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Isolation and Analytic Characterization of β-Sitosterol and GC-MS Analysis of Methanolic Leaves Extract of *Pongamia pinnata (L.)* pierre

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Abstract

In the course of present research venture, a phytosterol viz., β-sitosterol was secluded from the *in vivo* leaves of *P. pinnata* and identification of the secluded compound was accomplished through TLC, R_f value, IR spectroscopy and NMR. Furthermore, the leaf methanolic extract was also analyzed by GC/MS spectroscopy in *P. pinnata*. It was observed that 64 secondary compounds were isolated in *P. pinnata*, respectively.

Keywords: β-sitosterol; TLC-Thin Layer Chromatography; IR-InfraRed; NMR-Nuclear Magnetic Resonance; *P. pinnata* (L.)

Introduction

From the past few decennary, various records are available as well as investigations have been accomplished, showing the relevance of extracting, isolating, identifying, utilizing and exploring various medicinally bioactive compounds, from many plant species in *in vivo* and *in vitro* conditions such as in *Sericocalyx schomburgkii*, *Nerium oleander*, *Piper nigrum* and *Piper longum*, *Pavetta indica*, *Momordica charantia* etc. [1-5]. Hence, this field has opened up new vistas in the field of plant sciences and medicinal world. In the present research work, β-sitosterol has been extracted from the leaves of *P. pinnata*, and then purified and identified by some spectroscopic methods like IR, NMR etc. Besides this, GC/MS studies have been done using crude methanolic extracts of *P. pinnata*.

Pongamia pinnata (L.) belongs to family Leguminosae and sub-family Papilionaceae is a medium sized glabrous, perennial tree grows in South Eastern Asia and Australia [6]. This tree is 15-25 m in height, with straight or crooked trunk 50-80 cm or more in diameter and broad crown of spreading or drooping branches. Its bark is smooth and grey-brown; leaves are alternate, imparipinnate, hairless, pinkish-red when young, glossy dark green above and dull green with prominent veins beneath when mature. Flowers short-stalked, pea-shaped, 2-4 together and 15-18 mm long. Pods are smooth, oblique oblong to ellipsoid, 3-8 × 2-3.5 × 1-1.5 cm, flattened but slightly swollen, slightly curved with short, curved point (beaked), brown, thick-walled, thick leathery to subwoody, hard, indehiscent, 1-2 seeded, short stalked. Seed compressed ovoid or elliptical, bean-like, 1.5-2.5 × 1.2-2 × 0.8 cm, with a brittle coat long, flattened, dark brown, oily.

Root, bark, leaves, flower and seeds of this plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers [7]. In the traditional system of medicines, such as Ayurveda and Unani, this plant is used for anti-plasmodial, anti-inflammatory, anti-nonciceptive, anti-hyperglycamic, antidiarrhoeal, antioxidant, anti-hyperammonic and anti-ulcer [8].

Materials and Methods

Extraction of phytosterol

Shade dried leaves and the powder (100 gm) of *Pongamia pinnata* was defatted in a soxhlet apparatus in petroleum ether (60-80°C) for 24 hours on a water bath and extraction of steroids was done by using the protocol developed by Tomita et al. [9]. For the quantitative estimation TLC was done. Identification of the extracted compounds was done by using R_f value, NMR and IR spectroscopy.

GC-MS analysis

Shade dried leaves and the powder (50 gm) of *Pongamia pinnata* was taken into thimble and 750 ml of methanol was taken into the flask of soxhlet apparatus and cycled 10-15 times, then it was decanted into the beaker and was left open, so that the methanol gets evaporated. The qualitative and quantitative compositions of the methanol fraction were studied by GC/MS (QP 2010 Plus Shimadzu) at AIRF, JNU, Delhi. Mass was scanned in the range 38-300 amu. Compounds were identified by comparison of mass spectra with those in the Wiley 275 and NIST 02 libraries and mass spectra of standards.

Thin-layer chromatography

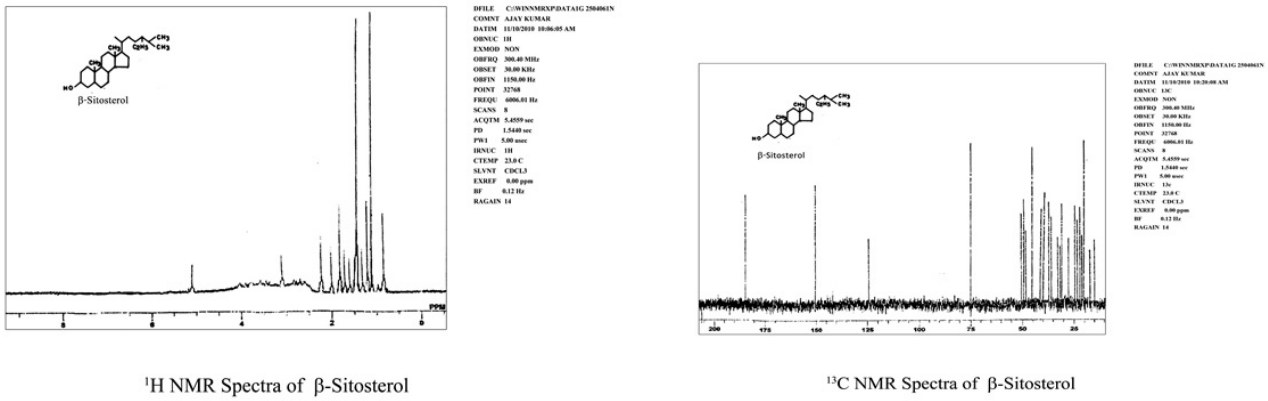
TLC Glass plates were coated with silica gel (30 gm/60 ml distilled water) dried at room temperature and activated before use at 100°C for 30 minutes in an oven and cooled at room temperature. Crude extract of leaf was applied 1.0 cm above from the lower edge of the silica gel plates along with the reference standard compound of β-sitosterol. Then plate was developed in an air tight chamber containing chloroform: methanol: water (60:25:15). The developed plates were air dried, sprayed with 50% sulphuric acid and heated (at 100°C for 15 min), it showed spots which harmonize with that of the reference standard β-sitosterol. When this plate placed in a chamber saturated with I_2 vapors, it also showed dull red color of β-sitosterol. R_f value come out with the R_f value of the standard β-sitosterol 0.83-0.86 [10]. The marked spots were scrapped and collected along with the silica gel and wash with ethanol then crystallized with chloroform. Finally, this purified material was subjected to its IR and NMR spectral analysis.

Results and Discussion

In the present research assay, phytosterol β-sitosterol was isolated from the leaves of *P. pinnata*. The characteristic IR, ^{1}H NMR and ^{13}C NMR spectral peaks of compound was found to be super imposable with those of their respective standard reference compound of β-sitosterol. Its IR and NMR spectra showed different peaks in Figure 1 and Table 1.

Name of Compound	UV light absorption band	IR: vcm^{-1}/max KBr	^{1}H NMR	^{13}C NMR
β-sitosterol	206 sh, 268 sh, 356 sh, 540 sh	3400 (O-H), 2700 (C-H), 1700 (C=O), 1640, 1610, 1570, 1510, 1450, 1400 (aromatic), 1385, 1310, 1270, 1180, 1010, 815	0.94 (H_1), 1.39 (H_2), 1.18 (H_3), 1.56 (H_4), 1.07 (H_5), 1.86 (H_6), 1.06 (H_7), 1.27 (H_8), 1.29 (H_9), 1.04 (H_{10}), 1.67 (H_{11}), 1.77 (H_{12}), 1.16 (H_{13}), 1.25 (H_{14}), 1.45 (H_{15}), 1.25 (H_{16}), 1.29 (H_{17}), 1.46 (H_{18}), 1.25 (H_{19}), 1.65 (H_{20}), 1.06 (H_{21}), 1.21 (H_{22}), 1.46 (H_{23}), 0.86 (H_{24}), 0.86 (H_{25}), 1.26 (H_{26})	13.3 (C_1), 21.6 (C_2), 34.5 (C_3), 42.7 (C_4), 30.5 (C_5), 20.1 (C_6), 20.1 (C_7), 30.0 (C_8), 35.7 (C_9), 29.7 (C_{10}), 18.5 (C_{11}), 48.2 (C_{12}), 20.3 (C_{13}), 20.4 (C_{14}), 40.1 (C_{15}), 27.4 (C_{16}), 295.35 (C_{17}), 36.9 (C_{18}), 36.8 (C_{19}), 32.1 (C_{20}), 26.9 (C_{21}), 36.2 (C_{22}), 364.81 (C_{23}), 269.86 (C_{24}), 25.2 (C_{25}), 257.24 (C_{26}), 34.5 (C_{27}), 19.3 (C_{28}), 23.4 (C_{29})

Table 1: Spectral studies of isolated phytosterol from *Pongamia pinnata* (L.).



^{1}H NMR Spectra of β-Sitosterol

^{13}C NMR Spectra of β-Sitosterol

Figure 1: ^{1}H NMR and ^{13}C NMR of β-sitosterol.

A range of compounds have been detected in *P. pinnata* including Saturated fatty acid (pentadecanoic acid), phytosterols (cholesterol, stigmasterol, β-sitosterol), tridecane, dodecane (alkane hydrocabon), glycerin (alcohol), tri-terpene (squalene, lupeol, betulin), sesquiterpenes (valerenol, alpha-caryophyllene, sclareolide), aromatic compound (myo-inositol), evonine (alkaloid), hexadecen-1ol (terpene alcohol), hexadecanoic acid (palmitic acid), phytol (diterpene), octadecadienoic acid (linoleic acid), and tocopherol (vitamin-E). In the methanolic leaf extract of *P. pinnata* the highest peak area (%) of 15.53 was obtained by 2-Thiopheneacetic acid, morpholide (retention-time 12.610) and the lowest peak area (%) of 0.12 was obtained by pentadecane (retention-time 17.252) (Figure 2). The detailed tabulation of the GC-MS analysis of the above plant species has been given in Table 2.

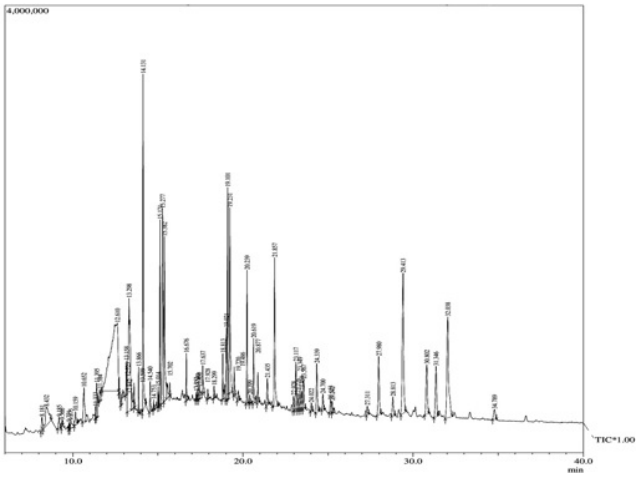


Figure 2: GC/MS spectrogram for the methanolic leaf extract of *Pongamia pinnata.*

S.No	R.T	Name of the Compound	Peak Area %	M.F	M.Wt	Compound Nature
1.	8.181	2,3-Dihydrobenzofuran	0.47	C_8H_8O	120	
2.	8.432	N,1-Dimethyl-4-piperidinamine	1.84	$C_7H_{16}N_2$	128	
3.	9.185	p-Vinylguaiacol	0.41	$C_9H_{10}O_2$	150.18	Aromatic flavoring agent
4.	9.366	2-Thiopheneacetic acid	0.13	$C_6H_6O_2S$	142.17	Organic acid
5.	9.771	Pentadecane	0.10	$C_{15}H_{32}$	212	Fragrance agents
6.	9.875	1-Ethyl-2-methylpyrrolidine	0.41	$C_7H_{15}N$	113	
7.	10.159	l-4-Hydroxylysine	0.50	$C_6H_{14}N_2O_3$	162.18	
8.	10.652	Trimethylpiperazine	1.42	$C_7H_{16}N_2$	128	
9.	11.333	2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (r)-	0.19	$C_{11}H_{16}O_2$	180.24	Flavoring agent
10.	11.395	Butyl hydroxy anisole	1.04	$C_{11}H_{16}O_2$	180.24	Antioxidant organic compound
11.	11.584	1-cyclohexyl-1-butanone	0.31	$C_{10}H_{18}O$	154.25	Flavoring agent
12.	12.610	2-Thiopheneacetic acid, morpholide	15.53	$C_{10}H_{13}NO_2S$	211.28	
13.	12.723	Tetradecanoic acid (Myristic acid)	0.16	$C_{14}H_{28}O_2$	228.37	Saturated fatty acid
14.	12.842	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	0.29	$C_{10}H_{12}O_3$	180.20	Alcohol
15.	13.158	2(4h)-benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6s-cis)-	0.66	$C_{11}H_{16}O_3$	196.10	Flavoring agent
16.	13.298	Tetradecanal	7.08	$C_{14}H_{28}O$	212.37	Flavor and fragrance agents
17.	13.599	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.40	$C_{20}H_{40}O$	296.54	Acyclic terpenoid
18.	13.866	Hexadecanoic acid, methyl ester	0.52	$C_{17}H_{34}O_2$	270.45	Saturated fatty acid.
19.	14.131	Pentadecanoic acid	5.97	$C_{15}H_{30}O_2$	242.4	Saturated fatty acid
20.	14.540	Benzenepropanoic acid, 2,5-dimethoxy	0.71	$C_{11}H_{14}O_4$	210.23	Fatty acid derivative
21.	14.753	Heptadecanoic acid (Margaric acid)	0.13	$C_{17}H_{34}O_2$	270.45	Saturated fatty acid
22.	15.014	8-Octadecenoic acid, methyl ester	0.30	$C_{19}H_{36}O_2$	296	fatty acid
23.	15.121	2-Hexadecen-1-ol,3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-;	2.79	$C_{20}H_{40}O$	296.60	Fatty acid
24.	15.277	9-Octadecenoic acid (Oleic acid)	5.44	$C_{18}H_{34}O_2$	282.46	Unsaturated fatty acid
25.	15.382	Octadecanoic acid (Stearic acid)	3.54	$C_{18}H_{36}O_2$	284.47	Saturated fatty acid
26.	15.702	Neophytadiene	0.17	$C_{20}H_{38}$	278.52	Hydrocarbon
27.	16.676	4,8,12,16-Tetramethylheptadecan-4-olide	0.70	$C_{21}H_{40}O_2$	324.54	
28.	17.252	2-methyl-3-hydroxy-2,3-dihydrobenzofuranone	0.12	$C_{10}H_{10}O_2$	162.18	Flavoring agent
29.	17.340	1,3,5-Trisilacyclohexane	0.19	$C_3H_{12}Si_3$	132.38	

30.	17.430	Trichloroacetic acid, hexadecyl ester	0.35	$C_{18}H_{33}Cl_3O_2$	387.81	Fatty acid
31.	17.637	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.58	$C_{19}H_{38}O_4$	330	fatty acid
32.	17.928	1,2-Benzenedicarboxylic acid (Phthalic acid)	0.17	$C_8H_6O_4$	166.13	Aromatic dicarboxylic acid
33.	18.299	Dichloroacetic acid, undec-2-enyl ester	0.22	$C_{13}H_{22}Cl_2O_2$	281.219	Fatty acid
34.	18.813	1,3,5-Trisilacyclohexane	0.75	$C_3H_{12}Si_3$	132	Organic Compound
35.	19.021	2-phenyl-furo[b]benzopyran-4(4h)-one (Karanjin)	1.78	$C_{18}H_{12}O_4$	292.29	Flavonoid
36.	19.101	2-[5-(2-Methyl-benzooxazol-7-yl)-1H-pyrazol-3-yl]-phenol	4.15	$C_{17}H_{13}N_3O_2$	291	Organic compound
37.	19.231	Octadecanoic acid, 2,3-dihydroxypropyl ester	4.30	$C_{21}H_{42}O_4$	358.55	Fatty acid
38.	19.486	2-phenyl-5-hydroxy-furo[b]benzopyran-4(4h)-one	0.60	$C_{17}H_{10}O_4$	278	
39.	19.720	2-propenoic acid, 3-(3-nitrophenyl)-, ethyl ester	0.29	$C_{11}H_{11}NO_4$	221	Fatty acid
40.	20.239	Squalene	2.27	$C_{30}H_{50}$	410.72	Triterpene
41.	20.399	Phenanthro[3,2-b]furan-7,11-dione, 1,2,3,4,8,9-hexahydro-4,4,8-trimethyl-, (+)-	0.14	$C_{19}H_{20}O_3$	296	
42.	20.619	2,5-Cyclohexadiene-1,4-dione, 2,5-dihydroxy-3,6-diphenyl-	1.44	$C_{18}H_{12}O_4$	292	
43.	20.877	1-Eicosanol	0.64	$C_{20}H_{42}O$	298.55	Fatty alcohol
44.	21.435	Sericic acid	0.76	$C_{30}H_{48}O_6$	504	Phenolic acid
45.	21.857	δ-Tocopherol	3.97	$C_{27}H_{46}O_2$	402	Vitamin- E
46.	22.978	Humulane-1,6-dien-3-ol	0.38	$C_{15}H_{26}O$	222.36	Monocyclic sesquiterpene
47.	23.117	Beta-tocopherol	1.18	$C_{28}H_{48}O_2$	416.68	Vitamin- E
48.	23.226	Boldine	0.45	$C_{19}H_{21}NO_4$	327.37	Alkaloid
49.	23.349	Tocopherol	0.51	$C_{28}H_{48}O_2$	416	Vitamin- E
50.	23.460	Solanesol	0.54	$C_{45}H_{74}O$	630	Terpene
51.	23.567	1-Triacontanol	0.86	$C_{30}H_{62}O$	438.81	Fatty alcohol
52.	24.022	Tetra-O-methylfisetin	0.24	$C_{19}H_{18}O_6$	342	Flavonoid
53.	24.339	Labetalol diacetate	1.51	$C_{23}H_{28}N_2O_5$	412	Anti hypertensive agent
54.	24.700	α-Tocopherol	0.46	$C_{29}H_{50}O_2$	430.71	Vitamin- E
55.	25.153	Cholesterol	0.38	$C_{27}H_{46}O$	386.65	Phytosterol
56.	25.242	3,5,6-Trimethoxyfurano-(7,8,2",3")-flavone	0.37	$C_{20}H_{16}O_6$	352.33	Flavonoid
57.	27.311	Campsterol	0.31	$C_{28}H_{48}O$	400.68	Phytosterol
58.	27.980	Stigmasterol	2.10	$C_{29}H_{48}O$	412.69	Phytosterol
59.	28.813	Tris(3-fluorophenyl)boroxin	0.70	$C_{18}H_{12}B_3F_3O_3$	365.71	Organic compound
60.	29.413	β-Sitosterol	6.01	$C_{29}H_{50}O$	414.71	Phytosterol

61.	30.802	B- amyrin	2.00	$C_{30}H_{50}O$	426.73	Phytosterol
62.	31.346	Lupeol	2.03	$C_{30}H_{50}O$	426.73	Triterpenoid
63.	32.038	Betulin	5.54	$C_{30}H_{50}O_2$	442.72	Triterpene
64.	34.789	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	0.49	$C_{20}H_{40}O$	296.53	Diterpene alcohol

Table 2: Activity of phytocomponents identified in the methanolic leaf extract of *Pongamia pinnata*.

Furthermore, the identification of (unknown) plant constituents is still of interest [10,11], as it is the first step to explain the benefits of traditionally used medicinal plants by scientific means [12]. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry.

Conclusion

The present research study states that, methanolic leaf extract sample of *P. pinnata* was first time analyze. The comparison of the mass spectrum with the NIST database library gave more than 90% match as well as a supportive compound structure match. This study will help to identify the compounds, which may be used in body products, drugs, pharmaceutical and therapeutic value. Based on the present inventory of biochemical compounds we conclude that it may be used for medical purposes. This knowledge can be used for the further development of phytomedicines from this plant species.

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