoside

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