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Research Article

ANTIFUNGAL ACTIVITY OF *CHEILANTHES GRISEA* BLANFORD

Rachana Mishra[1*] and D. L. Verma[2]

1. Department of Chemistry, Kumaun University, DSB Campus, Naini Tal-263002, (Uttarakhand) India.
2. Department of Chemistry, Kumaun University, SSJ Campus, Almora-263601, (Uttarakhand) India.

***Corresponding Author: Email**: 09411102476m@gmail.com

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ABSTRACT

Cheilanthes *grisea* Blanford, a rare fern of Kumaun hills, is a member of psinopteridaceae family of leptosporangiate group of ferns. Aq. methanolic extract (50%) of this fern was screened for antifungal activity by thin layer autobiochromatographic methods. Chemical investigation of the fern fronds of C. *grisea* Blanford revealed the presence of a flavonol glycoside, quercetin-3-O-β-glycosyl (1→2) rhamnoside from an antifungal active fraction of n-butanol soluble of aqueous ethanolic extract. Its structure was elucidated by UV, ^{1}HNMR, and hydrolytic methods.

Keywords: *Cheilanthes grisea* Blanford, ethanolic extract, UV, ^{1}HNMR, hydrolytic method.

INTRODUCTION

C. *grisea* Blanford, a group of laptosporangiate ferns of family psinopteridaceae, distributed widely in temperate and humid regions of Western Central Himalaya. *Cheilanthes grisea* 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. Various species of *Cheilanthes* have widely been recommended as medicines of traditional uses (Chopra *et al*., 1958). *Cheilanthes* species have been screened for various biological activities (Banerjee and sen, 1980). Therefore, present chemical investigation reveals the antifungal activity examination of C. *grisea* Blanford. There are few studies reported by Verma and his co-workers, which are on important medicinal plants and have widely been referred by various workers of medicinal chemistry (Khetwal and Verma, 1983, 1984, 1986, 1990; Khetwal *et al*., 1985, 1986, Mishra, 2008, Mishra and Verma, 2009a, 2009b and 2009c).

MATERIAL AND METHODS

Plant material and authentification: C. *grisea* Blanford, family Psinopteridaceae, an endemic fern of Kumaun Himalaya was collected from morainic environes of Pindari and Milam glaciers of Kumaun Himalaya. It was identified by authorities of B.S.I. Deharadun and Botany Department of Kumaun University Nainital, Uttarakhand. The Vouch. Specimen no. 18 has been deposited in the plant taxonomy laboratory of Botany Department of Kumaun University at Almora Campus, Uttarakhand, India.

Extraction and isolation: About 300gm air dried and powdered sample of C. *grisea* Blanford was extracted with aqueous ethanol (1:1) by cold percolation method for three days. The extract was filtered and concentrated under

reduced pressure in Rota evaporator at 60^{0}C. The residue was dissolved into CH_2Cl_2:H_2O (1:1). After separating dichloromethane layer, H_2O layer was further partitioned with n-BuOH.

The butanol soluble fraction was evaporated to dryness and residue was examined for antifungal tests (Homans and Fusches, 1970; Pero and Owens, 1971). A part of BuOH soluble residue was dissolved in MeOH and it was banded in a continuous streak on silica gel TLC with the help of glass capillary. The plates were developed with $CHCl_3$-MeOH (3:1) solvent system for 40 minutes and were dried in oven at 60^{0}C for 10 minutes. The dried plates were sprayed with conidial suspension of *Aspergillus niger* and *Aspergillus flavus* in a medium prepared as follows:

7gm $K_2H_2PO_4$, 39gm $NaHPO_4.2H_2O$, 4gm KNO_3, 1gm $MgSO_4.7H_2O$, 1gm NaCl per gm of tap water. The solution was autoclaved at 120^{0}C for 20 minutes. Just before making conidial suspension 10ml of 30% aq. solution of glucose is added per 60ml of this solution. The dried and developed plates were also sprayed with the conidial suspension of *Aspergillus niger* in Brassica agar medium (Khulbe *et al*., 1983). The sprayed plates were incubated in moist atmosphere for 2-3days at 37^{0}C and the zones of inhibition were inspected in Visible and UV light (360nm). Two zones of inhibitions were observed on TLC at Rf 30 and 75 with Visible and UV light.

BuOH fraction which gave antifungal test was examined for active principles. Major portion of BuOH soluble was adsorbed on ten sheets of Whatman No.3 chromatographic papers and developed with 30% HOAc. After development a broad purple UV fluorescent band observed on PC and it was eluted with 70% MeOH. All the elutes were combined and evaporated to dryness. The residue was adsorbed on Saphdex LH-20 CC and eluted with different ratio of MeOH and H_2O. On eluting column with 90% MeOH, earlier eluting fractions gave a purple UV fluorescent compound while later eluting fraction gave two blue green UV fluorescent compounds. Elute of purple UV fluorescent compound on concentration gave a gray amorphous powder, Compound [A], m.p. 270^{0}C. It was hydrolyzed with 2N-HCl for an hour and gave dull yellow UV fluorescent aglycone on PC under UV light and was identified as quercetin by its m.p., UV, ^{1}HNMR, MS and CoPC with its authentic sample by using three solvent systems: (i) BAW (n-BuOH:AcOH:H_2O:: 4:1:5, V/V, upper layer), (ii) BEW (n-BuOH:EtOH:H_2O::4:1:2.2, V/V, upper layer) and (iii) 50% HOAc. The hydrolysate was neutralized and it gave two sugar on PC were identified as glucose and rhamnose by Co-PC with their respective authentic.

The glycoside appeared as a purple UV fluorescent spot on PC but its hydrolyzed aglycone gave dull yellow colour under UV light, indicated release of sugar moieties from 3-position (Sayed *et al.*,1999). On the basis of colour reactions, UV spectral studies and shift obtained with various diagnostic reagents (as given in table no. 1) indicates that the compound is flavonol-3-O-oligosaccharride (Nawwar *et al.*, 1989). The colour reactions and Rf values of the compound closely resembles with rutin [quercetin-3-O-rhamnosyl (1→6) glycoside] and neohesperidoside [quercetin-3-O-rhamno syl (1→2) glucoside].

Table [1]: UV spectra of compound [A] in MeOH (λ_{max}, nm)

Shift Reagent	Shift (λ_{max}, nm)			
	Band II			band I
MeOH	256	265sh		358
$AlCl_3$	265	300sh	363sh	420
$AlCl_3$+HCl	260	298sh		380
NaOAc	256sh	271		380
NaOAc+H_3BO_3	264			380
NaOMe	272	325		409(dec)
$ZrOCl_2$+Citric acid	256	265sh		365

^{1}HNMR study of the compound (as shown in table no. 2) gave two anomeric proton signals at δ 4.23 (1H, d, J=7.5Hz) and δ 5.58 (1H, d, J=2.0Hz) were assigned for glucose and rhamnose respectively.

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

Shift	Multiplicity	H-attributed
6.18	1H, d, J=1.9Hz	H-6
6.40	1H, d, J=1.9Hz	H-8
7.32	1H, d, J=2.0Hz	H-2'
6.88	1H, d, J=8.3Hz	H-5'
7.20	1H, dd, J= 2.0 and 8.3 Hz,	H-6'
5.30	1H, d, J=2.0Hz	Rha H-1"
4.00	1H, d, J=3.1Hz	Rha H-2"
3.53	1H, dd, J=8.8 and 3.1Hz	Rha H-3"
3.18	1H, dd, J=9.3 and 9.0Hz	Rha H-4"
3.24	1H,(m)	Rha H-5"
0.85	1H, d, J=5.3Hz	Rha CH_3
4.23	1H, d, J=7.5Hz	Glc H-1"'
3.00	1H, d, J=3.5Hz	Glc H-2"'
3.25	1H, d, J=5.6 and 3.2Hz	Glc H-3"'
3.24	1H, d, J=5.6 and 3.0Hz	Glc H-4"'
3.39	1H, d, J=2.0Hz	Glc H-5"'

It is well known that the anomeric proton of primary sugar always resonates at low field in comparison to terminal sugar (Overend, 1972). Thus, the compound [A] was identified as quercetin-3-O-glycosylrhamnoside. On comparing the proton signals in ^{1}HNMR spectra of authentic quercetin-3-O-rhamnoside with compound [A], quercetin-3-O-glucosyl-rhamnoside, it was found that rhamnose-H-2" proton of this quercetin-3-O-glucosyl rhamnoside appears δ 4.00 while H-2" proton of rhamnose sugar of compound [A] appeared at δ 4.40. On the basis of which the structure of glycoside was identified as quercetin-3-O-glucosyl (1→2) rhamnoside. Coupling constant of anomeric protons of rhamnose and glucose were 2.0Hz and 7.5Hz respectively, indicating both the sugars are in pyranose form. Thus, the compound was identified as quercetin-3-O-α-L-β-D-glucopyranosyl (1→2) rhanopyranoside [Fig. 1].

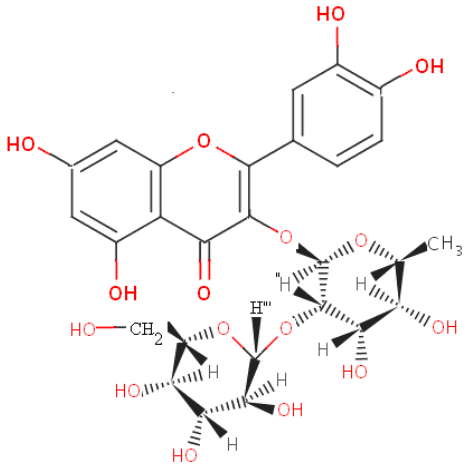

Fig. 1: Quercetin-3-O-α-L-β-D-glucopyranosyl (1→2) rhanopyranoside

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