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Triterpenoid and sterol constituents of *Strobilanthes ciliatus* Nees

P.Reneela*, Shubashini K.Sripathi

Department of Chemistry, Avinashilingam University for Women, Coimbatore, Tamilnadu-641043, (INDIA)

E-mail: reneela@rediffmail.com

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ABSTRACT

Phytochemical investigation on the stems of *Strobilanthes ciliatus* Nees (Family Acanthaceae) was conducted. The separation of the chemical components was carried out by chromatography and structures of the compounds were elucidated by spectroscopic methods including nuclear magnetic resonance as well as mass spectrometry. The compounds were identified as Lupeol, Stigmasterol, Betulin, and Stigmasterol glycoside.

KEYWORDS

Strobilanthes ciliatus;
Acanthaceae;
Lupeol;
Stigmasterol;
Betulin;
Stigmasterol glycoside.

INTRODUCTION

Strobilanthes is one of the most interesting genera in the family *Acanthaceae* known for its diversified habits, gregarious nature and infrequent but elegant flowering. This is the second largest genus in the family with approximately 300 species entirely restricted to hills in tropical Asia. The Indian subcontinent has nearly 150 species out of which 59 are seen in peninsular India. The genus is not greatly explored for economic utility. Out of the 59 species of *Strobilanthes* seen in south India 39 are endemic to peninsular India. *Strobilanthes ciliatus* is one of the species endemic to Western Ghats, India. This plant has got several therapeutic properties. *Strobilanthes ciliatus* has a strong aroma and is used medicinally. It is widely used in Ayurveda as a source of the drug 'Sahachara'[9]. The plant is used for a variety of ailments like rheumatalgia, lumbago, sciatica, limping, chest congension, strangury, fever, leucoderma, skin diseases, inflammations, cough, bronchitis, odontalgia and general debility[10]. The roots are bitter, sweet, thermogenic, emollient, diurectic, febrifuge, diaphoretic, depurative, anti inflammatory, expectorant and tonic. In this paper, the isolation and characterization of four compounds from Strobilanthes ciliatus stems are reported.

EXPERIMENTAL

Strobilanthes ciliatus stems (2 Kg) was collected from the reserve forests of Nilambur, Malappuram District, Kerala State, India in the month of May 2007. Air dried pieces of stems were thoroughly percolated separately and extracted with petroleum ether (60^0-80^0 C) for 12 hours and the residual plant material was then extracted with acetone (2.5 L) for a period of (2×12) hours. The acetone extract (20g) was then subjected to column chromatography and with petroleum ether containing increasing percentages of ethylacetate. Fractions eluted with 100 percentage petroleum ether yielded lupeol (1.2 g), fractions eluted with 49:1 petroleum ether: ethylacetate yielded stigmasterol (500mg), fractions eluted with 19:1 petroleum ether: ethylacetate yielded betulin (300mg) and fractions eluted with 1:1 petroleum ether: ethylacetate yielded stigmasterol glycoside (350 mg).

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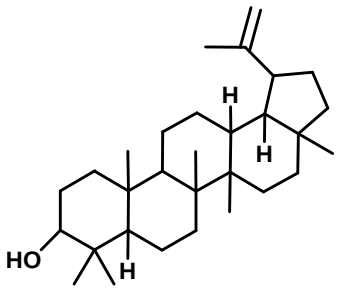
Melting points were measured on electrical melting point apparatus (Joshiba) and were uncorrected. Infrared (IR) spectra were recorded on Shimadzu FT-IR (4000-400) spectrophotometer. Mass spectra were recorded in GC-8000^{TOP} CE instrument. ^{1}H, ^{13}C-NMR and DEPT spectra were recorded on Bruker Advance 400 Spectrometer. Deuterated chloroform ($CDCl_3$) was used as solvent. Acme silica gel of 60-120 mesh activated at 120^0C was used as adsorbent in column chromatography. Silica gel (100 mesh) with 13% $CaSO_4$ ½ H_2O binder was used as solid stationary phase in thin layer chromatography. The spots were visualized under UV light at wavelength 254 nm and 365 nm and in iodine vapours.

RESULTS AND DISCUSSION

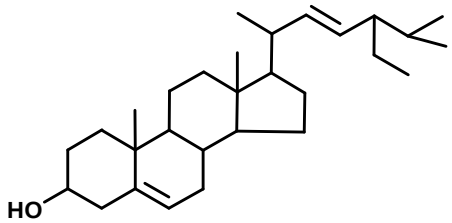
The acetone extract of the stems of *Strobilanthes ciliatus* yielded four compounds which were identified by spectroscopic analysis as well as by comparison of their spectral data with previously reported values.

Compound **(1)** was isolated as white crystals, mp 206^0C. Its IR spectrum showed characteristic absorptions at 3326 (OH), 2941(CH), 1458, 1379, 1035, 879 cm^{-1}. The mass spectrum showed the molecular ion peak at *m/z* 426 corresponding to $C_{30}H_{50}O$. The ^{1}H- NMR spectrum revealed signals for six tertiary methyls (δ 0.76, 0.78, 0.83, 0.94, 0.96 and 1.030), a singlet at δ 1.68 for the allylic methyl group and two broad singlets at δ 4.57 and δ 4.69 for the exomethylene protons. A secondary carbinol at δ 3.18 (1 dd, J = 5 Hz and 4.5 Hz) is also seen. The remaining protons appeared as complex multiplets between δ 1.0 to 2.6. These data indicated a pentacyclic triterpenoid of lupane type and comparison of its physical and spectral data with published values confirmed the identity of compound 1 as lupeol. The ^{13}C-NMR spectrum of compound **(1)** showed seven-methyl groups at δ 27.9, 19.4, 17.9, 16.1, 15.9, 15.3, 14.5 and an exomethylene group at δ 150.9 (C- 20) and 109.3 (C-29). A secondary hydroxyl bearing carbon is seen at δ 79.0 (C-3) in addition to ten methylene, five methine and five quaternary carbons. These data confirmed the identity of compound **(1)** as lupeol.[1,7].



1

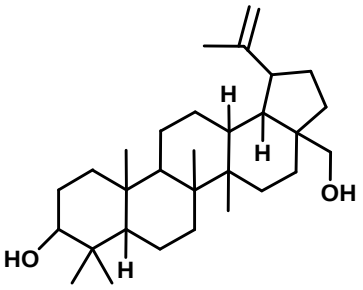
Compound **(2)** was isolated as a white powder. The IR spectra showed characteristic peaks at 3384(OH), 2958(CH), 1458, 1380, 1051, 970 cm^{-1}. The mass spectral values suggested a molecular formula $C_{29}H_{48}O$ (m/z 412). The proton nmr spectrum showed the presence of six methyls that appeared at δ 0.68, 0.79, 0.82, 0.86, 0.92 and 1.02. The olefinic protons are seen at δ 5.35, 5.15 and 5.03. ^{13}C-NMR and DEPT spectra inferred twenty nine carbon signals including six methyls, nine methylene, eleven methine and three quaternary. Olefinic carbons were seen at 140.7(C-5), 138.3(C-22), 129.2(C-23) and 121.7(C-6). The hydroxyl bearing carbon was seen at 71.8(C-3). These data showed confirmed compound **(2)** as stigmasterol.[3,6].



2

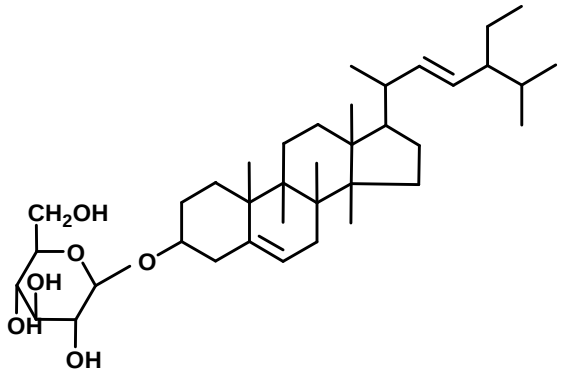
Compound **(3)**, obtained as white crystals, mp 246^0C. IR spectrum showed characteristic peaks at 3384, 2933, 1458, 1375, 1026, 883 cm^{-1} suggesting that it contained a hydroxyl group and a terminal double bond. The mass spectrum showed the molecular ion peak at *m/z* 426 corresponding to the molecular formula $C_{30}H_{50}O_2$. The ^{1}H-NMR spectrum exhibited signals for six methyl groups at δ 0.76, 0.82, 0.96, 0.98, 1.0 , 1.68 and one isopropenyl moiety at δ 1.41 indicating a lupane skeleton[5]. Two diastereotopic protons of the methylene group (attached to hydroxyl) at δ 3.32(d, J = 11 Hz, H-28) and 3.78 ppm (d, J = 10.5 Hz, H-28') and two exocyclic methylene protons at δ 4.68, 4.58 (H-29 and 29', s) were also seen. The ^{13}C-NMR spectrum revealed thirty carbon signals, which were confirmed by DEPT experiment to be six methyl groups, twelve methylene groups, six methine carbons,

six quaternary carbons. Further the structure of 3 was confirmed by comparing the NMR data with those of betulin reported by earlier researchers[2,8].



3

Compound **(4)** was obtained as a pale white solid eluted with 1:1 petroleum ether: ethylacetate yielded stigmasterol glycoside. IR showed intense absorption bands at 3384, 2956, 2933, 1458, 1072, 1024 cm^{-1}indicating the presence of OH (3384), CH stretch (2956, 2933). The molecular mass of 3 was found to be 574 from mass spectrum indicating a molecular formula of $C_{33}H_{58}O_6$. The ^{13}C NMR spectrum showed the presence of thirty five signals which were resolved as six methyl, ten methylene, sixteen methine and three quaternary carbons. Six anomeric carbon in ^{13}C-NMR spectra showed resonance absorption between 60 and 71 and a methine carbon at 101.7 indicated the presence of sugar moiety in the molecule. These values were found similar to that previously reported in the literature[4] for stigmasterol glycoside **(4)**.



4

ANALYTICAL DATA

Lupeol **(1)** was obtained as white powder from petroleum ether (100%); 1.2 g; mp 206°C; IR(KBr) 3326 (OH), 2941(CH), 1458,1379,1035,879 cm^{-1}; ^1H-NMR ($CDCl_3$, 500 MHz), δ: 0.76, 0.78, 0.83, 0.94, 0.96, 1.03 (each 3H,s, Me-28, Me-23,Me-24, Me-25, Me-26, Me-27),1.68 (3H, br s, Me-30), 3.18 (1H, dd, *J* = 5,4.5Hz,H-3); ^{13}C-NMR data, see Table 1; EIMS m/z 426 [M+] (4), 411 [M+-CH3] (10), 408 [M+-H2O] (3), 218 (10), 207 (8), 189 (50), 163 (70),135 (60), 107 (67), 105 (50), 79(40),41

TABLE 1 : ^{13}C NMR data of compounds 1-4

Position	Compound			
	1	2	3	4
1	38.8	37.2	38.6	39.2
2	27.4	28.1	27.3	29.0
3	79.0	71.8	78.9	79.5
4	38.0	39.6	38.8	40.4
5	55.3	140.7	55.2	140.0
6	18.3	121.7	18.2	122.4
7	34.2	31.7	34.2	32.4
8	40.8	31.9	40.9	32.6
9	50.4	51.2	50.3	51.8
10	37.1	36.5	37.3	37.2
11	20.9	21.1	20.8	21.5
12	25.1	40.4	25.2	40.2
13	38.7	42.2	37.1	42.8
14	42.7	56.8	42.7	57.3
15	27.4	24.3	27.0	25.9
16	35.6	26.0	29.1	26.7
17	43.0	55.9	47.9	56.5
18	47.9	12.2	47.7	12.1
19	48.3	19.8	48.8	19.6
20	150.9	35.9	150.5	36.6
21	29.8	18.9	29.7	19.2
22	40.0	138.3	34.0	138.4
23	27.9	129.2	28.1	128.9
24	15.3	50.1	15.3	46.6
25	16.1	29.2	16.1	29.7
26	15.9	21.3	15.9	20.0
27	14.5	19.3	14.7	19.4
28	17.9	23.0	60.5	23.4
29	109.3	12.3	109.6	12.3
30	19.4		19.1	
1’				101.7
2’				70.8
3’				76.5
4’				74.1
5’				77.1
6’				62.2

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(100) calcd for $C_{30}H_{50}O$.

Stigmasterol **(2)** was obtained as white powder from petroleum ether–ethyl acetate (49:1); 500mg; mp169°C; IR (KBr) 3384(OH), 2958(CH), 1458, 1380, 1051, 970 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.68, 0.79, 0.82, 0.86, 0.92, 1.02 (each 3H, s, (Me-18, Me-29, Me-27, Me-26, Me-21, Me-19), 3.53 (1H, m, H-3), 5.35(1H, s, H-6), 5.03 (1H, m, H-23), 5.15 (1H, m, H-22); ^{13}C-NMR data, see Table 1; EIMS m/z 412 [M+] (39.7), 351 (13.5), 314 (7.0), 300 (25.5), 271 (38.4), 229 (8.6), 203 (100) calcd for $C_{29}H_{42}O$.

Betulin **(3)** was obtained as white powder from petroleum ether–ethyl acetate (19:1); 300mg; mp 245°C; IR (KBr) 3384(OH), 2933, 1458, 1375, 1026, 883 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.76, 0.82, 0.96, 0.98, 1.0 , 1.68 (each 3H,s, Me-23,Me-24, Me-25, Me-26, Me-27, Me-30), 3.32(d, J = 11 Hz, H-28), 3.78 ppm (d, J = 10.5 Hz, H-28'), δ 4.68, 4.58 (H-29 and 29', s); ^{13}C-NMR data, see Table 1; EI-MS: m/z = 442 [M]+, 412 (50), 411 (100), 399 (31), 385 (37), 207 (72), 203 (87) calcd for $C_{30}H_{50}O_2$.

Stigmasterol glycoside **(4)** was obtained as white powder from petroleum ether–ethyl acetate (1:1); 350mg; mp 268-270°C; IR (KBr) 3384, 2956, 2933, 1458, 1072, 1024 cm^{-1}; ^{1}H- NMR ($CDCl_3$, 500 MHz): δ0.70, 0.72, 0.81, 0.85, 0.94, 1.02 (Me-18, Me-29, Me-27, Me-26, Me-21, Me-19), 3.22 (H-3), 4.35 (J = 8Hz, anomeric carbon), 4.54 (H-23), 5.16 (m,H-22), 5.31 (br.s,H-5); ^{13}C-NMR data, see Table 1; EI-MS: m/z = 573 [M]+, 515 (4) 435(3), 395(6), 297(11), 255(16), 203 (100), 181(27) calcd for $C_{35}H_{58}O_6$.

CONCLUSION

Phytochemical analysis of *Strobilanthes ciliatus* has led to the isolation of four compounds lupeol, stigmasterol, betulin and stigmasterol glycoside.The isolation and identification of these compounds from the stems of *Strobilanthes ciliatus* is herein reported for the first time from this plant. This is also the first attempt of any phytochemical investigation from *Strobilanthes ciliatus* and hence is of phytochemical significance. Further isolation and purification of other fractions of this plant is being carried out.

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