RESEARCH ARTICLE

β-Sitosterol Glucoside from *Pisonia grandis* R.Br. Stem Bark in Ethyl Acetate Extract

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Abstract

Aim: Plants are ancient source of medicine due to the presence of various bioactive molecules present in different parts of the system. In this study, we investigated the biologically active extract and isolated one pure compound from the stem bark of *Pisonia grandis* R.Br. **Materials and Methods:** ^{1}H and ^{13}C nuclear magnetic resonance (NMR), mass spectroscopy, F*ourier*-transform *infrared* spectroscopy, thin-layer chromatography, potassium bromide, and DMSO-d6 were used. **Results and Discussions:** The pure compound was isolated *and identified* as *β-sitosterol* glucoside, *and the structure was characterized by ^{1}H and ^{13}C NMR spectroscopy analysis*. Later on with the structural elucidation with ^{13}CNMR, the mass spectrum *was also revealed*, the m/z ratio of β-sitosterol glucoside *was found* to be 613[M+2$H_2$0+1]$^{+}$. **Conclusion:** The study implies that though the compound isolated is abundantly known and is present in *majority* of the plants, the work held out is first *to report* for the presence of β-sitosterol glucoside from the ethyl acetate extract in the stem bark of *P. grandis* R.Br.

Key words: Column chromatography, isolation, *Pisonia grandis*, spectral analysis, β-sitosterol glucoside

INTRODUCTION

Pisonia grandis R.Br. is a dominant plant belonging to the family Nyctaginaceae commonly known as the lettuce tree and named as "Saeng Chan," is an ornamental plant grown widely in the garden. It is one of the most widespread large shrubs in the forests of India, usually occurring in deciduous forests. The plant is among three species of Pisonia, including *P. aculeata* and *Pisonia umbellifera*, reported to be found in Thailand[1] and having a synonym as *Pisonia spanoghe*. It is popularly known as "Leechai kottai keerai" in Tamil.

The Ayurvedic literature reveals that *P. grandis* has tremendous traditional and medicinal uses including analgesic, anti-inflammatory, and diuretic activity.[2] *P. grandis* has a protective wound healing potential on Wistar rats-excision wound and incision wound. Literature survey states that it has potent anti-bacterial activity[3],possess anti-fungal activity against various microorganisms[4], anti-diabetic activity[5],anxiolytic activity in mice[6], anti-oxidant activity[7,8], anti-plasmodial activity[9], anti-pyretic activity[10], hepatoprotective[11], anti-arthritic activity[12], and is also used in the treatment of various aliments like analgesia, ulcer, dysentery and snake bite[13,14], anorexia, jaundice[15]. The analytical techniques including HPTLC fingerprinting analysis were performed and reported.[16-18] Phytosterols are extensively present in all the plant species of the genus *Pisonia* and have important pharmacological activities which include lowering the cholesterol levels and acts as a potent anti-tumour agent. β-sitosterol is used in the treatment of immune dysfunctions, inflammatory disorders and rheumatoid arthritis, breast cancer, colon cancer and benign prostatic hypertrophy and also used as a precursor in the synthesis of steroidal drugs.[19] In the present study an attempt is made in the isolation

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of the active chemical marker from *P. grandis* using conventional column chromatography.

P. grandis has numerous bioactive compounds which include pinnitol, allantoin, β-sitosterol, α-spinasterol, β-sitosterolglucoside, octacosanol, dulcitol, flavonoids, and quercetin. These are the compounds which have been isolated from the leaves of the plant.[20]

In the study, the conventional column chromatography was used for the isolation of the compound, characterization, and structural elucidation of the isolated compound from the stem bark of *P. grandis* R.Br.

MATERIALS AND METHODS

General Experimental Procedure

All the reagents used were of analytical grade. The ^{1}H and ^{13}CNMR spectra were recorded by a Bruker (500 MHz) instrument using DMSO-d6as solvent and the chemical shift was reported in δ ppm with respect to TMS. The mass spectrum was recorded on Shimadzu prominence liquid chromatography–mass spectrometry (LC-MS) 2020.

Sample Collection

The plant was collected in and around SRM University, Kattankulathur campus, and authenticated by Dr. P.Jayaraman, Director, Plant Anatomy Research Centre Medicinal Plants Research Unit, Tambaram, Chennai - 45, India. The plant material collected was shade dried and coarsely powdered. The powdered plant material (whole stem, 1 kg) of *P. grandis* R.Br was extracted successively using non-polar to polar solvents, i.e., hexane, ethyl acetate, and ethanol by cold maceration method. In each solvent, the plant material was soaked for 72 h at 30 ± 2°C, filtered, and to the residue, the respective solvent was added and repeated thrice until it becomes pale in color till all the extraction is done. All the filtrates of the individual extracts were pooled, and the solvent was reduced in a rotary evaporator under vacuum.[21]

Extraction and Isolation

Isolation was performed by conventional column chromatography for the separation of the pure compound from ethyl acetate crude extract. The column was prepared using silica slurry by wet packing method. The column prepared for the separation and isolation of the phytoconstituents should be properly grasped till the complete analysis. The sample was mixed uniformly and was introduced into the column using a long Pasteur pipette followed by addition of the eluent slowly from the top without disturbing the column. The eluent strength was changed from least polar solvent (hexane) system to polar solvent (methanol). The fractions obtained were analyzed by thin-layer chromatography. The same and pure fractions were collected in 10% methanol in ethyl acetate and pooled, dried using Rotavac, and the compound obtained was white crystalline solid in appearance, which was characterized using IR, nuclear magnetic resonance (NMR), and mass spectroscopic analysis.

Characterization of the Isolated Compound by Spectral Analysis

The isolated compounds were identified using infrared (IR), NMR, and mass spectroscopy. The IR spectroscopy was performed with KBr on Bruker Alpha E&T, fourier-transform IR (FT-IR) instrument and the spectral characterization was tabulated under [Table 1].

^{1}HNMR, ^{13}CNMR, and DEPT-135 were recorded on Bruker 500 MHz AVANCE. The chemical shifts were reported as parts per million where tetramethylsilane as reference standard. Solvent used was DMSO-d6. Mass spectroscopy was performed on Shimadzu LC-MS.

Isolated compound: White crystalline solid.

^{1}H NMR(500 MHz, DMSO-d6, δ, ppm): 0.52(3H, s, H-18), 0.96(3H, s, H-19), 0.91(3H, m, H-21), 0.82(3H, m, H-26), 0.78(3H, m, H-27), 0.67(3H, m, H-29), 3.64(1H, m, H-3), 5.33(1H brs, H-6).

^{13}C NMR(500 MHz, DMSO-d6, δ, ppm): 37.30(C-1), 29.56(C-2), 77.42(C-3), 42.22 (C-4), 138.51(C-5), 129.46(C-6), 31.85(C-7), 31.80(C-8), 51.07 (C-9), 37.02 (C-10), 20.19(C-11), (C-12), 43.29(C-13), 55.68(C-14), 24.36(C-15), 25.34(C-16), 54.95(C-17), 12.59(C-18), 19.32(C-19), 36.70(C-20), 19.58(C-21), 34.38(C-22), 28.60(C-23), 49.15(C-24), 29.72(C-25), 21.41 (C-26), 21.72(C-27), 23.09(C-28), 13.26 (C-29), 101.32, 73.95, 77.22, 77.16, 70.57, and 61.58 (six carbons–glucose unit).

FT-IR spectrum for the Compound Isolated

IR (cm^{-1}): 3437.90 (OH), 2926.67 (CH_2-CH), 1588.47 (-C=C-), 1422.91 (-C=C-H), 1095.04 and 965.82 (CH), and 801.42 and 672.14 (-C=C-).

Table 1: Characterization and spectral data of isolated compound PG-1

Nature	Solid
Color	White
Molecular formula	$C_{35}H_{60}O_6$
Molecular weight	576
Mass	613(M+2$H_2$0+1)$^{+}$
IR (KBr) cm^{-1}	3413(OH), 2940, 2870(CH2, CH),1635(C=C),1454(C=C) 1277 (C-C), 801 (C-C)

RESULTS AND DISCUSSIONS

The ethyl acetate extract of P. *grandis* stem bark was column chromatographed over silica gel using hexane to methanol, as an eluent to yield a white colorless compound.[22] The compound isolated and recovered was subjected to thin-layer chromatography for the identification, and an R_f value of 0.60 was found using the mobile phase system, i.e., isopropyl alcohol:ethyl acetate (3:2).

The ^{1}H NMR spectrum of the isolated compound shows 6 signals at δ_1 0.52 (3H, s, H -18), 0.67(3H, m, H -29), 0.78(3H, m, H -27), 0.82 (3H, m, H -26), 0.91 (3H, m, H -21), and 0.96 (3H, m, H -29) ppm (-CH3) at C-18, C-29, C-27, C-26, C-21, and C-19 corresponds to methyl hydrogen. One proton at C-3 shows multiplet at 3.64 ppm assigned to carbon connected oxygen and doublet at 5.33 ppm is a characteristic peak for double bond in the ring between quaternary carbon (C-5) and methine carbon (C-6). The carbon NMR shows 35 carbon signals and its structure is matching with literature report. From the DEPT-135 NMR analysis, aglycone part of β-sitosterol glucoside contains 29 carbons, of which six methyl carbons (6-CH_3), eleven methylene carbons (11-CH_2), nine methine carbons (9- =CH-), and three quarternary carbons(3- =C=) and the interpreted results are tabulated in Table 2.

Methyl carbons(-CH_3): C-18, C-19, C-21, C-26, C-27, C-29, and signal appeared at 12.59, 19.32, 19.58, 21.41, 21.72, and 13.26 ppm.

Methylene carbons(=CH_2): C-1, C-2, C-4, C-7, C-11, C-12, C-15, C-16, C-22, C-23, C-28, and signal appeared at 37.30. 29.56, 42.22, 31.85, 20.19, 38.79, 24.36, 25.34, 34.38, 28.60, and 23.09 ppm

Methine carbons(=CH-): C-3, C-6, C-8, C-9, C-14, C-17, C-20, C-24, C-25, and signal appeared at 77.42, 129.46, 31.80, 51.07, 55.68, 54.95, 36.70, 49.15, and 29.72 ppm.

Quarternary carbons (=C=): C-5, C-10, C-13 and signal appeared at138.51, 37.02, 43.29 ppm.

Glucose unit: It contains six carbons of which oxygenated carbon (C-1') signal appeared at 101.32 ppm and methylene carbon (C-6) appeared 61.58 and other four carbon signals appeared at 73.95, 77.22, 77.16, and 70.57 ppm. The ^{1}H NMR and ^{13}C NMR spectroscopic analysis confirms that the isolated compound was found to be as beta-sitosterol glucoside that was confirmed by the reported data [13]. The structure of the compound is shown in Figure 1.

Table 2: NMR spectral data of isolated compound PG-1

Atom	^{13}C NMR (δ ppm)	^{1}H NMR (δ ppm)
1	37.30	1.39-2.36 (m, 2H)
2	29.56	1.39-2.36 (m, 2H)
3	77.42	3.64 (m, 1H)
4	42.22	1.39-2.36 (m, 2H)
5	138.51	-
6	129.46	5.33 (m, 1H)
7	31.85	1.39-2.36 (m, 2H)
8	31.80	1.39-2.36 (m, 1H)
9	51.07	1.39-2.36 (m, 1H)
10	37.02	-
11	20.19	1.39-2.36 (m, 2H)
12	38.79	1.39-2.36 (m, 2H)
13	43.29	-
14	55.68	1.39-2.36 (m, 1H)
15	38.79	1.39-2.36 (m, 2H)
16	24.36	1.39-2.36 (m, 2H)
17	54.95	1.39-2.36 (m, 1H)
18	12.59	0.52 (s, 3H)
19	19.32	0.96 (s, 3H)
20	36.70	1.39-2.36 (m, 1H)
21	19.58	0.91 (m, 3H)
22	25.34	0.82-1.24 (m, 2H)
23	34.38	0.82-1.24 (m, 2H)
24	49.15	1.51 (m, 1H)
25	29.72	1.94 (m, 1H)
26	21.41	0.82 (m, 3H)
27	21.72	0.78 (m, 3H)
28	28.60	0.82-1.24 (m, 2H)
29	13.26	0.67 (m, 3H)
1'	101.32	5.03 (m, 1H)
2'	73.95	3.06-3.64 (m, 1H)
3'	77.22	3.06-3.64 (m, 1H)
4'	70.57	3.06-3.64 (m, 1H)
5'	77.16	3.06-3.64 (m, 1H)
6'	61.58	3.06-3.64 (m, 2H)`

NMR: Nuclear magnetic resonance

CONCLUSION

In the study, the conventional column chromatography was implemented for the isolation of the pure compound from the stem bark of *P. grandis* in the ethyl acetate extract. As per literature review, much isolation and phytochemical analysis were performed, but no steroidal presence was confirmed. The research has laid sufficient background information for the identification of the isolated compound by IR, NMR, and mass spectroscopy. The isolated compound shows solid matrix and white in nature. The actual molecular weight of the compound is 576, and in mass spectrum, it shows a peak at 613 $(M+2H_20+1)^+$

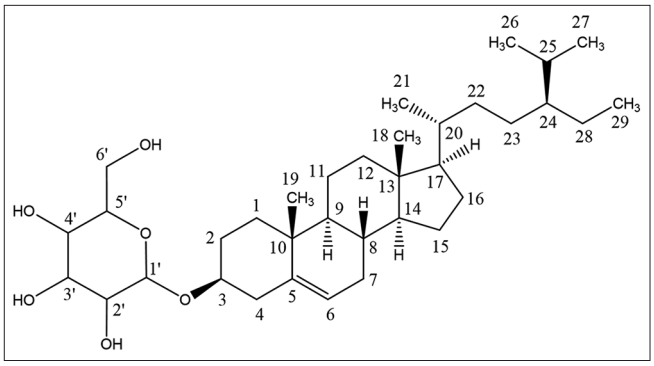

Figure 1: β -sitosterol glucoside

which was due to adduct of water molecule. From all the above spectral data evidence, the compound isolated from the stem bark of *P. grandis* R.Br. ethyl acetate extract elucidated is confirmed to be β-sitosterol glucoside.[23] This is to first report of the steroidal glucoside compound in the plant stem bark.

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