Antifungal Activity And Flavonoid Composition Of *Wiesnerella denudata* steph

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Abstract:*Wiesnerella denudata* Steph, a liverwort of family Marchantiacea and is an endemic species of bryoflora to Indian Himalayan Region. Aqueous extract of the liverwort has been used as a medicine to cure cough, bronchitis and asthma by the local inhabitants of Kumaun Hills. The Botanically identified liverwort, specimen No. 3 extracted with 50% H_2O-MeOH by cold percolation method. The concentrated H_2O layer of the extract was partitioned with CH_2Cl_2. 50% HOAc fractionation of CH_2Cl_2 soluble on cellulose CC gave a dark fluorescent band and it was eluted and collected separately. An eluate of the fraction was evaporated to dryness and it gave antifungal test against the conidial suspension of *Aspergillus flavus* and *Aspergillus niger* by the standard method of thin layer autobiography. An antifungal active fraction afforded four flavone glycosides, apigenin-7-O-β-D-glucoside, acacetin- 7 – O -α- L- rhamnoside, luteolin -3- O- glucuronide and luteolin -5- O- β- D-glucopyranoside. [Academia Arena, 2009;1(6):42-45]. (ISSN 1553-992X)

Keywords: *Wiesnerella denudata* Steph, medicine, cough, bronchitis, asthma

Introduction

Wiesnerella denudata Steph, a liverwort of family Marchantiacea and an endemic to Indian Himalayan Region (IHR), has widely been used as a medicine to cure cough, bronchitis and asthma (Tewari, 1984). *Marchantia*, a prominent flavonoid producing genus of family Marchantiacea, is characterized by the presence of glucosides and glucuronides of apigenin, luteolin, acacetin, chrysoeriol and genkwanin. The dominant flanoids have been reported from the genus *Marchantia* are apigenin -4'- 7-di- O-glucoronide, apigenin -7- O- glucoside, apigenin -7- O-β -D- glucoronide, apigenin-4'-O-glucoronide, chrysoeriol-7-O-β-D-glucoronide and luteolin-6-8-di-C-glucoside (Markham and Porter, 1974; 1975 and 1978). The flavonoids isolated from various members of genus *Marchantia* have widely been employed to chemotaxonomic and phylogenetic studies (Markham *et al*., 1977, Campbell *et al.*, 1979, Schier, 1974). There are few studies reported by Verma and his co-workers, which are for 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).

Documenting the flavonoid composition from various species of Marchantiacea to chemotaxonomic and phylogenetic studies, the genus *Wiesnerella* has been separated from the family Marchantiacea and new family Weisnerellaceae has been created for the members of genus *Wiesnerella* (Campbell *et al.*, 1979, Schier, 1974). The flavonoids, luteolin-5-O-glucuronide, apigenin-7-O-rhamnoside and acacetin-7-O-rhamnoside have previously been isolated from *Wiesnerella denudata* (Campbell *et al.*, 1979). The terpenoids, germacranolides and guainolides have also been isolated from *Wiesnerella denudata* (Asakawal, 1982). Present communication reveals the presence of flavone glycosides and antifungal activity from CH_2Cl_2 soluble of *Wiesnerella denudata.* This is first report on chemical constituents of *Wiesnerella denudate* native to India Himalayan region.

Material and method

1. Plant material and authentification: *Wiesnerella denudata* Steph was collected from Dhobi ghat of Naini Tal at the altitude 2100m. It was identified by Prof. K. R. Verma, Department of Botany, Kumaun University, S. S. J. Campus, Almora (Uttarakhand). Its vouch. Specimen no. 3 has been deposited in the plant taxonomy laboratory of Botany Department of Kumaun University at Almora Campus, Uttarakhand, India.

2. Extraction and isolation of flavonoid positive fraction: About 400gm air dried and powdered sample of *Wiesnerella denudata* was extracted with aqueous methanol (50:50) by cold percolation method for six days. The extract was filtered and concentrated under reduced pressure in Rota evaporator at 60^0 C. The residue was partitioned between CH_2Cl_2:H_2O. The CH_2Cl_2 soluble was evaporated to dryness. It was chromatographed on cellulose (Merck CC) and eluted with 50% HOAc. A single broad dark purple fluorescent band was observed on CC and it was eluted and collected separately by

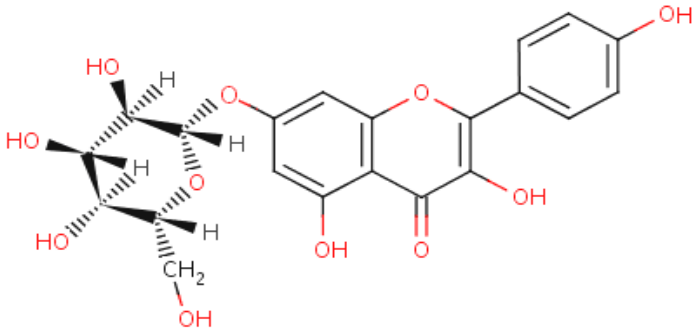
monitoring with UV light. An elute of the fraction was evaporated to dryness and residue was dissolved in MeOH. It was examined on 2DPC using t-BAW (3:1:1) and 30% HOAc as a developing solvent. A total of seven spots were discernible on 2DPC after spraying with aqueous solution of $FeCl_3$ and $K_4Fe(CN)_6$ (1:1). Out of seven spots four were identified as flavonoids after spraying PC with NH_3, $ZrOCl_2$ and NA (Naturstoffreagenz, A) reagents (Mabry *et al.*, 1970).

3. Screening of antifungal activity: The flavonoid positive fraction, an eluate of dark purple fluorescent band observed on CC after fractionation of CH_2Cl_2 soluble with 50% HOAc, was screened for antifungal activity by the standard method of thin layer autobiography using SiO_2 an absorbent and conidial suspension of *Aspergillus flavus* and *Aspergillus niger* in sugar salt medium as a spraying reagent (Homans and Fush, 1970; Pero and Owens, 1971). The TLC plate was developed with CH_2Cl_2: MeOH (90:10). The dried and developed plate was sprayed with conidial suspension of *Aspergillus flavus* in sugar salt medium. The plate was incubated at 27^0 C for three days. Two zones of inhibition were observed on TLC.

4. Isolation of flavonoids from antifungal active fraction: The flavonoid positive fraction, a bioassay guided antifungal active, was dissolved in MeOH. It was banded on Whatmann No. 3 PC (8 sheets) and repeatedly developed in BAW (BuOH: AcOH: H_2O, 4:1:5, upper layer). After three times repeated development, four fluorescent bands were observed on PC at Rf 65, 60, 58 and 55 which representing Frac-I, Frac-II, Frac-III and Frac-IV respectively. Each band was cut and eluted separately by monitoring with UV light. Four compounds A, B, C and D were isolated from Frac-I, Frac-II, Frac-III and Frac-IV respectively. An eluate of each fraction was finally purified on Sephadex LH-20 using 50% aqueous MeOH as an eluent.

Result and discussion

Compound [A], a dark purple fluorescent on paper chromatogram under UV light, gave positive tests for HCl, $FeCl_3$ and α-naphthol. Complete acid hydrolysis of compound [A] gave apigenin (CoPC) and glucose. UV spectrum gave MeOH (270, 341); $AlCl_3$ (277, 300, 386); NaOMe (267, 389); $NaOAc+H_3BO_3$ (268, 339) and NaOAc (272, 350). It was identified as apigenin-7-O-glucoside.

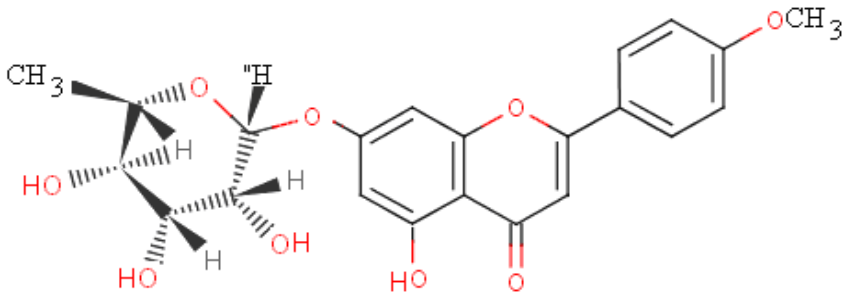


Compound [A]

Compound [B], a dark purple fluorescent on paper chromatogram under UV light, gave positive tests for $FeCl_3$, Mg+HCl and α-naphthol. Complete acid hydrolysis of the compound gave acacetin (CoPC) and rhamnose. FABMS (-ve) of the compound B gave a molecular ion at m/z 429 $[M-H]^-$ and other prominent ions observed at m/z 283 $[m/z\ 429\text{-rham}]^-$ indicating release of one mole of rhamnose from apigenin-4'-OCH_3. It was identified as acacetin-7-O-rhamnoside on the basis of ^{1}HNMR studies (see, table no. 1).

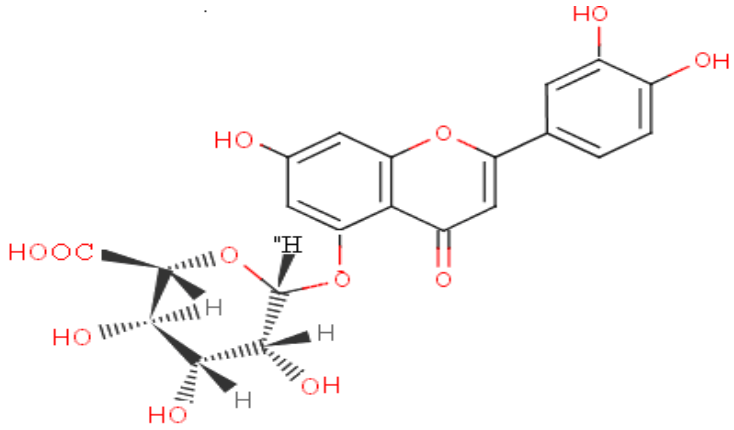
Table [1]: ^{1}HNMR of compound [B] in DMSO-d_6 (400MHz)

Shift	Multiplicity	H-attributed
6.87	1H(s)	H-3
6.35	1H, d, J=2.0Hz	H-6
6.75	1H, d, J=2.0Hz	H-8
7.12	2H, d, J= 8.8Hz	H-3'/5'
8.05	2H, d, J= 8.8Hz	H-2'/6'
5.70	1H, d, J=1.2Hz	H-1"
3.10-4.10	(m)	Remaining protons of rhamnose
1.20	3H,d,J=6.0Hz	-OCH_3



Compound [B]

Compound [C] is a dark purple fluorescent on paper chromatogram under UV light, gave positive test for $FeCl_3$, Mg+HCl and α-naphthol. Normal acid hydrolysis with 1.5N HCl of the compound afforded luteolin (CoPC) and glucuronic acid (CoPC). The position of attachment of glucuronic acid to luteolin was assigned on the basis of ^{1}HNMR studies (see, table no. 2). The compound [C] was identified as luteolin-3-O-glucuronide.



Compound [C]

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

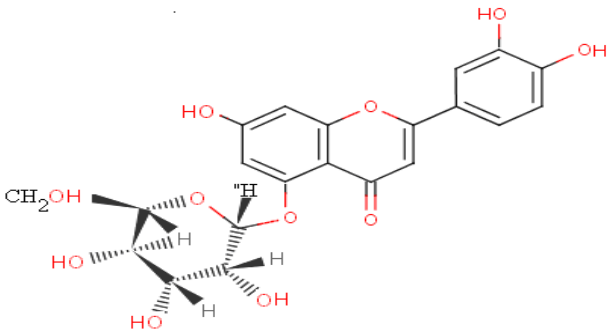
Shift	Multiplicity	H-attributed
6.20	1H, d, J=2.0Hz	H-6
6.53	1H, d, J=2.0Hz	H-8
6.81	1H(s)	H-3
6.98	1H, d, J= 8.0Hz	H-5’
7.65	1H, dd, J= 2.0, 8.0Hz	H-6’
7.80	1H, d, J=2.0Hz	H-2’
5.10	1H, d, J=7.5Hz	H-1”
3.10-4.10	(m)	Remaining proton of glucuronic acid

Compound [D] is a blue fluorescent on paper chromatogram under UV light and a slower moving component on PC in BAW. FABMS(-ve) of the compound gave a molecular ion at m/z 447[M-H]$^-$ and prominent ion at m/z 285 [m/z 447-glucose] indicating the release of glucose moiety from luteolin. Complete acid hydrolysis of compound [D] with 2NHCl supported the presence luteolin (CoPC) and glucose (CoPC). The compound [D] appeared as a blue fluorescent on PC under UV light with and without the presence of NH_3 vapours indicating the release of glucose moiety from 5 position.

It has further been supported by ^{1}HNMR studies (in DMSO-d_6 and 400MHz), see table no. 3. The compound [D] was identified as luteolin-5-O-β-D-glucopyranoside.

Table [3]: [1]HNMR of compound [D] in DMSO-d_6 (400MHz)

Shift	Multiplicity	H-attributed
6.55	1H(s)	H-3
6.69	1H, d, J=2.0Hz	H-6
6.79	1H, d, J=2.0Hz	H-8
6.87	1H, d, J= 8.8Hz	H-5
7.35	1H, d, J= 2.0Hz	H-2'
7.37	1H, dd, J=2.0, 8.8Hz	H-6'
5.10	1H,d,J=7.5Hz	H-1"
3.0-4.0	(m)	Remaining protons of glucose



Compound [D]

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