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Original Article

ISOLATION AND IDENTIFICATION OF A NEW PHYTOSTEROL FROM *HOLOPTELEA INTEGRIFOLIA* (ROXB) PLANCH LEAVES

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ABSTRACT

Objective: The Plant *Holoptelea Intergrifolia* (Roxb.) Planch is being used for the treatment of various disorders since time immemorial in the indige nous system of medicine in India. The main objective of the work was to isolate a new phytosterol from petroleum ether extract of *Holoptelea Integrifolia* leaves using Preparative Thin Layer Chromatography (TLC).

Methods: As per ICH guidelines we have Prepared Thin Layer Chromatographic plates for separation of a new phytosterol from Petroleum ether extract of leaves of *Holoptelea Intergrifolia* (Roxb.) Planch. The mobile phase used for separation of phytosterol consisted ofChloroform: Ethyl acetate, in the volume ratio of 4:6 (v/v), UV, LC/MS, IR and NMR spectral analytical techniques were used for identification and confirmation of structure of a new Phytosterol by Preparative TLC.

Results: Preliminary phytochemical analysis of petroleum ether extract of *Holoptelea integrifolia* leaves showed the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins and carbohydrates. The isolated phytosterol designated as17-(6-(diethylamino) decan-3-yl)-10,13-dimethyl-12,13-dihydro-10H-cyclopenta[a] phenanthren-3-ol. It responded positively to Liebermann Burchard test indicating steroidal nature of the molecule.

Conclusion: On the basis of spectral data analysis and chemical reactions, the structure of a new phytosterol isolated by preparative TLC from petroleum ether extract of leaves of *Holoptelea Integrifolia* (Roxb.) Planch has been formulated by UV, LC/MS, IR and NMR spectral analysis as 17-(6-(diethylamino) decan-3-yl)-10,13-dimethyl-12,13-dihydro-10H-cyclopenta[a] phenanthren-3-ol. This is a new phytosterol isolated from plant source and being reported for the first time.

Keywords: *Holoptelea Integrifolia* leaves, UV, LC/MS, IR, NMR, Phytosterol.

INTRODUCTION

The Plant Holoptelea Intergrifolia (Roxb.) Planch is being used for the treatment of various disorders since time immemorial in the indigenous system of medicine in India.

The bark and leaves of Holoptelea Integrifoila used as bitter, astringent, acrid, thermogenic, anti inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism [1,2].

The plant Holoptelea integrifolia is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea [3]. The mucilaginous bark is boiled and the juice squeezed out and applied to rheumatic swellings [4]. The stem bark contains the triterpenoidal fatty acid esters, holoptelin-A (epi-friedelinol palmitate) and holoptelin-B (epi-friedelinol stearate), friedelin and epi-friedelinol. sitosterol and stigmasterol are isolated from dried seed. Histamine and 5- hydroxytryptamine are isolated from pollens [5]. 2-aminonaphthoquinone, Friedlin, -sitosterol, -D-glucose, are also isolated from stem bark [6].

The leaves of Holoptelea integrifolia ethanolic extract showed the presence of terpenoid, steroids, tannins, saponins, carbohydrates and protein. 1,4 naphthalene- dione has been isolated from leaves of Holoptelea integrifolia and is reported to possess antibacterial activity against Staphylocopccus aureus[7], hexacosanol, octacosanol, Beta sitosterol Beta amyrin are isolated from leaves. Beta-sitosterol, 2",3"-dihydroxyoelan12-en-28 oic acid and hederagenin are isolated from heartwood [8]. The leaves contain friedelin or friedelin type compounds has been considered for treatment of cancer of bladder, convulsions, inflammation, topical ulcers, rheumatic inflammation, fever and dysentery [9].

MATERIALS AND METHODS

Plant Introduction and Collection

Holoptelea Integrifoila belongs to the family ulmaceae commonly called as Indian Elm and commonly used in India by the tribal people for it's medicinal properties. Leaves of Holoptelea Integrifoila were collected in the Month of August from the agricul-tural fields of Tirunelveli district, Tamil Nadu, India The plant was identified and leaves of Holoptelea Integrifolia were authenticated and confirmed from Dr.V.Chelladurai, Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of India by compairing morphologicalfeatures(leaf and stem arrangement, flower /inflorescence arrangement ,fruit and seed morphology etc.). The collected plant material was shade dried to retain its vital phytocons-tituents and then subjected to size reduction for further extraction process.

Instruments and Chemicals used:

The solvent used for extraction was Petroleum ether. Other reagents used were of laboratory grade and obtained from various other commercial sources. All the reagents used were of laboratory and analytical grade. Solvents were obtained from SD Fine Chem Ltd. (Mumbai). UV spectra was recorded using CAMAG TLC Scanner - IV, LCMS was recorded using SHIMADZU- LC/MS 2020, IR spectra was recorded using SHIMADZU-IR PRESTIGE-21, NMR spectra was recorded using Mercury plus 300 MH_Z NMR Spectrometer.

Preparation of petroleum ether extract

The powder of *Holoptelea Integrifoila* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using petroleum ether. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the

previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50° C to obtain petroleum ether extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The yield of the dried petroleum ether extract of leaves was found to be 4.8 % w/w. The perfectly dried extract was then stored in an air tight container in a refrigerator below 10°C. The Petroleum ether extract of *H. Integrifolia* leaves was subjected to the following investigations,

1. Preliminary phytochemical screening.

2. Isolation of phytosterol by TLC.

Preliminary phytochemical testing of extract

The extract was subjected to following chemical tests to detect the chemical constituents present in it. 0.5 gm of extract was dissolved in 5 ml of distilled water and filtered. The filtrate was used to determine the presence of various phytoconstituents[10].

Isolation of Phytosterol by preparative TLC

High Performance Thin Layer Chromatography (HPTLC) instru mentation

Chromatographic conditions

The petroleum ether extract sample was prepared in petroleum ether as a sample solution was spotted in the form of bands of width 8.0 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F254 (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists,Mumbai) using a Camag Linomat V (Switzerland). The petroleum ether extract sample Volume applied was 250 µl for recording on each plate. A total of 40 plates were recorded. A constant application rate of 1.0 µl/s was employed and space between two bands was 5 mm. The slit dimension was kept at 6.0mm × 0.45 mm and 10 mm/s scanning speed was employed. The slit bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used.

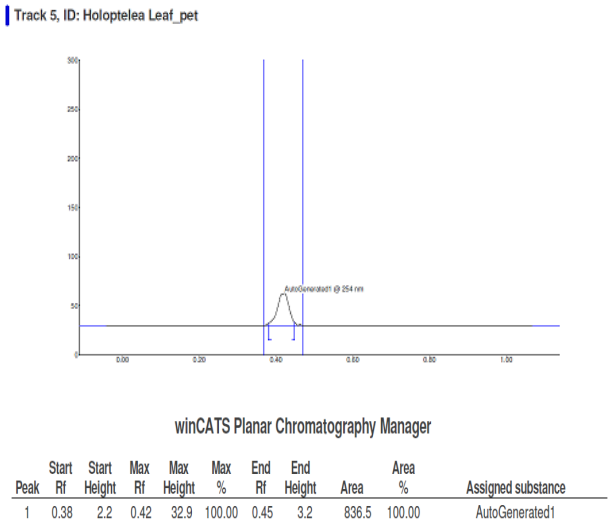
The mobile phase for phytosterol consisted of Chloroform:Ethyl acetate, in the volume ratio of 4:6 (v/v) for isolation of phytosterol and 20 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with filter paper whatman no:1 in the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature (25° C ± 2) at relative humidity of 60% ± 5. The length of chromatogram run was 8.0 cm. Subsequent to the scanning, TLC plates were dried in a current of air with the help of an air dryer.

Densitometric scanning was performed with Camag TLC scanner IV in the reflectance absorbance mode at 540 nm and operated by Win CATS software (1.4.6 Camag) with the help of tungstant lamp. Subsequent to the development; TLC plate was dried in oven at 110°C. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was carried out by comparing peak areas with linear regression [11-19].

RESULTS

Preliminary phytochemical examination of petroleum ether extract

Preliminary phytochemical analysis of petroleum ether extract of Holoptelea integrifolia Leaves showed the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins and carbohydrates.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.38	2.2	0.42	32.9	100.00	0.45	3.2	836.5	100.00	AutoGenerated1

Fig. 1: HPTLC chromatogram of a new phytosterol obtained by preparative TLC from petroleum ether extract of leaves of *Holop telea Integrifolia* (Roxb.) Planch

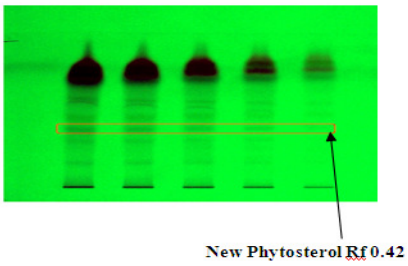
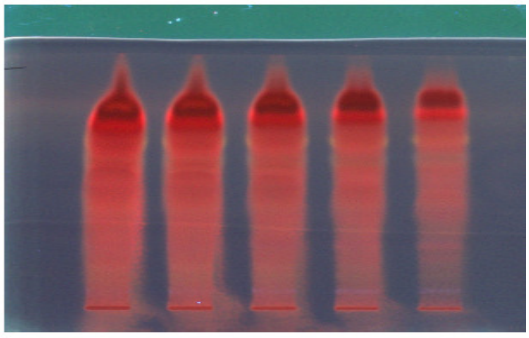
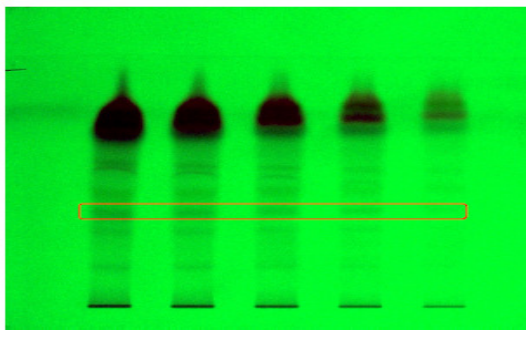


Fig. 2: UV Spectra of a new phytosterol at 254 nm, isolated from Petroleum ether extract of leaves of *Holoptelea Integrifolia* (Roxb.) Planch using Preparative TLC

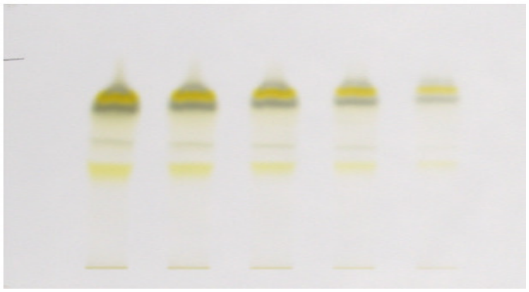
After observing the Chromatoram and spectra (Fig.1 and 2 respectiv ely) for HPTLC of phytosterol isolated by preparative TLC in petroleum ether extract of leaves of *Holoptelea Integrifolia* (Roxb.) Planch the spectra showed Rf value 0.42 for new phytosterol.



A) at 366 nm



B) at 254 nm



C) Visible light

Fig. 3: HPTLC spectra of a new phytosterol in Petroleum ether extract of leaves of *Holoptelea Integrifolia* (Roxb.) Planch, Volume applied 250 µl.

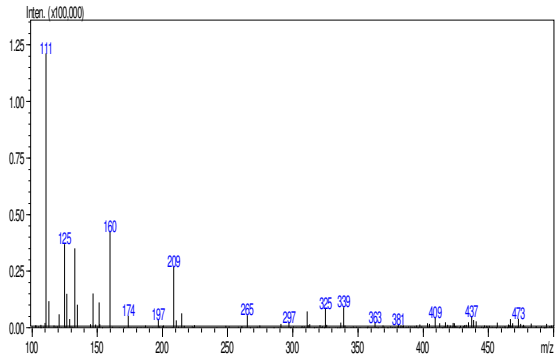


Fig. 4: Liquid Chromatography/ Mass Spectrometry (LC/MS) of i solated new phytosterol (Rf: 0.42)

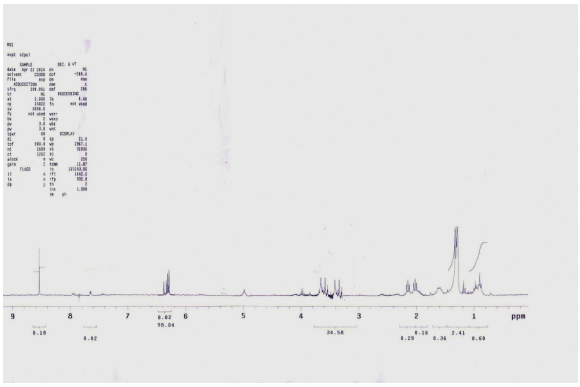
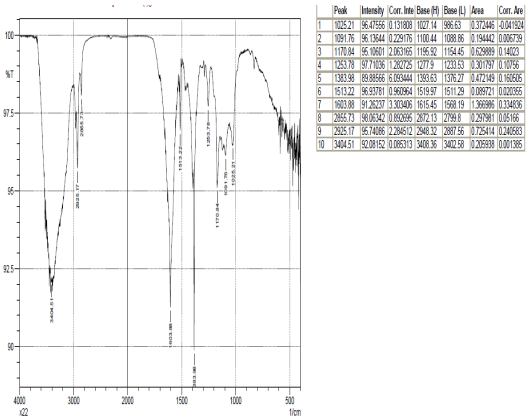
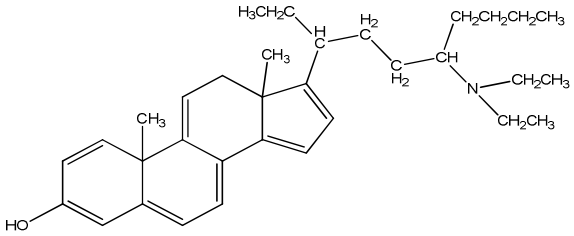


Fig. 6: NMR Spectrum of isolated new Phytosterol (Rf: 0.42)



Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	1025.21	96.47566	0.131808	1027.14	986.63	0.372446	-0.0419324
2	1091.76	96.13644	0.229176	1100.44	1089.86	0.194442	0.0067135
3	1170.84	95.19081	2.063165	1196.92	1154.45	0.639869	0.14023
4	1253.79	97.71836	1.262725	1277.9	1233.53	0.301797	0.10756
5	1383.98	89.88566	6.693444	1393.63	1376.27	0.472149	0.1628265
6	1513.22	96.93781	0.960964	1519.97	1511.29	0.089721	0.0202695
7	1603.88	91.26237	3.303406	1615.45	1598.19	1.366986	0.3348396
8	2855.73	98.06342	0.852695	2872.13	2799.8	0.297981	0.051166
9	2925.17	95.74086	2.284512	2948.32	2897.56	0.725414	0.2405693
10	3404.51	92.08152	0.085313	3408.36	3402.58	0.205938	0.0001385

Fig. 5: IR Spectrum of isolated new phytosterol (Rf: 0.42)



Chemical Formula: $C_{33}H_{47}NO$
Molecular Weight: 473.73
m/z: 473.37 (100.0%), 474.37 (36.3%), 475.37 (6.5%)
Elemental Analysis: C, 83.67; H, 10.00; N, 2.96; O, 3.38

IUPAC NAME: 17-(6-(diethylamino) decan-3-yl)-10,13-dimethyl-12,13-dihydro-10H-cyclopenta[a] phenanthren-3-ol

Fig. 7: Probable structure and IUPAC name of a new phytosterol from elemental analysis, LC/MS, IR and NMR may be

Table 1: FT-IR and NMR Spectral data analysis of a new phytosterol (Rf: 0.42) isolated from petroleum ether extract of leaves of *Holoptele a Integrifolia* (Roxb) Planch using preparative TLC

S. No.	IR range (cm^{-1})	Functional group	NMR value (δ ppm)	Protons
01	3404	-OH str.	5.0	1H of –OH, singlet
	2925, 2855	-CH alkyl str.	6.2-6.8	10H of aromatic, multiplet
	1603	-C-N str.	3.2-3.6	4H of –CH-CH-, triplet
	1513	-C=C- aromatic bend	1.8-2.2	19 H of alkyl, unidentified
	1170, 1253, 1091&1025	-C-O-C- str.	0.8-1.4	13 H of alkyl, unidentified

DISCUSSION

Fig.1 Shows HPTLC chromatogram of phytosterol obtained by Prepa rative TLC from

petroleum ether extract of leaves of Holoptelea Integrifolia (Roxb.) Planch with Rf value of 0.42. Fig.2 Shows UV Spectra of Phytosterol at 254 nm, isolated from Petroleum ether ext-ract of leaves of Holoptelea Integrifolia (Roxb.) Planch. Fig. 3 shows HPTLC spectra of phytosterol in Petroleum ether extract of leaves of Holoptelea Integrifolia (Roxb.) Planch, the volume applied was 250 ml at 366 nm, 254 nm and in visible light. Fig 4,5,6 Show LC/MS, IR and NMR Spectra of the isolated new Phytosterol from Petroleum ether

extract of leaves of Holoptelea Integrifolia (Roxb) Planch respectively. Table 1 Shows FT IR and NMR Spectral data analysis of the isolated Phytosterol. While the Fig 7 shows the Probable structure and IUPAC name of the isolated new phytosterol on the basis of spectral data analysis from LC/MS, IR and NMR and chemical reaction studies from Petroleum ether extract of leaves of Holoptelea Integrifolia (Roxb) Planch by using Preparative TLC. The isolated phytosterol designated as17-(6-(diethylamino) decan-3-yl)-10,13-dimethyl-12,13-dihydro-10H-cyclopenta[a] phenanthren-3-ol. It responded positively to Liebermann Burchard test indicating steroidal nature of the molecule.

CONCLUSION

On the basis of spectral data analysis and chemical reactions, the structure of a new phytosterol isolated by preparative TLC from petroleum ether extract of leaves of *Holoptelea Integrifolia* (Roxb.) Planch has been formulated by UV, LC/MS, FT-IR and NMR analysis as 17-(6-(diethylamino) decan-3-yl)-10,13-dimethyl-12,13-dihydro-10H-cyclopenta[a] phenanthren-3-ol. This is a new phytosterol isolated from plant source and being reported for the first time.

CONFLICT OF INTERESTS

Declared None

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