3-Methoxy Flavones From *Cheilanthes Bicolor*

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ABSTRACT: ***Cheilanthes bicolor*** (*Roxb),* a rare fern of Kumaun hills, is a member of psinopteridacea family of leptosporangiate group of ferns. The fern fronds of *cheilanthes bicolor* (Voucher Species No. 12) collected from Ranikhet and Jageshwar sites of Almora Uttarakhand (India). The air dried and powdered sample (1 kg) of botanically identified species of cheilanthes bicolor (VS No-12) was extracted with aqueous methanol (1:1) by cold percolation method for 6 days. The major part of aqueous methanolic extract was evaporated to dryness under reduced pressure in rota evaporator to dryness at 65^0c until only H_2O layer (approx 50ml) remained. It was partitioned with dichloromethane (50 ml) after separation of CH_2Cl_2 soluble (lower layer) the Upper layer of H_2O further partitioned with n-BuOH. In order to catalog all the flavoniods present in the aerial parts of cheilanthes bicolor, 2 DPC was applied by fresh aqueous methanolic extract on whatman No-1 paper using BAW (n-BuOH-AcOH-H_2O-4:1:5 v/v, Upper layer) and 30% ACOH solvent system.After developing chromatogram the spots were studied in visible and UV light both with and without the presence of ammonia vapour and after spraying with NA reagent.On the basis of UV, MS and ^{1}HNMR the two compound (1) with Rf value (83) kaempferol 3, 5 dimethyl ether and compound (B) with Rf value (81) kaempferol -3- methyl ether. [Nature and Science 2010;8(10):369-371]. (ISSN: 1545-0740).

Keywords: *Cheilanthes bicolor* rare species, Kumaun Himalaya

Introduction

Cheilanthes Swartz, a group of leptosporangiate ferns of family psinopteridacea, 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 cleilanthes have widely been recommended as medicines of traditional uses (Chopra etal, 1958). Therefore, cheilanthes species have been screened for various biological activities (Banerjee and Sen 1980). C. bicolor (Roxb) 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 bicolor. The extract derived from other medicinal plants have widely been investigate for various biological activities (Khetwal and Verma, 1983, 1984).

Material and Method

cheilanthes bicolor (Roxb) family psinopteridaceae was collected from the hills of Uttarakhand Ranikhet and Jageshwar sites of Almora. 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. 12 has been deposited in the Chemistry Department of Kumaun University, SSJ Campus, Almora Uttarakhand (India).

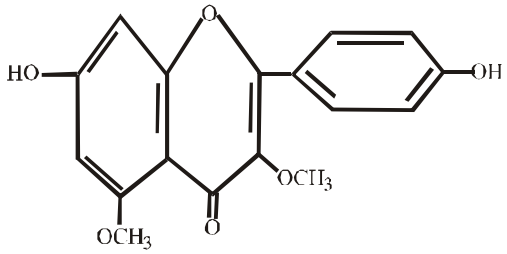
Extraction and Isolation of Flavonoidal Compounds

The air dried and powdered sample (1 kg) of botanically identified species of cheilanthes bicolor (VS No-12) was extracted with aqueous methanol (1:1) by cold percolation method for 6 days. The extract was partitioned with dichloromethane. The dicholoromethane fraction was evaporated to dryness and dissolved in MeOH and the methanolic extract was analysed by 2 DPC on whatman No-1 paper using BAW (n-BuOH-AcOH-H_2O-4:1:5 v/v, Upper layer) and 30% AcOH solvent system.After developing chromatogram the spots were studied in visible and UV light both with and without the presence of ammonia vapour. On inspecting developed and dried 2DPC with UV light (360 nm) the compound (A) at Rf value (83) appeared as fluorescent blue

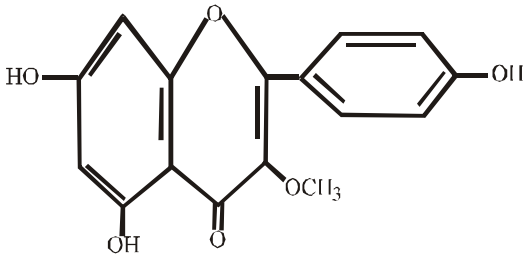
and at Rf (81) dark purple representing compound B respectively. The eluent of each fraction was re chromatographed in BAW and finally purified on Sephadex LH-20 cc.

RESULTS AND DISCUSSION

A blue fluorescent band derived from 30% HOAC , isolated by the RPPC followed by their purification on Sephadex LH-20 CC.The compound (A) a blue fluorescent on PC and faster moving component at Rf (83) in (BAW) was isolated from dicholoromethane fraction of aqueous methanolic extract of aerial parts of cheilanthes bicolor. The methanolic solution of the compound (A) gave positive colour reaction with $FeCl_3$ and Mg + HCl and negative response to α-napthol indicating a flavonoid aglycone. Its blue fluorescent spot on cellulose TLC turn to yellow with NH_3 supporting a flavonol compound with free hydroxyl group at 4` and hydroxyl group at 3 and 5 positions are substituted. The MS of compound (A) exhibited a molecular formula $C_{17}H_{14}O_6$ in accord with a flavone containing two hydroxyls and two methoxyl groups. Finally the structure of compound (A) is identified by `HNMR studies in DMSO – d_6 (400 MHz). δ6.35 (1H, d=2.0) for H-6, δ6.48 (1H, d=2.0) for H-8, δ6.92 (2H, d=8.5) for 3`/5`and three signals δ7.88 (2H, d=8.5) for H-2`/6` and δ3.83 (3H, S), δ3.70 (3H, S) for H-2`/6`, OCH_3 at 3 and 5 respectively. The compound (A) identified as Kaempferol – 3, 5, Dimethyl ether



The compound (B), a dark purple fluorescent on paper chromatogram at Rf (81) (BAW) under UV light was isolated from Dicholoromethane fraction of aqueous methanolic extract of the fern fronds of cheilanthes bicolor.EIMS of the compound gave a molecular ion m/e at 300 (100%) $[M^+]$ and other prominent ions are m/e 299, 137 $[M-H]^+$, 121 $[B_2^+]$and 105 $[B\text{-}COCH_3]^+$ indicating a flavonoid compound with three hydroxyl groups and one mthoxyl group. On cellulose TLC of the compound sprayed with NH_3 and NA reagent, $ZrOCl_2$ reagent the dark purple fluorescence of compound (B) turn to yellow indicating a flavone with 4`, 5, free hydroxyl group and a hydroxyl group at 3 – position is substituted. The methanolic solution of compound gave green colour with $FeCl_3$ and pinkish red colour with Mg + HCl and vanilline + HCl supporting a flavonoid compound 5, 7 dihydroxyl system in the A ring (Hillis and Urbach 1958) (Dean 1963, Geissman 1953). On the basis of colour reactions the hydroxyl groups are substituted at the position C-4`, C-5 and C-7.`HNMR of compound in (DMSO – d_6) gave two coupled doublets each with (J=2.0 Hz) at δ6.20 and δ6.40 representing H-6 and H-8 respectively of A ring. Two symmetrical doublets each with J=8.5 Hz appeared at δ7.06, (2H, d) and δ8.10 (2H, d) were assignable to H-3`/5` and H-2`/6` of B ring. A singlet for 3 protons appeared at δ3.88 was identified to the OCH_3 group attached at 3-position. On the basis of `HNMR spectra the compound (B) was identified as Kaempferol-3-methyl ether.



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References:

1. Altona, C. and Haasnoot, C.A.G. Org. Mag. Resonance, (1980), 13, 417.
2. Benerjee, R.D. and Sen, S.P. Economic Botany, 1980, 34(3), 284-98.
3. Chopra R.N.,Chopra I. C., Handa K. L. and Kapoor L. D. Medicinal ferns .In chopra,s Indigenous drugs of India. (11) Edition, U.N. Dhar and sons, Calcutta;1958.
4. Geissman, T. A. (1955), In "Modern method of plant analysis" eds. (Peach, K. and

Tracey. M. V., vol (III), springer verlag, Berlin, P. 450.
5. Khetwal K.S and Verma DL.Indian J. of Pharmaceutical Sciences.1984; 46 (1);25-26.
6. Khetwal K.S, AND Verma DL.Natural and Applied Science bulletin. 1983,34(4);337-338.
7. Mabry, T.J. Markham, M.B. and Thomas, M.B. The systematic Identification of Flavonoids, Springer Verlay, Berlin, 1970.
8. Markham, K. R (1982) Techniques of flavonoid identification, A. P. London.
9. Overend, W.G. A Carbohydrate Chemistry and Biochemistry, ed. Pigman, W and Horton, D, A.P 1972, 308.
10. Pande, D.C., Dashila, R.S. and Kandpal, M.M., Eco. And Taxo. Botany, 1989, 8(1), 221-223.

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