WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 7, Issue 2, 806-818 Research Article ISSN 2278 – 4357

PHYTOCHEMICAL INVESTIGATION & CHARACTERIZATION OF HEARTWOOD OF *PTEROCARPUS MARSUPIUM* ROXB. (FAMILY: LEGUMINOCEAE).

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Article Received on
25 Nov. 2017,
Revised on 15 Dec. 2017,
Accepted on 05 Jan. 2018
DOI: 10.20959/wjpps20182-10835

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ABSTRACT

***Pterocarpus marsupium* Roxb.** (Family Leguminoceae) is found throughout India. The plant is reputed to possess a diverse range of medicinal benefits, among these its hypoglycemic potential is of importance.[1] Qualitative phytochemical evaluation for ethyl acetate & methanol extract was carried out and a Compound Pterostilbene was separated by Preparative TLC Method & the presence of Pterostilbene was confirmed by HPLC and HPTLC techniques using standard Pterostilbene. The isolated compound was characterized by UV, IR, ^{13}C NMR & H^{1}NMR.

KEYWORDS: *Pterocarpus marsupium*, Pterostilbene, HPTLC, HPLC, ^{13}C NMR, H^{1}NMR.

INTRODUCTION

Pterocarpus marsupium Roxb. (Family Leguminoceae) commonly known as Bijasar, is well known drug in Indian system of medicine and is widely cultivated in India, for its medicinal properties in Ayurvedic and Unani system for treatment of diabetes.[1] *Pterocarpus* is a rich source of polyphenolic compounds such as Stilbene, Pterostilbene catechin, epicatechin, the flavonoids, pseudobaptigenin, liquiritigenin, the chalcone, isoliquiritigenin etc.[2]

The plant shows strong antihyperlipidemic activity[3], hepatoprotective activity[4], antioxidant properties[5] and useful in treatment of liver damage[6] and antidiabetic activity.[7, 8] Bark and heartwood of *Pterocarpus marsupium* have been used in various Ayurvedic formulations.

The plant and the gum are hot and bitter with a sharp taste, laxative anthelmintic. The flowers are sweet, bitter; improving the appetite, cause flatulence. The gum is bitter with a bad taste, useful in all diseases of the body, styptic, vulnerant, tonic to the liver, antipyretic, anthelmintic. The gum is used for toothache on the Coromandel Coast. It is a good astringent in diarrhea and pyrosis. Bark is used as an astringent. The bruised leaves are useful as an external application to boils, sores and skin diseases.[1]

MATERIAL AND METHODS

The commercial plant extract obtained from Amsar Goa Pvt Ltd, was extracted with petroleum ether, ethyl acetate and methanol. Color, pH, nature and yield of both extract were recorded and compared (Table 1) fractions of commercial extract were obtained in very good yield than plant extract; (hence commercial extract was selected for further studies).

Table 1: Comparison of yield of extracts.

Tests	Fractions of Plant extract			Fractions of Commercial extract		
	Petroleum Ether (60-80°C)	Ethyl acetate	Methanol	Petroleum ether	Ethyl acetate	Methanol
Color	Yellow	Brown	Brown	Yellow	Brown	Brown
PH	6.1	6.5	6.56	6.1	6.54	6.56
Nature	Sticky	Powder	Powder	Sticky	Powder	Powder
Yield (%)	0.41	5.5%	14.9%	0.52%	8.8%	45.9%

Both extracts were evaluated qualitatively & showed presence of carbohydrates, steroids, saponin, tannins and phenolics compounds. (Table 2).

Table 2: Qualitative evaluation of extracts[9]

Tests	Methanol solution of Commercial extract	Methanol solution of Plant material
Carbohydrates	+	+
Proteins	-	-
Fats and Oils	-	-
Amino acids	-	-
Steroids	+	+
Glycosides (Saponin glycosides)	+	+
Alkaloids	-	-
Tannins and Phenolic compounds	+	+

- Indicates negative test,

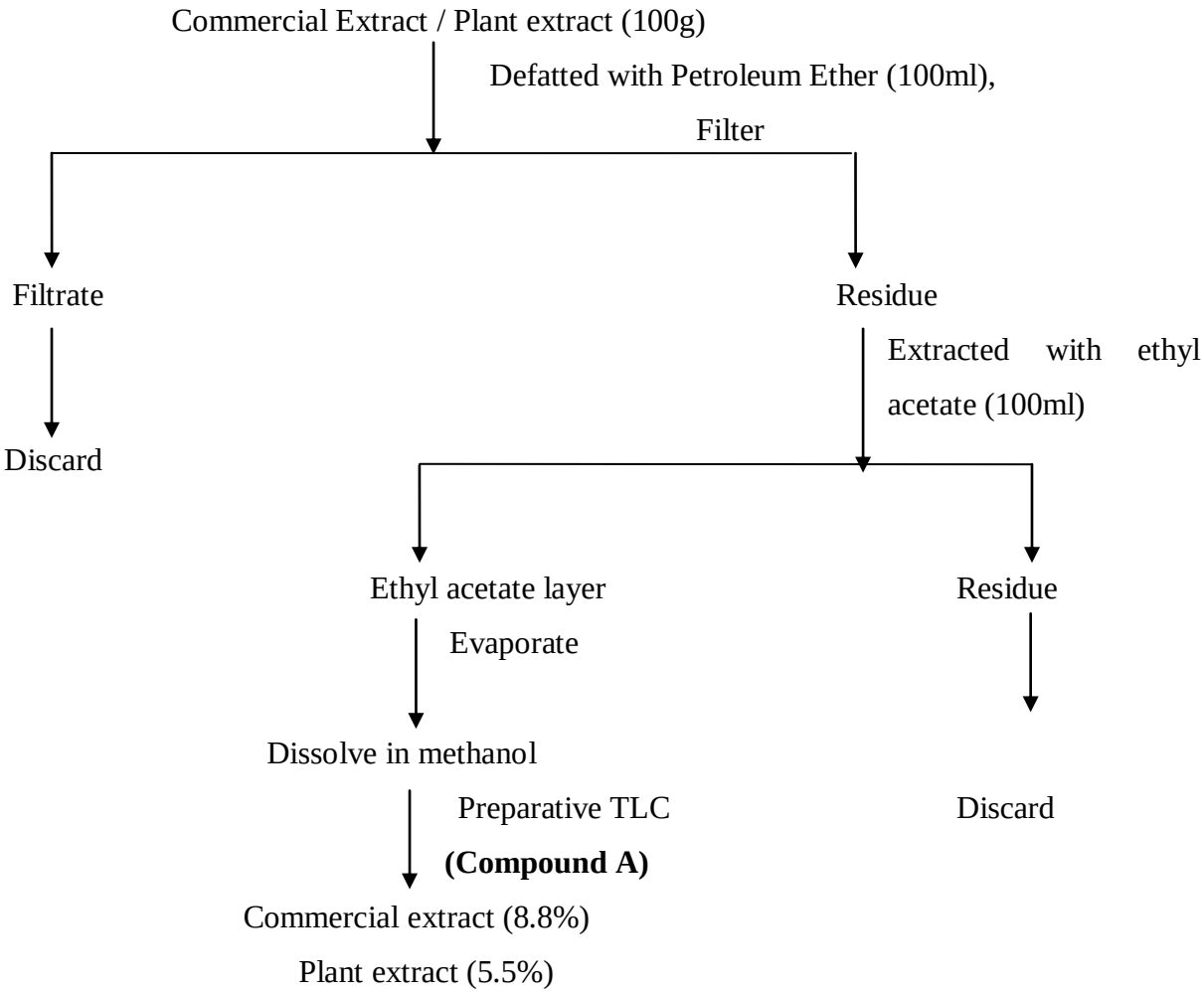
+ Indicates positive test.

TLC / HPTLC studies

Preparation of Ethyl acetate extract

The commercial extract was defatted with petroleum ether (60-80^{0}c) (100 ml). Petroleum ether layer was then discarded and residue was extracted with ethyl acetate for 1 hr. The ethyl acetate layer was evaporated and dissolved in methanol and used for spotting on prepared TLC plates. Yield of ethyl acetate extract was calculated. Various mobile phases were tried for ethyl acetate extract. Chloroform and methanol (8:2) gave maximum resolution for ethyl acetate extract. So it was selected for further isolation studies.

SCHEME: For Isolation of Phytoconstituent (Stilbene) (Compound A)



HPTLC studies of Ethyl acetate extract

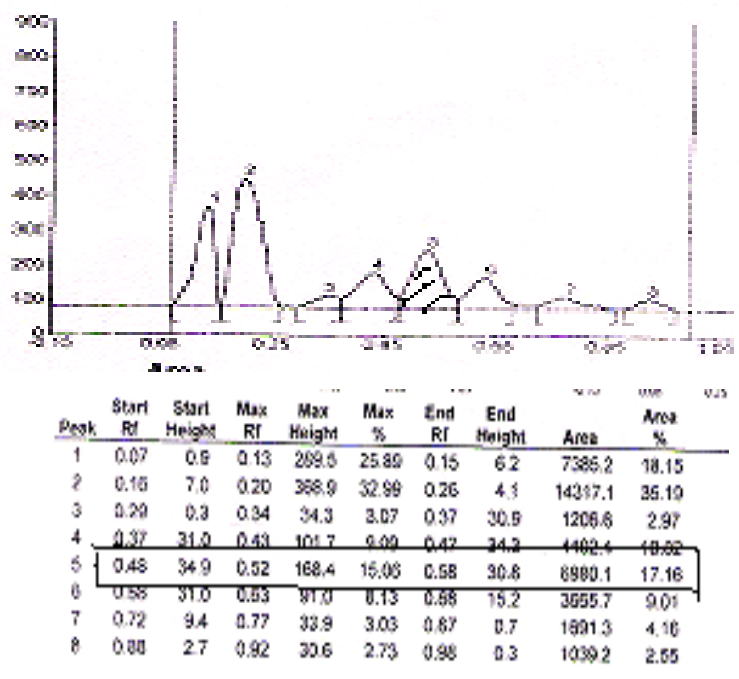
For HPTLC, precoated silica gel GF 254 (thickness 0.2 mm) plates were used. The plates were activated in an oven at 105^{0}C for 20 min. Regular chromatographic glass chamber (12 X 12 X 5 cm) with glass lid filtered from inside with filter paper were used for development of plates.

10 mL of solvent system was poured into the chamber and allowed to equilibrate for 30 min. The commercial ethyl acetate extract was dissolved in methanol and applied as a band by CAMAG LINOMAT IV applicator. The plates were developed by ascending technique using, chloroform: methanol (8:2) as the mobile phase. After development the plates were dried, at R.T.

Spots were located under UV light at 254 nm, 366 nm and 540 nm. The plates were scanned using CAMAG SCANNER III with WINCATS III software (Fig 1) (Table 3). Component A was isolated from ethyl ether extract, by preparative TLC (Fig 6&7). Video images of ethyl acetate extract were taken, at 254, 366 and 540 nm (Fig.2&3).

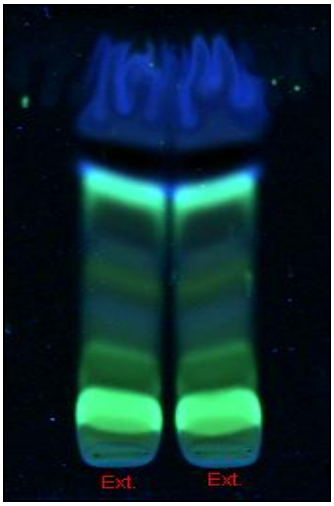
Table 3: HPTLC analysis of Ethyl acetate extract.

Description	At 366nm
No. Of spots	8
Rf	0.13,0.20,0.34,0.46,0.52, 0.63,0.77,0.92



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.07	0.9	0.13	269.6	25.89	0.15	6.2	7386.2	18.15
2	0.16	7.0	0.20	368.9	32.99	0.26	4.1	14317.1	35.19
3	0.29	0.3	0.34	34.3	3.07	0.37	30.9	1206.6	2.97
4	0.37	31.0	0.43	101.7	9.09	0.47	34.2	1402.4	18.02
5	0.46	34.9	0.52	168.4	15.05	0.58	30.8	6980.1	17.16
6	0.58	31.0	0.63	91.0	8.13	0.68	15.2	3655.7	9.01
7	0.72	9.4	0.77	33.9	3.03	0.87	0.7	1691.3	4.16
8	0.88	2.7	0.92	30.6	2.73	0.98	0.3	1039.2	2.55

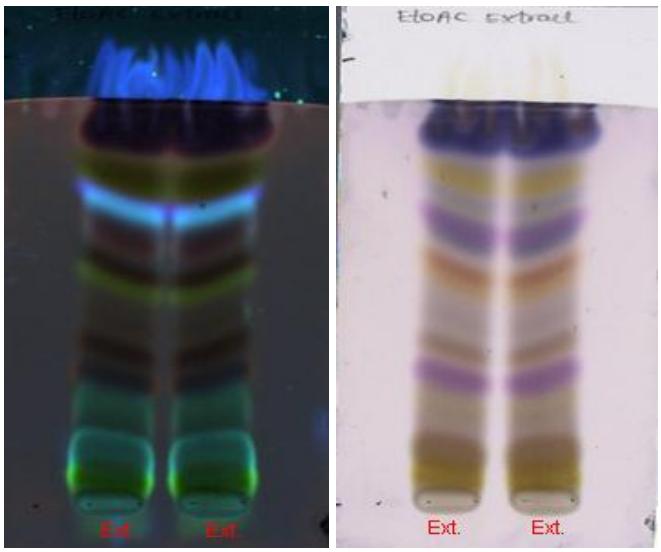
Fig 1: HPTLC Fingerprint of Ethyl acetate extract at 366nm



At 366nm

Fig. 2. Photodocumentation of Ethyl Acetate Extract.

Before Derivatisation



At 366nm **at 540nm**

Fig. 3. Photodocumentation of Ethyl Acetate Extract After Derivatisation with ASR.

Preparative TLC method

For preparative TLC glass plates of (10×10cm) were coated with silica gel of GF254 (Merck). Slurry of silica gel was prepared in d/w by weighing about 45 g of silica gel and suspending it in 100 ml of distilled water. Preparative plates were prepared by pouring about 25 ml of slurry of Silica gel on the plates taking precautions to make a layer with even thickness on the surface of the plate. The plates were air-dried and were activated in an oven at 105ºC for 15min.

Rectangular chromatographic glass chamber (25×25×10 cm) with a glass lid and lined from inside with filter paper were used for the development of plates. 100 ml of the solvent system was poured into the chamber and allowed to equilibrate for 30min such as saturated with filter paper. The Ethyl acetate fraction of commercial extract of *Pterocarpus marsupium* was dissolved in methanol and applied by means of glass capillary in form of band. The plates were developed by ascending technique using chloroform with varying proportions of methanol. The plates were taken out and were air dried at Room Temperature.

Ten isolated bands were seen under UV light at 366 and 540 nm. Component having Rf 0.46 was isolated as it was exactly matching with the standard, marker compound.

The band was scrapped off and extracted with methanol. Purity of HPLC and components A was checked by HPLC and HPTLC method. Spots were located under UV Light at 254 and 366nm and 540nm. This band was scrapped off and extracted with methanol. Purity of both the components was checked by HPLC and HPTLC methods.

The plates were derived with 1) Anisaldehyde sulphuric acid. (ASR)(Table 4) 2) Vanillin sulphuric acid. (VSR)

RESULTS

Derivatisation

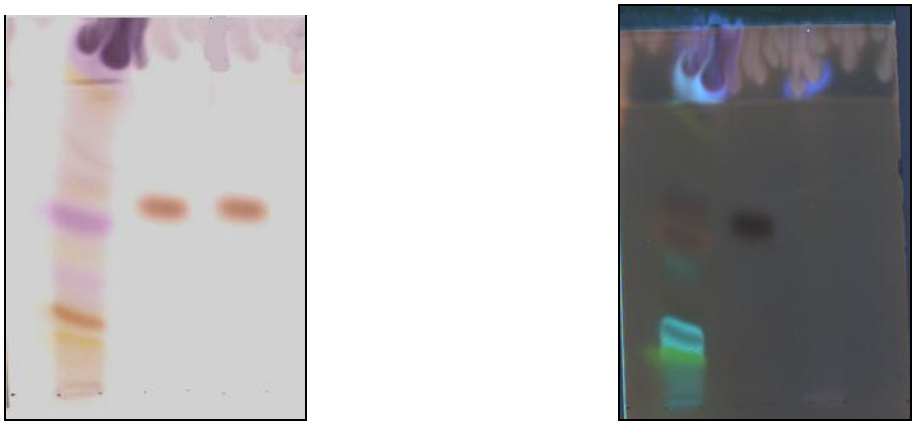
Table 4: Derivatisation of Compound A by TLC.

Sr. No.	Rf	Derivatising agent	Color before derivatisation		Color after derivatisation	
			At 254nm	At 365nm	At 365nm	At 540nm
A	0.46	ASR	Blue	Fluorescent blue	Fluorescent blue	Brownish pink

Photo documentation of Compound A

- When derivatised with ASR, a single well-isolated brownish pink color band was observed at Rf 0.46 for standard and Compound A respectively (fig. 4).
- When derivatised with VSR, a single well-isolated violet color band was observed at Rf 0.46 for standard and respectively (fig. 5).

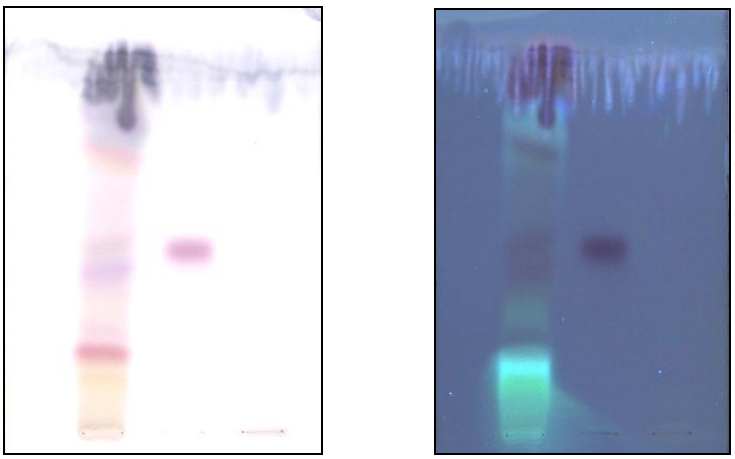
TLC/ HPTLC Studies: Compound A



Etract P1 Std **Ext P1**

At 540nm **at 366nm**

Fig 4: Photo documentation of isolated Compound A after Derivatisation with ASR.



At 540nm **at 366nm**

Fig 5: Photo documentation of isolated Compound A after Derivatisation with VS Reagent.

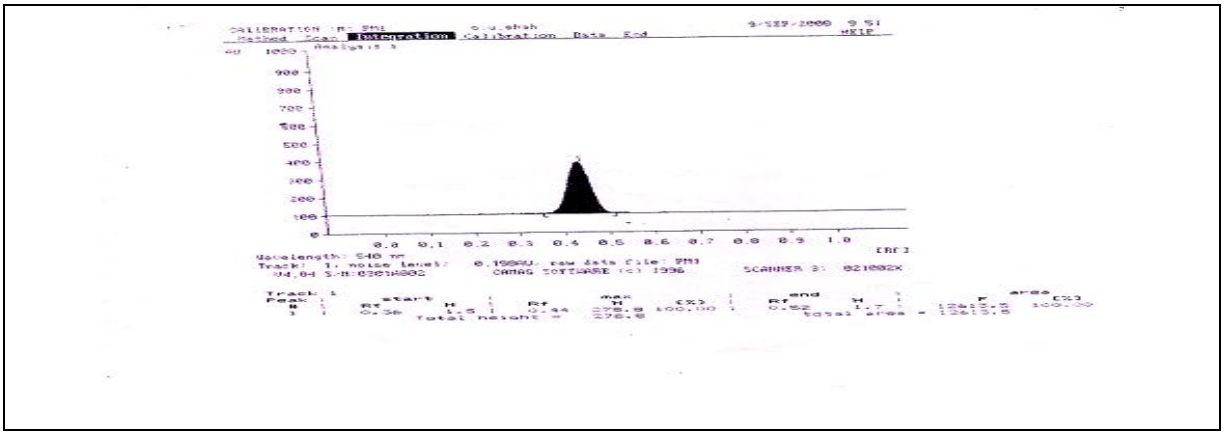


Fig 6: HPTLC Fingerprint of Standard at 540nm (Rf- 0.44)

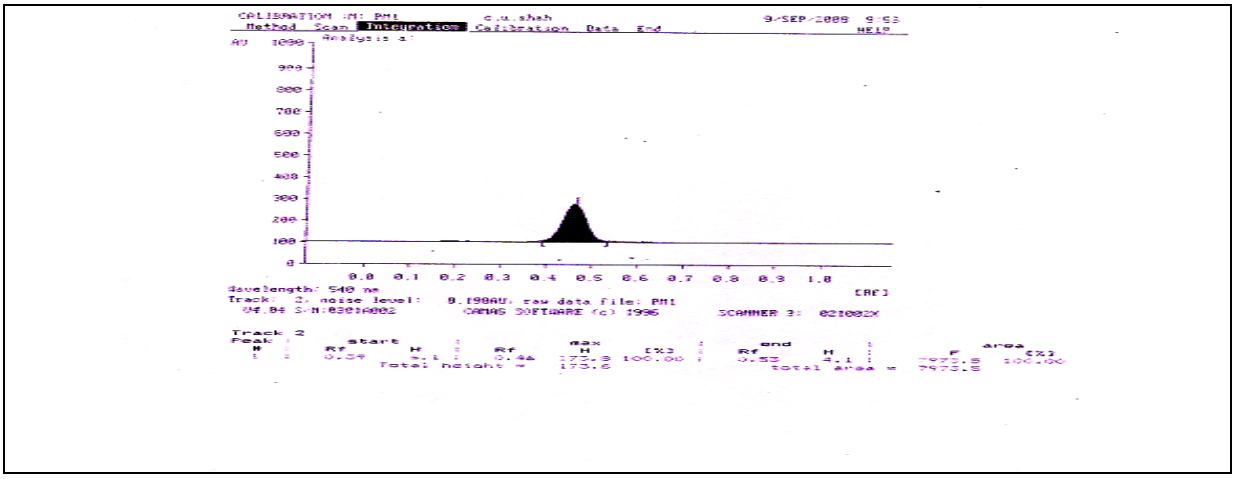


Fig 7: HPTLC Fingerprint of isolated component A at 540nm (Rf-0.46)

HPLC Studies of Compound A

The HPLC analysis was done on a TOSOH – CCPM SYSTEM USING UV- Visible detector. Column used was Phenomenex (250 x 4.60 mm). Stock solution was prepared by dissolving 0.1 gm of standard & isolated pterostilbene in methanol. Flow rate was 1.5 ml/min, wavelength used was 315nm & injection loop capacity was 100 µl.(Fig 8&9).

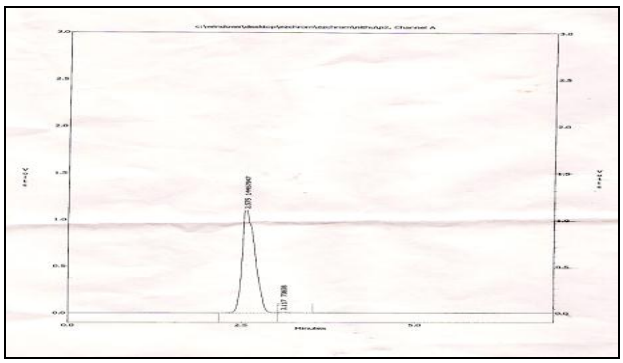


Fig 8: HPLC Fingerprint of isolated Compound A (R.T.-2.57).

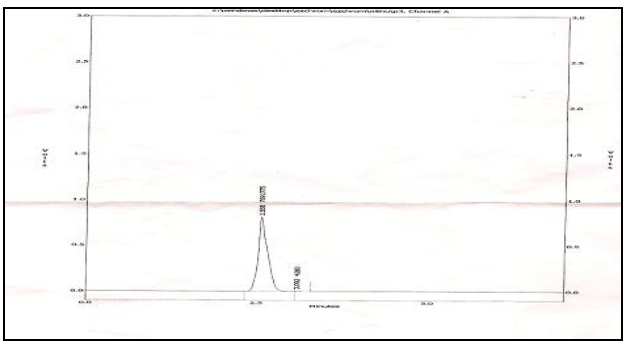


Fig 9: HPLC Fingerprint of Standard (R.T.-2.56).

The UV spectrum was recorded using JASCO UV visible spectrophotometer. UV analysis was done by dissolving small quantity of sample in methanol. Scanning was done for this sample. UV studies of Compound A showed UV λ max 317 nm [Fig.1].

The IR spectra were recorded using JEAL–FT NMR systems (300 MHz) spectrophotometer. IR spectra showed presence of hydroxyl group at 3441 cm^{-1}, alkenes at 1632 cm^{-1} and ether linkage at 1016 cm^{-1}. [Fig.2] [Table 3].

The NMR Spectra was recorded using JEAL FT NMR SYSTEM (300MHz) spectrophotometer.

H1 NMR spectra shows presence of aromatics protons δ=7.340, 7.312, 7.157, 7.103, 6.776, 6.721, 6.242, 6.214 ppm. and binary protons (S=6.320ppm) and hydroxyl group at 6.052 ppm.[Fig.3]

[Table 4]

^{13}C NMR shows presence of hydroxyl carbon at 158.404, 158.116, 158.012 ppm. Methoxy carbon at 166.404 ppm, C=C unsaturated carbon at 145.403, 140.203,140. 008 ppm and unsaturated carbon at 127.271, 124.522, 123.543, 116.228, 106.931, 107.197, 103.864, 102.558, 101.338 ppm. [Fig.4] [Table5].

.

Fig 10: UV Spectra of Standard and isolated compound A

_____ Standard

_____ Isolated

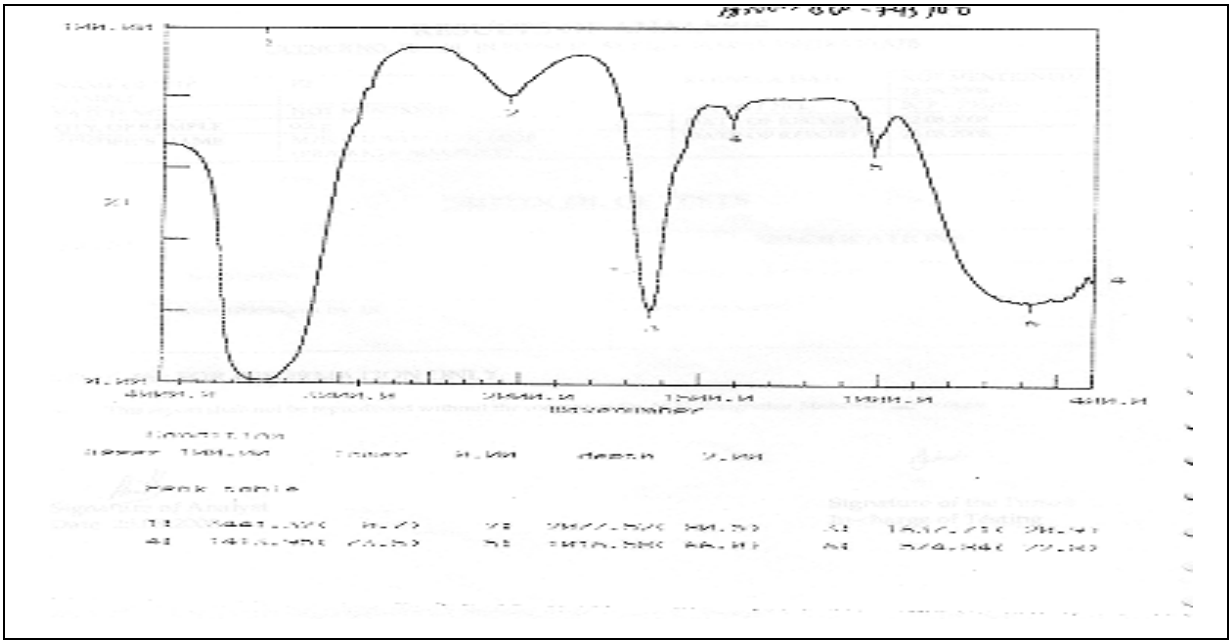


Fig.11: IR Spectra of Isolated compound A.

Table 5: IR Interpretation of isolated compound A.

IR Values (cm^{-1})	Interpretation	Absorption Range
3441	Aromatic OH stretch	3550-3200
1632	C=C stretch	1650-1200
1413	C=C Aromatic ring stretch	1600-1200
1016	-C-O-C-, ether stretch	1200-1000

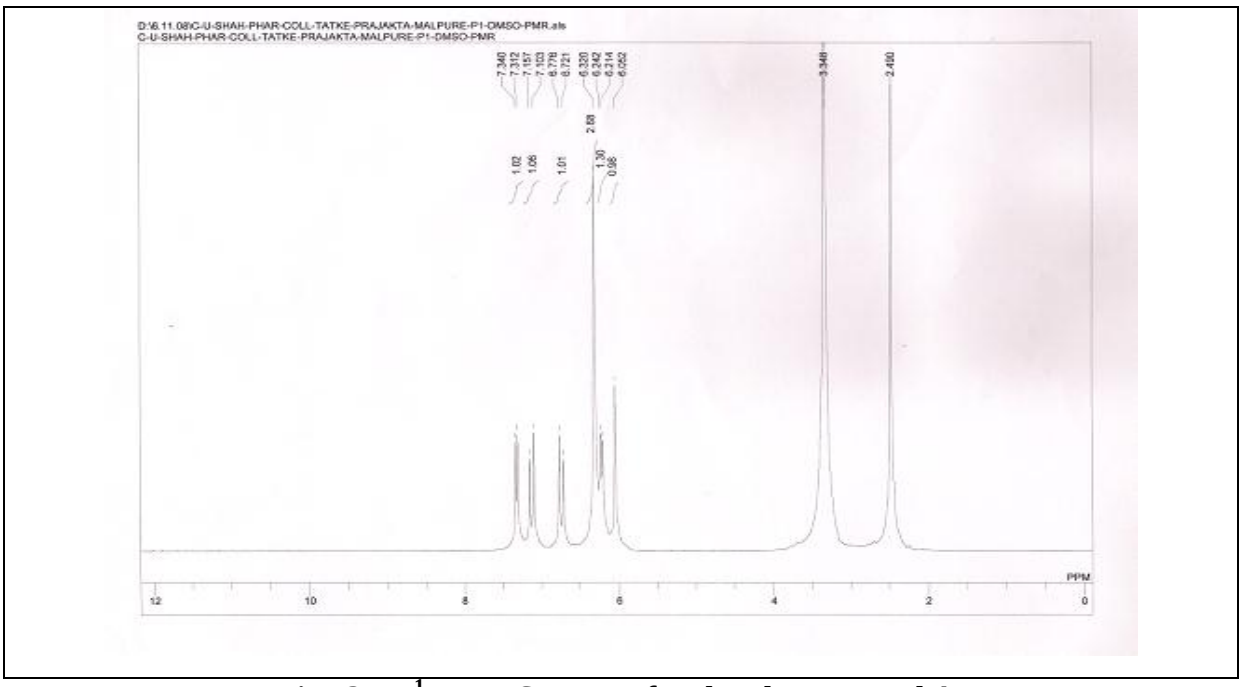


Fig.12: H^1NMR Spectra of Isolated compound A.

Table 6: H^1NMR spectra Interpretation of isolated compound A.

Chemical shift values (δ ppm)	Inference
2.490	(s, 3H)
7.340,7.312	(d, 2H)
7.157,7.103	(d, 2H)
6.776,6.721	(d, 2H)
6.242,6.214	(d, 2H)
6.320	(s, binary Protons)
6.052	(s, OH)

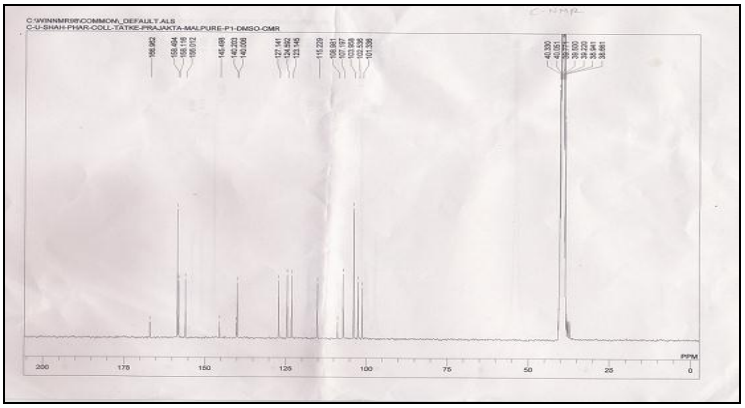


Fig 13: ^{13}C NMR Spectra of Isolated compound A.

Table 7: ^{13}C NMR Spectra Interpretation of isolated compound A.

Chemical shift (δ ppm)	Inference
166.404	C=O, Aromatic ketone
158.404, 158.116, 158.012	Carbon attached Hydroxyl group
145.403, 140.203, 140.008	C=C, unsaturated carbon
127.271, 124.522, 123.543	Unsaturated carbon
116.228, 106.937, 107.197, 103.864, 102.558, 101.338	Unsaturated carbon

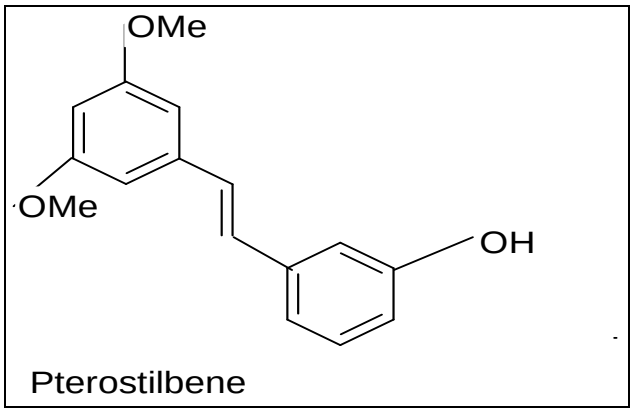
Pterostilbene

UV spectra showed λ max at 317 nm which is matching with standard. (Fig.10)

IR spectra showed Aromatics OH stretch (3441 cm^{-1}), C=C stretch (1632 cm^{-1}). (Fig.11) (Table 5). H^1 NMR spectra showed presence of aromatic protons, binary protons and hydroxyl group. (Fig. 12) (Table 6). ^{13}C NMR spectra revealed presence of Carbon attached to hydroxyl carbon, methoxy carbon, and unsaturated carbons (Fig.13) (Table 7).

DISCUSSION

- The methanolic fraction of commercial extract was compared with methanolic fraction of plant extract for yield and intensity of spots. Commercial extract showed better yield and hence selected for further studies.
- Preliminary Phytochemical screening of methanolic solution of extracts showed presence of carbohydrate, glycosides, tannins and phenolic compounds.
- HPTLC method was developed for ethyl acetate fraction of *Pterocarpus marsupium.*
- One compound (Compound A) was isolated successfully by Preparative TLC Method.
- HPTLC & HPLC Chromatograms were taken for isolated compound A which was matching with that of Standard Pterostilbene.
- Melting point of Compound A was obtained at 122-124°C, which matched with the melting point of Standard pterostilbene, 123°C and UV λmax at 317nm, which was matching with standard with UV λ max at 317nm.
- UV analysis of a Compound A showed λ max at 317 nm which is matching with the standard, Melting point of compound A was found to be 122-124°C, IR spectra showed presence of hydroxyl group, alkenes and ether linkage. H1 NMR spectra showed presence of aromatics protons, binary protons and hydroxyl group.
- ^{13}C NMR showed presence of carbon attached to hydroxyl carbon, methoxy carbon and unsaturated carbons.
- From the above chemical &spectral studies of the isolated compound A, it was confirmed as pterostilbene.



Pterostilbene

CONCLUSION

The Commercial ethyl acetate extract of *Pterocarpus marsupium* was investigated both qualitatively & quantitatively. From ethyl acetate extract Pterostilbene was identified by

HPLC, HPTLC and isolated in pure form by preparative TLC, then Pterostilbene was characterized by, UV, IR and ^{13}C *NMR,* H^{1}*NMR*. From the above chemical & spectral studies of the isolated compound A, it was confirmed as pterostilbene.

ACKNOWLEDGEMENT

We are grateful to Amsar Goa Pvt. Ltd. for providing us Commercial extract and standard Pterostilbene as a gift sample.

We acknowledge the help of Anchrom Laboratories, Mulund for providing us Video images of samples.

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