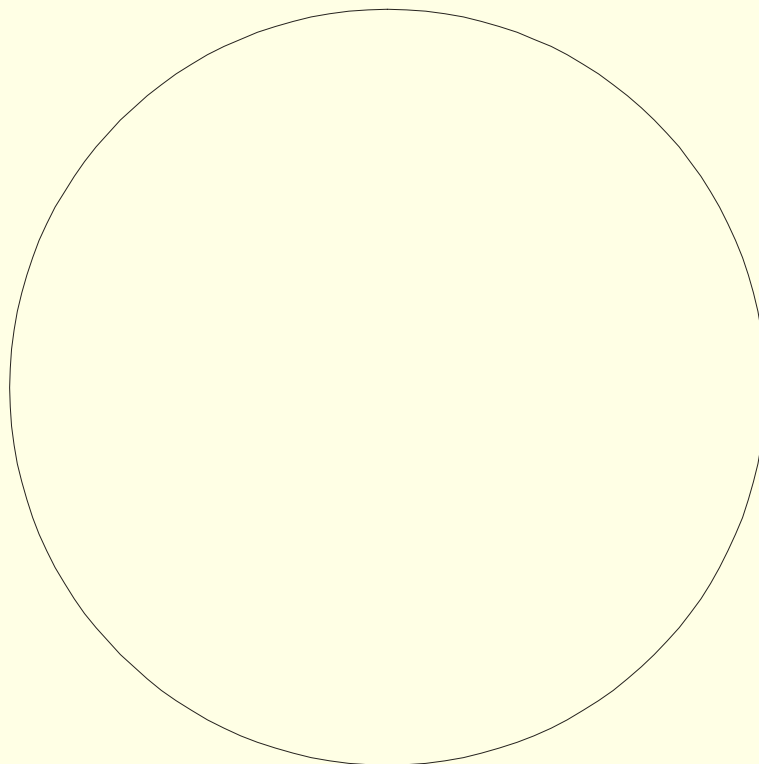


Phyllanthus amaru



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Hosur Road, Bangalore - 561 229



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Phyllanthus amarus Schum. and Thonn.

(a) Classification:

| | | |
|-----------------|---|-------------------------------|
| Kingdom | : | Plantae |
| Division | : | Angiospermae |
| Class | : | Dicotyledoneae |
| Order | : | Tubiflorae |
| Family | : | Euphorbiaceae |
| Genus | : | <i>Phyllanthus</i> |
| Species | : | <i>amarus</i> Schum. & Thonn. |

(b) Vernacular name:

| | | |
|-----------|---|--------------------------|
| Hindi | : | Jamgli amla, Jaramla |
| Kannada | : | Kirunelli |
| Malayalam | : | Kilarnelli, Kilukanelli |
| Tamil | : | Kilanelli, Kilakkainelli |
| Sanskrit | : | Bhumyamalaki |
| Bengali | : | Bhuiamla, sadahazurmani |
| Marathi | : | Bhuivali |
| Telugu | : | Nela usirika |



(c) Part used : Whole plant

(d) Botanical description: A herb that grows upto 10-60 cms tall, erect, stem terete, younger parts rough, cataphylls 1.5-1.9 mm long, deltoid acuminate; leaf 3.0-11.0 x 1.5-6.0 mm, elliptic oblong to ovate, obtuse or minutely apiculate at apex, obtuse or slightly inequilateral at base; Flowers axillary, proximal 2-3 axils with unisexual 1-3 male flowers and all succeeding axils with bisexual cymules. Male flowers -pedicel 1mm long, calyx 5, sub equal 0.7 x 0.3 mm, oblong, elliptic, apex acute, hyaline with unbranched mid rib; disc segments 5, rounded, stamens 3, filaments connate. Female flowers-pedicel 0.8-1.0 mm long, calyx lobes 5, 0.6 x 0.25 mm, ovate-oblong, acute at apex; disc flat deeply 5 lobed, lobes often toothed at apex, styles 3, free, shallowly bifid at apex. Capsule 1.8 mm in diameter, oblate and rounded, seeds about 0.9mm long, triangular with 6-7 longitudinal ribs and many transverse striations on the back¹.

(e) Geographical distribution: Widespread throughout the tropics and subtropics in sandy regions as a weed in cultivated and wastelands¹.

(f) Traditional use: The plant is bitter, astringent, cooling, diuretic, stomachic, febrifuge and antiseptic. It is useful in dropsy, jaundice, diarrhoea, dysentery, intermittent fevers, diseases of urino-genital system, scabies ulcers and wounds¹⁻³.



(g) Anatomy of the Plant¹:

Leaf : Epidermal walls wavy, stomata anisocytic, which is distributed mainly on the lower epidermis. Upper epidermis has a thin cuticle. Stomata are followed by respiratory cavities beneath. There is a single layer of palisade cells, which occupy nearly half of the space between the two epidermis. Below the palisade there is a row of broad collecting cells, each of which occur in relation to 3 or 4 palisade cells. Reduced vascular elements are clearly seen running on long stretch beneath the collecting cells. The palisade ratio has been determined to vary between 13 and 17.



Upper Epidermis

Fig.1



Lower Epidermis

Fig.2

Branchlet :- Rounded in transverse section, cortex 6-8 cells thick most of the cells contain chloroplast and few druse crystals. After 3 -4 rows, there is a row of cells containing starch grains followed by 2-3 layers of fiber cells which are interrupted by cortex parenchyma. Phloem 5 - 7 cell thick, Xylem vessels 8 - 33 μm in diameter, pith cells contain chloroplasts.

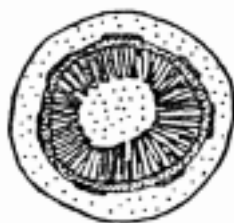


Fig.3

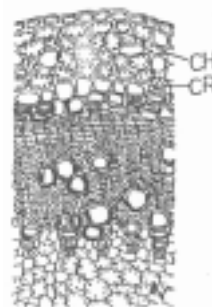


Fig.4

Transverse section of Branchlet

Stem :- Epidermal cells and some of the cortical cells contain tannin, cortex about 15 cells thick, some contain calcium oxalate druse crystals, inner cortex contains groups of 7 -10 thick walled cells interrupted at regular intervals by parenchyma cells on the outer side of the group of thick walled cells there is a row of parenchymatous cells containing starch grains. Phloem 7 - 10 cells



thick, thin walled, without any contents. Xylem vessels 16 -54 μm in diameter, pith cells thin walled may contain a few druse like crystals.

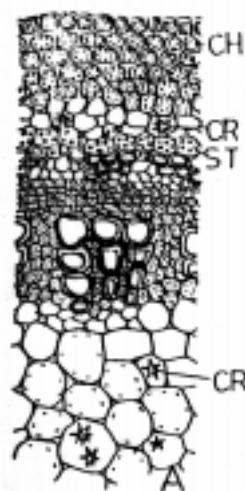


Fig.5 CH - Chloroplast, CR - crystals, ST - Starch

Transverse section of stem

Root :- Cork cells 6 - 8 cells thick, contain dark brown tannin, cortex 10 - 15 cells thick, some filled with tannin and some with starch and some with tannin, Phloem 4 - 6 cells thick, xylem vessels 12 - 53 μm in diameter.

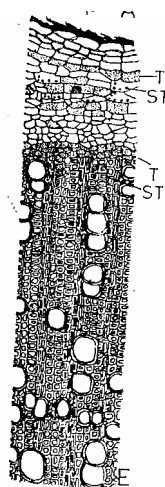


Fig.6 T - Tannin, ST - Starch

Transverse section of Root

(h) Pharmacology and Clinical studies :

Anti viral activity : *Phyllanthus amarus* and *Eclipta alba* were tested for their invitro inactivation property of Hepatitis B surface antigen (HBsAg). Crude extracts as well as the purified active principles were mixed and incubated with 30 HBsAg positive sera (1:64 CEP titra) brought about by invitro inactivation of HbsAg as tested by both counter immunoelectrophoresis(CEP)and reversed passive



haemagglutinin (RPHA) methods¹. Further studies proved the Anti Hepatitis-B virus surface active antigen in preliminary study, carriers of Hepatitis B virus were treated with a preparation of the plant for 30 days, 22 of 37 (59%) treated patients had lost Hepatitis B surface antigen compared with 1 of 23 (4%) placebo treated control². An aqueous extract of the plant inhibited endogenous DNA Polymerase of Hepatitis-B virus and binds to the surface antigen of Hepatitis-B virus invitro. Further studies were conducted on mice infected with Woodchuck Hepatitis virus (WHV) when administered with extract i.p. was effective in three out of four animals in reducing WHV within 3-6 weeks eliminating both the surface antigen titer and DNA polymerase activity in serum³. In another study alcoholic, hexane, chloroform, butanol and water extract of the plant were tested for invitro effects on HbsAg, HBeAg and HBV-DNA in serum samples positive for HBV antigen followed by the screening of the respective antigen by Elisa. The extract were effective against HBV antigen, the butanol extract being most potent⁴.

The beneficial use in the treatment of acute and chronic Hepatitis B patients were proved at cellular level using aqueous extract of *Phyllanthus amarus* on human hepatocellular carcinoma derived cell at 1mg/ml concentration on a single dose. Inhibition of the secretion of HBsAg for a period of 48 hours was observed^{5,6}. Disruption of hepatitis B virus polymerase activity, mRNA transcription and replication supported the role of *Phyllanthus amarus* being used as an antiviral agent^{7,8}.

Anti hyper glycaemic activity : An aqueous extract of the leaves of *Phyllanthus niruri* was administered at a dosage of 5 mg/kg by oral route. The observation showed a lowering a blood glucose level in normal and alloxan diabetic rabbits, the extract lowered blood sugar level even after the administration of glucose⁹. In another study the aqueous extract of aerial plants at a dosage of 0.1 and 1g/kg body weight significantly enhanced clearance of glucose from the blood as compared to control during an oral glucose tolerance test (OGTT) using normal fasted albino rabbits ($p \leq 0.05$). Only the aqueous extract contain hypoglycaemic activity¹⁰.

In a clinical trial conducted on nine mild hypertensive patients (four of them suffering from Diabetes Mellitus) were treated with a preparation of the whole plant of *Phyllanthus amarus* for 10 days. The observations indicated that *Phyllanthus amarus* as a potential diuretic hypotensive and hypoglycaemic drug for human. Blood glucose was significantly reduced in the treated group¹¹.

In another clinical trial 25 patients in the age group of 35 - 55 with moderate and severe diabetic blood sugar level (250 - 400mg/100ml) showed statistically significant ($P < 0.05$) lowering of blood sugar levels at a dose of 1gm thrice daily for a period of 3 months¹².



Anti hepatotoxic activity : Albino rats were treated with ethanol orally for 30 days, while the control was feed with sucrose. At the end of 30 days whole plant powder was administered at a dosage of 200mg/rat per day for 45 days. The increased deposition of triglyceride, cholesterol and phospholipids found in liver, brain, kidney and heart due to ethanol administration was brought back to normal values on administration of *Phyllanthus amarus* powder¹⁵. Administration of whole plant powder for 7 days at dosage of 35 mg and 70mg per kg body weight helped in restoring the levels of biochemical parameters to normal within next 48 hours in calves. AST, ALT, bilirubin and icteric were elevated with in 24 hours indicating liver parenchymal damage¹⁶. The butanol fraction of the whole plant also exhibited anti hepatotoxic activity¹⁷. In another study *Phyllanthus amarus* whole plant powder administered at a dosage of 0.66g/kg in rat showed hepatoprotective activity against CCl₄ induced liver damage¹⁸.

(i) Toxicity : The maximum tolerated dose in mouse was 0.1mg /animal in mouse.

(j) Phytochemistry : The major lignans Phyllanthin and Hypophyllanthin has been reported to exhibit antihepatotoxic activity.

Major : Lignans - a diarylbutane, Phyllanthin (~0.5%) and an aryltetrahydronaphthalene, hypophyllanthin (~0.2%)^{1,2}.

Minor : Hydrolysable tannins viz., phyllanthusiin D³, amariin⁴, amarulone⁵ and amarinic acid⁶; alkaloids viz., ent - norsecurinine⁷ sobubbialine, epibubbialine⁸; diarylbutane, nyrphyllin⁶ and a neolignan, phyllnirurin⁷.

(k) Active principles:

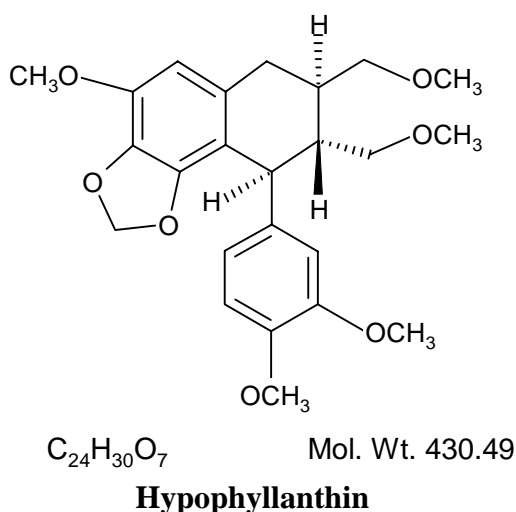


Fig.7

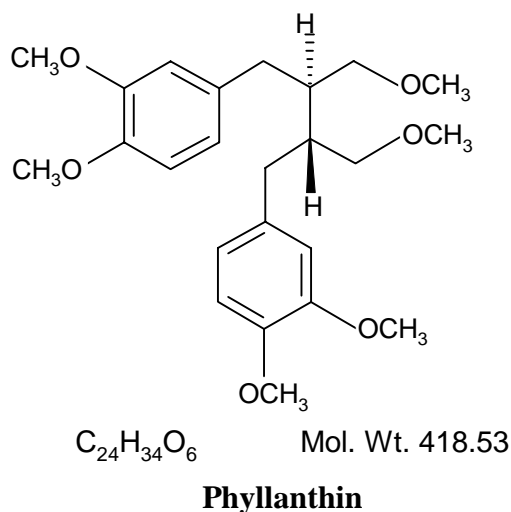


Fig.8

All these information provided has been collected from sources considered reliable, but has not been independently verified by Natural Remedies Pvt. Ltd.



ANALYTICAL SPECIFICATION OF THE CRUDE DRUG

Macroscopic Characters:

| | | |
|---------------|---|--|
| Colour | : | Greenish to brown colour when completely dried |
| Odour | : | No distinct odour |
| Taste | : | Slightly bitter |

| TESTS | LIMITS | PROTOCOLS |
|--|------------------|---|
| <u>Tests for extraneous material</u> | | Quality Control Methods for Medicinal Plant Materials -WHO |
| Foreign matter | < 1.0% | -do- |
| Sand & Silica | Absent | -do- |
| Insect infestation | Nil | -do- |
| Rodent contamination | Nil | -do- |
| <u>Physico-chemical analysis</u> | | |
| Ash content | 7.0 % w/w | -do- |
| Acid insoluble ash | 4.5 % max | -do- |
| Moisture content (By Loss on drying at 105°C) | <8.0 % w/w | -do- |
| <u>Successive extractive value</u> | | |
| Petroleum ether extractive value | 1.5 - 3.2 % w/w | -do- |
| Chloroform extractive value | 0.3 - 1.0 % w/w | -do- |
| Methanol extractive value | 10.0 - 18.0% w/w | -do- |
| <u>Alcohol soluble extractive value</u> | 12 - 20 % w/w | |
| <u>Phytochemical analysis</u> | | |
| Phyllanthin & Hypophyllanthin | > 0.3 % w/w | BY HPLC |



IDENTIFICATION OF CRUDE DRUG BY TLC

Sample detail : *Phyllanthus amarus* crude drug
Adsorbent : Silica gel 60 F₂₅₄
Mobile Phase : Hexane : ethyl acetate 2: 1
Sample preparation : 2 gms of *Phyllanthus amarus* whole plant powder was extracted with petroleum ether. The extract was concentrated and diluted with chloroform. 10µl was applied on different TLC plates.
Solvent front run upto : 9 cms
Application : CAMAG Linomat IV
Detection : Spray with 10% Sulphuric acid in methanol



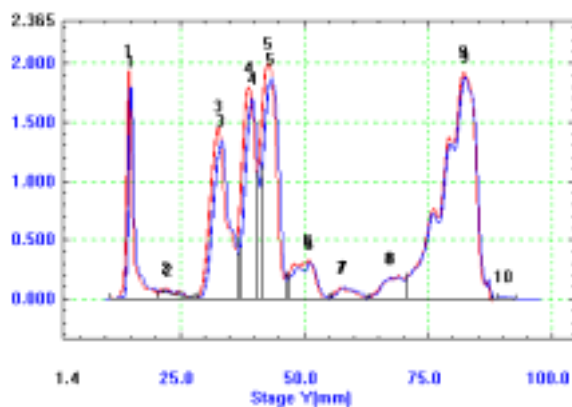
S T

Pet ether before spray



S T

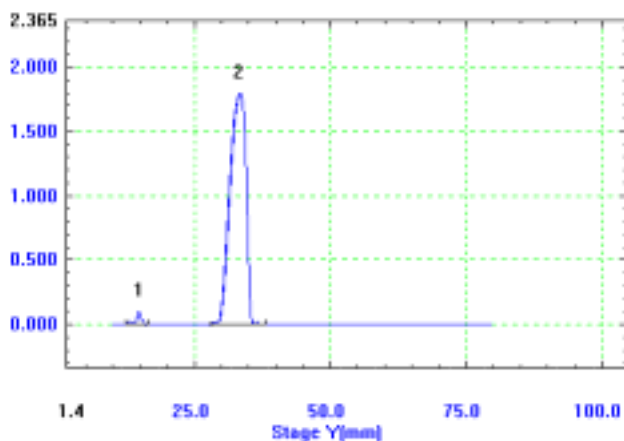
Pet ether after spray



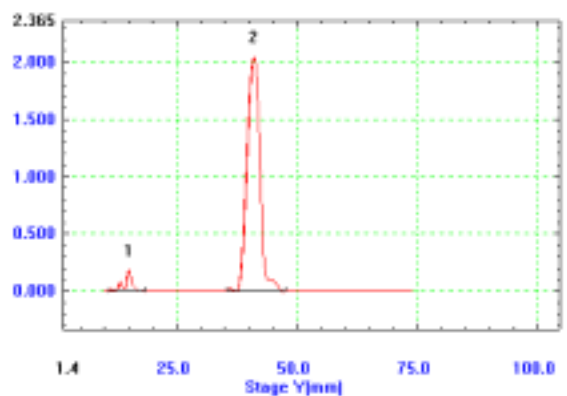
S - Reference Standard

T- Sample

HPTLC
Pet ether



Phyllanthin Ref Standard



Hypophyllanthin Ref Standard



IDENTIFICATION OF CRUDE DRUG BY TLC

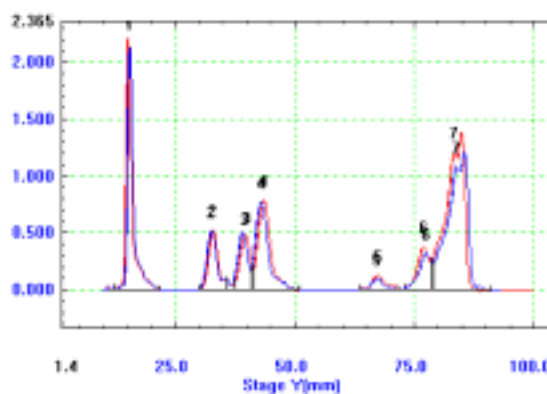
Sample detail : *Phyllanthus amarus* crude drug
Adsorbent : Silica gel 60 F₂₅₄
Mobile Phase : Hexane : ethyl acetate 2: 1
Sample preparation : 2 gms of *Phyllanthus amarus* whole plant powder was extracted with petroleum ether. The marc obtained after petroleum ether extract was further extracted with chloroform. 10µl was applied on different TLC plates.
Solvent front run upto : 9 cms
Application : CAMAG Linomat IV
Detection : Spray with 10% Sulphuric acid in methanol



S **T**
Chloroform
before spray

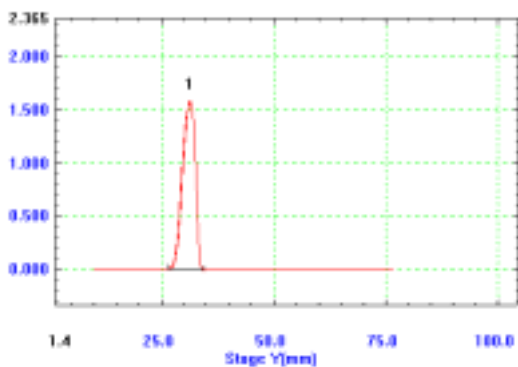


S **T**
Chloroform
after spray

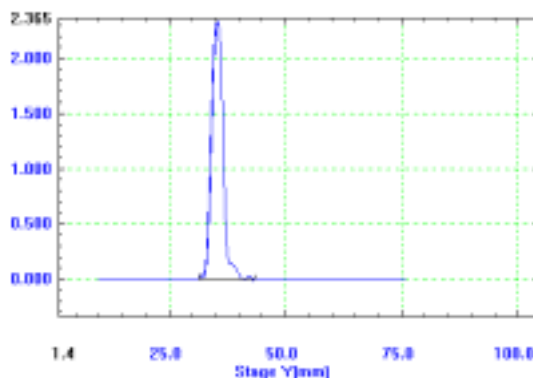


S - Reference Standard
T - Sample

HPTLC - chrom
Chloroform



Phyllanthin Ref Standard



Hypophyllanthin Ref Standard



IDENTIFICATION OF CRUDE DRUG BY TLC

| | | |
|-------------------------------|---|--|
| Sample detail | : | <i>Phyllanthus amarus</i> crude drug |
| Adsorbent | : | Silica gel 60 F ₂₅₄ |
| Mobile Phase | : | Hexane : ethyl acetate 2: 1 |
| Sample preparation | : | 2 gms of <i>Phyllanthus amarus</i> whole plant powder was successively extracted with petroleum ether and chloroform. The mark obtained after chloroform extraction was further extracted with methanol. 10µl was applied on different TLC plates. |
| Solvent front run upto | : | 9 cms |
| Application | : | CAMAG Linomat IV |
| Detection | : | Spray with 10% Sulphuric acid in methanol |



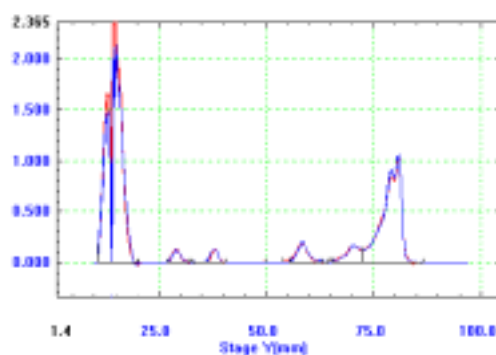
S **T**

S. Methanol before
spray



S **T**

S. Methanol after
spray



S - Reference Standard

T - Sample

HPTLC
S.Methanol

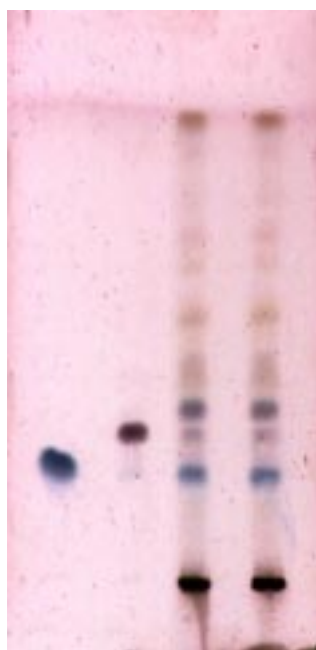


IDENTIFICATION OF CRUDE DRUG BY TLC

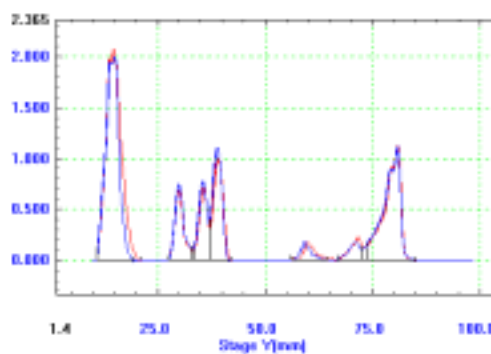
| | | |
|-------------------------------|---|--|
| Sample detail | : | <i>Phyllanthus amarus</i> crude drug |
| Adsorbent | : | Silica gel 60 F ₂₅₄ |
| Mobile Phase | : | Hexane : ethyl acetate 2: 1 |
| Sample preparation | : | 2 gms of <i>Phyllanthus amarus</i> whole plant powder was extracted with methanol. The extract was concentrated and diluted with chloroform. 10 μ l was applied on different TLC plates. |
| Solvent front run upto | : | 9 cms |
| Application | : | CAMAG Linomat IV |
| Detection | : | Spray with 10% Sulphuric acid in methanol |



S T
Complete Methanol before spray



P H S T
Complete Methanol after spray



S - Reference Standard
T - Sample
P - Phyllanthin Ref. Standard
H - Hypophyllanthin Ref. Standard

HPTLC
Complete Methanol



Estimation Of Phyllanthin & Hypophyllanthin In *Phyllanthus amarus*

Abstract :

A HPLC procedure for standardising the plant *phyllanthus amarus* by using two bioactive lignans Phyllanthin and Hypophyllanthin as reference standards.

Experimental

Extraction of Plant Material :

Extraction of the plant was carried out using 2 methods :

1. 7gms of plant was size reduced and was macerated with 50ml of CHCl_3 for 1 day⁴ and separated, this process was repeated 3 times. Combined CHCl_3 extract was evaporated to complete dryness and dissolved in HPLC grade methanol and volume made upto 50ml
2. 7gms of plant was size reduced and mixed with 2.1gms of lime and 30ml of water and macerated for 1 day and marc was extracted with 20ml of 3% Methanolic KOH for 1 hour, process was repeated 3 times. Combined the Methanolic KOH extract, concentrated to 20ml and was re-extracted with 40ml petroleum ether (60-80) 3 times in a separating funnel. Combined Petroleum ether extract was evaporated to dryness and was dissolved in HPLC grade Methanol and volume made upto 50ml.

HPLC Analysis :

Instrument :

A Shimadzu Chromatographic system comprising of L.C.8A model dual pump, A photo diode array detector (SPD-M10A vp model). Lichocart 250-4, Lichrosphere 6 μ

Column :

E-Merck Nitrile Column (250mm x 4mm)

Mobile Phase :

Buffer(pH 2.8) : Acetonitrile (83:17)

Mobile phase flow rate = 1.9ml/min

Wave length :

The detector was operated at 230nm.

Caliberation Curves :

Phyllanthin and Hypophyllanthin were dissolved separately i.e 25mg in 25ml of HPLC Methanol. (The two lignans were isolated from *Phyllanthus amarus* and identity was confirmed by comparing with the UV, IR, ¹HMR and ¹³CNMR of the isolated with those reported in the literatures). From that 5ml of Phyllanthin and Hypophyllanthin were pipetted into a single 25ml volume flask and the volume was made upto 25ml with HPLC Methanol, a final concentration of 200mcg/ml of Phyllanthin and Hypophyllanthin, this is taken as



working stock solution. From the solution 10 and 20 μ l were injected and the area under the peak was noted.

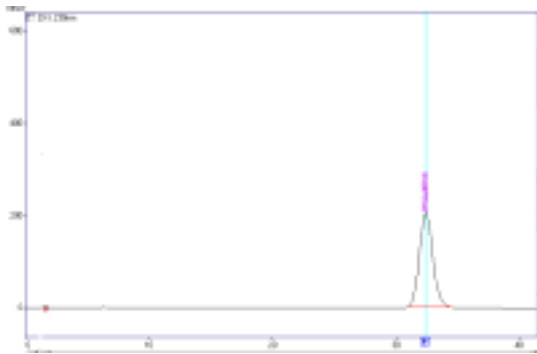
Estimation of Phyllanthin and Hypophyllanthin in samples

Comparison of procedures for maximum extraction of Phyllanthin and Hypophyllanthin. The whole fresh plant obtained from Madras was size reduced and extracted as mentioned above the solution were pooled and final volume was made upto the mark with HPLC Methanol.

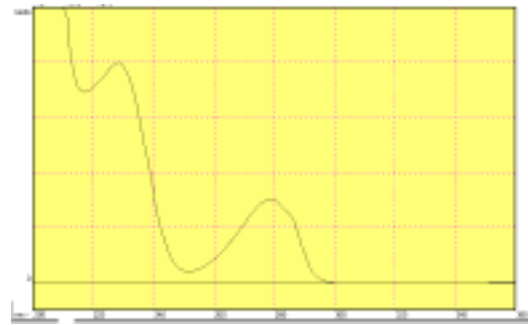
The HPLC estimation was carried out using 20 μ l of the samples and the concentration of Phyllanthin and Hypophyllanthin was estimated using the area under the curve obtained from the sample and compared with the standard.



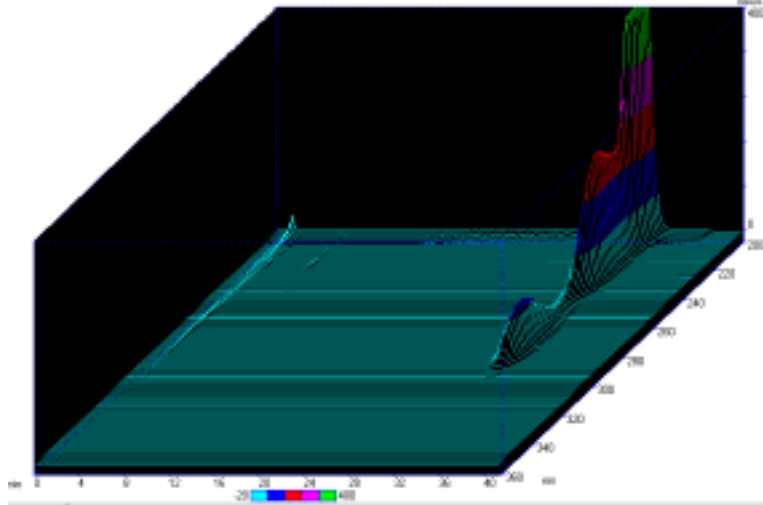
HPLC PROFILE



Chormatogram of Phyllathin



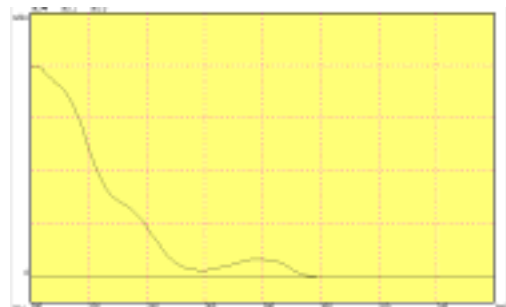
PDA of Phyllathin



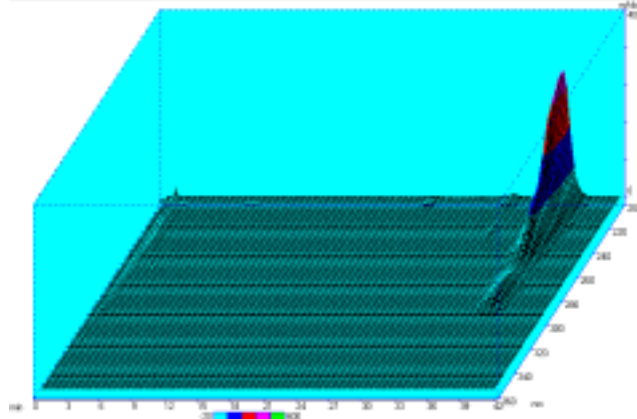
3D VIEW



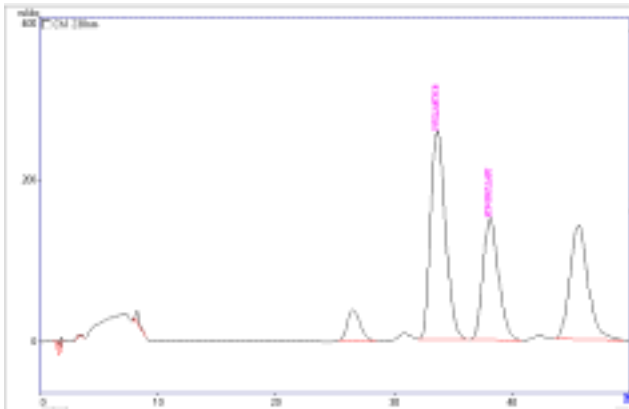
Chormatogram of Hypophyllathin



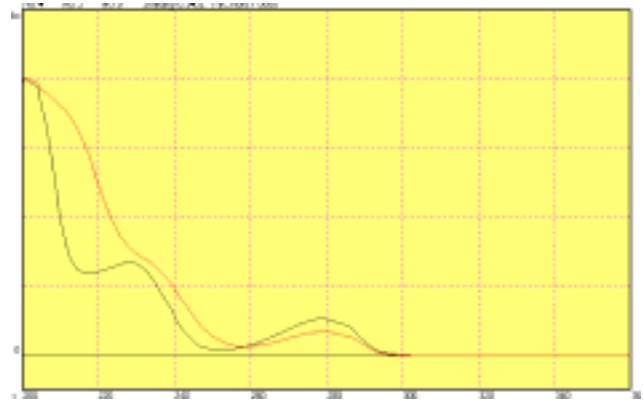
PDA of Hypophyllathin



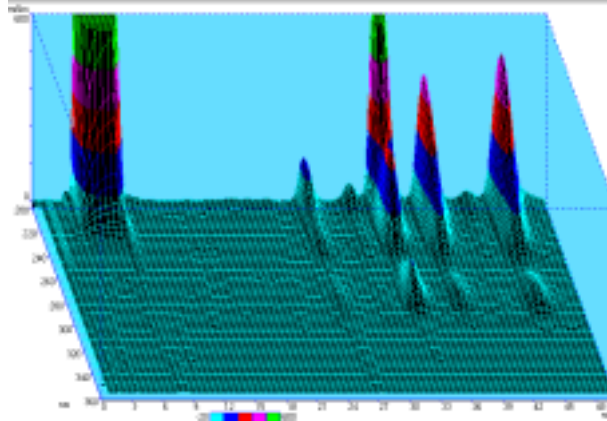
3D VIEW



Chormatogram of P.amarus Sample



PDA of P.amarus Sample



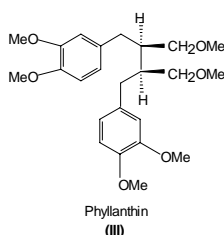
3D VIEW



Analytical Specification of Phyllanthin

(+) 3,4,3',4',9,9', Hexa Methoxy - 8,8` bytyro lignan

Phyllanthin is an lignan isolated from the whole plant of *Phyllanthus amarus* Schum & Thonn.



TESTS

RESULTS

Description

White Crystalline powder

Solubility

Soluble in Methanol, Chloroform, acetone, ether.
Sparingly soluble in petroleum ether, almost in soluble
in water

Identification

(by Spectroscopy & Chromatography)

UV absorption

Exhibits maxima at 280, 229nm

TLC & HPTLC

Gives a single spot

FTIR

Characteristic of phyllanthin

¹H NMR

Characteristic of phyllanthin

¹³C NMR

Characteristic of phyllanthin

MASS SPECTRUM

The base peak of the isolated Phyllanthin
corresponded to its mass number phyllanthin

HPLC

matches with the phyllanthin

UV spectra of the principal peak

Loss on drying at 105°C

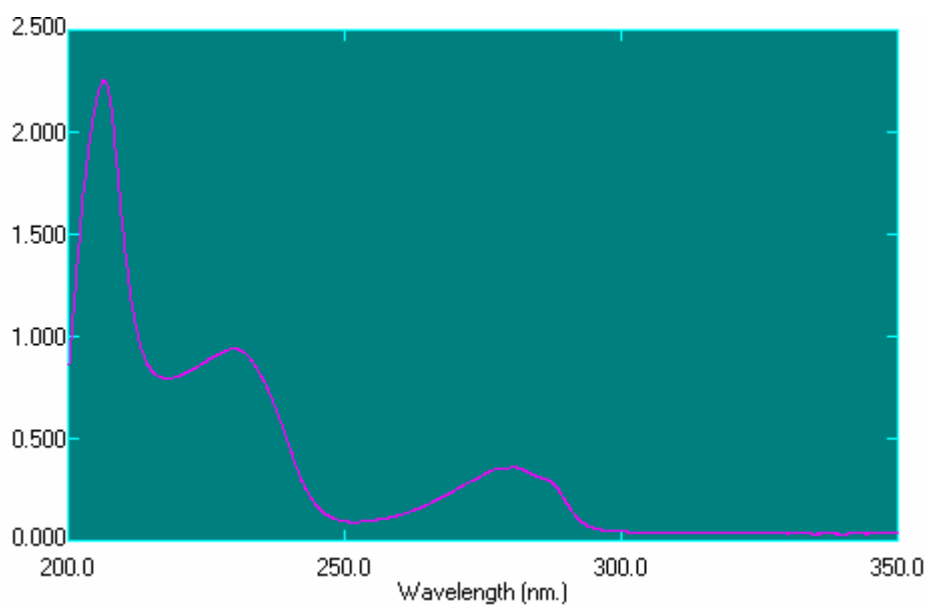
1.0% w/w

Purity (HPLC)

> 98% w/w



UV ABSORPTION SPECTRA



UV - Absorption of 0.0192% w/v solution in methanol

Created : 16:48 23/01/2000
Data : Original
Measuring Mode : Abs
Scan speed : Medium
Slit width : 1.0
Sample Interval : 0.2

| No | wave length (nm) | Abs |
|----|------------------|-------|
| 1 | 280 | 0.354 |
| 2 | 229 | 0.965 |
| 3 | 206 | 2.194 |



TLC PROFILE

| | | |
|-------------------------------|---|---|
| Sample detail | : | Phyllanthin |
| Adsorbant | : | Precoated silica gel 60F ₂₅₄ |
| Solvent system | : | n-Hexene : Ethyl Acetate 2 : 1 |
| Sample preparation | : | 10 mg of phyllanthin dissolved in 1ml of methanol 10 ml applied on TLC Plate. |
| Solvent front run upto | : | 9 cms |
| Detection | : | 10% sulphuric acid in methanol (fig.1) |
| Scanning | : | 205 nm (fig.2) |
| Application | : | Camag Linomat IV |



Fig.2

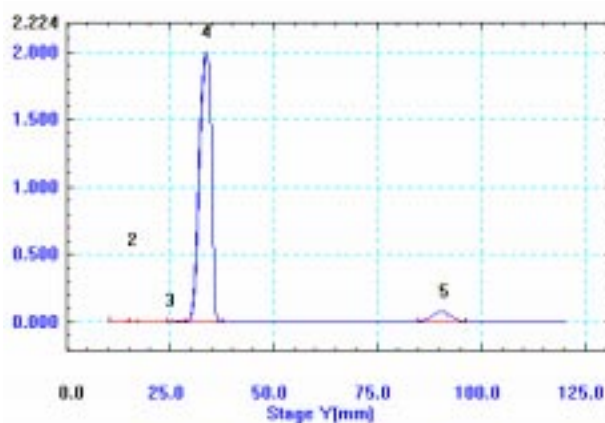
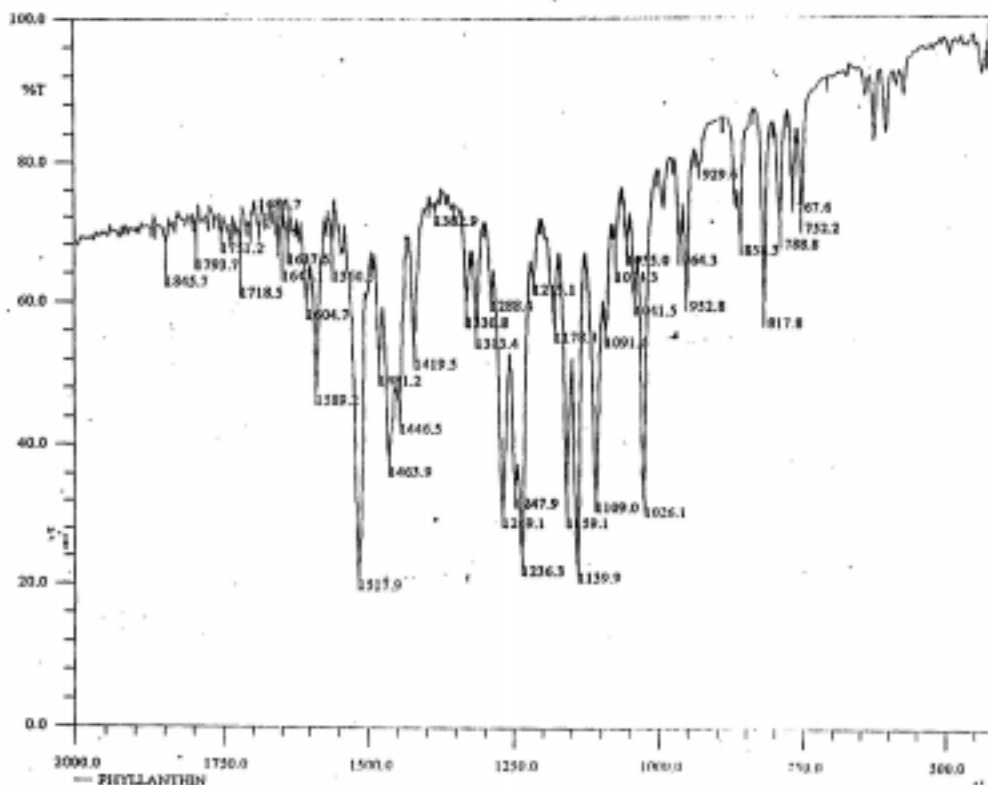


Fig.3



FTIR in KBr dispersion



Sample

Instrument : Fourier Transmission Infrared Spectra

Make : SHIMADZU

Isolated phyllanthin exhibited the following peaks

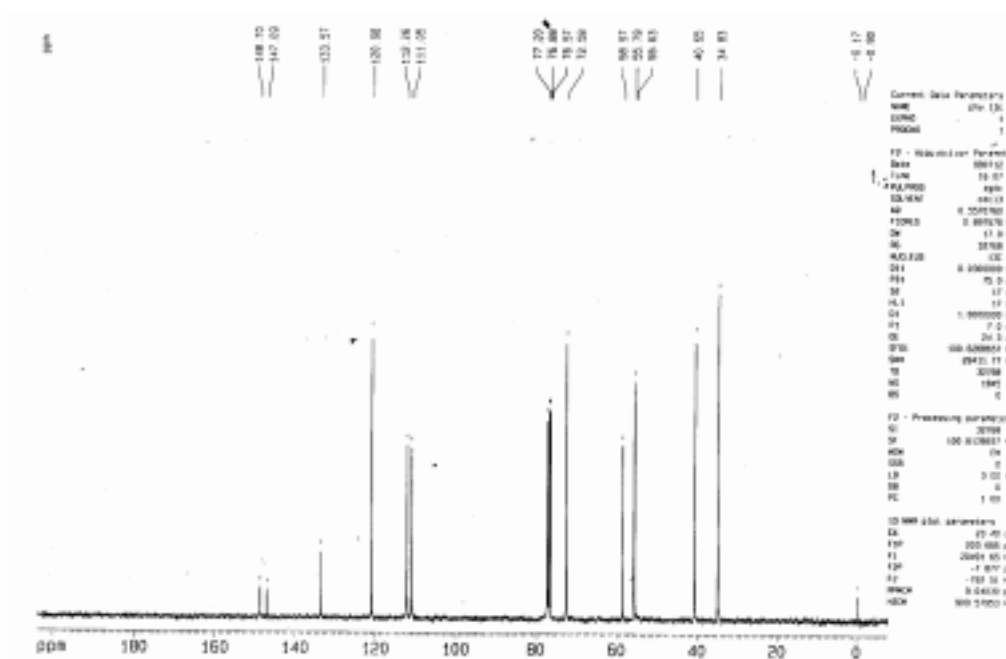
1517.9, 1481.2, 1463.9, 1446.5, 1419.5, 1313.4, 1269.1, 1247.9, 1236.3, 1178.4, 1159.1, 1139.9, 1109, 1055, 1041.5, 1026.1, 964.3, 952.8, 858.3, 817.8, 788.8, 767.6 & 752.2.

Ref : (New liznamj from Phyllanthin nirurilin - the constituents of phyllanthin).

Terahedron 1966 vol -22 pp-2899 to 2908 L.Ramachandra Row et al.



¹³C NMR SPECTRUM in CDCl₃



Instrument : ¹³CNMR Spectrometer

Solvent : CDCl₃

Nucleus : ¹³C

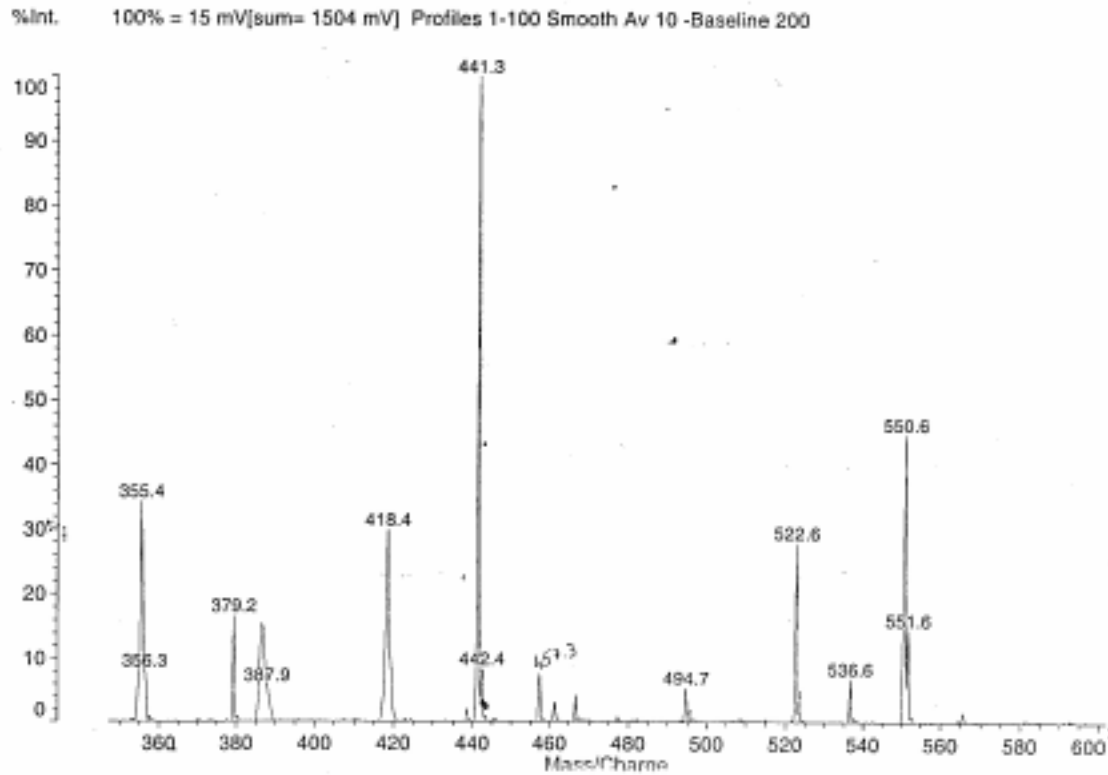
Reported Values : 133.6, 112.2, 148.7, 147.1, 111.0, 121.0, 34.9, 40.7, 72.8, 55.9, 55.7, 58.7,

Values of isolated : 133.57, 112.26, 148.7, 147.09, 111.05, 120.98, 34.83, 40.65, 72.58, 55.79, 55.63, 58.57



MASS SPECTRUM

Data: phyllanthin.15 14 Jul 99 14:59 Cal: S4 11 Jan 98 12:34
Kratos PC-Kompact MALDI 4 V1.0.3: + Linear High Power: 80, P.Ext. @ 500 (bin 83)



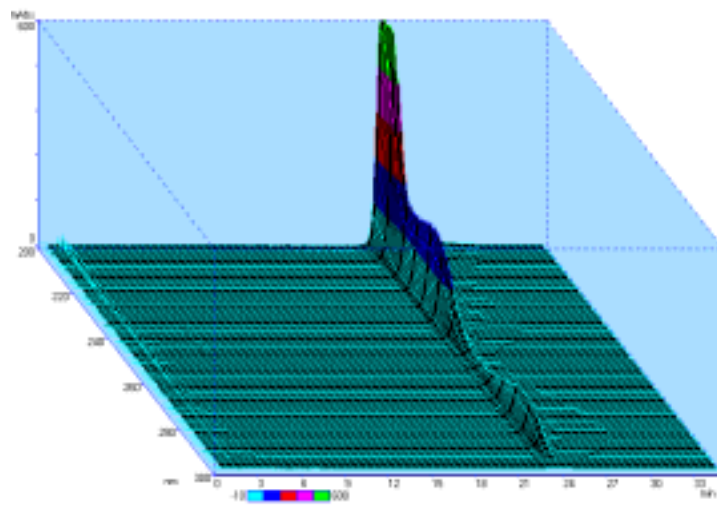
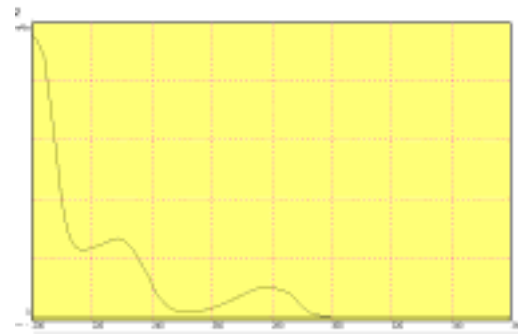
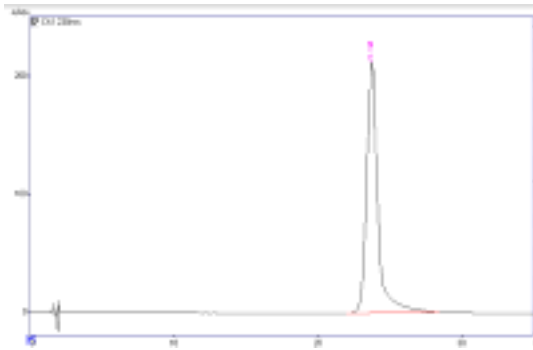
Mol Wt of Phyllanthin is 418

Isolated compound gave peak at 418.4.

Peak at 441.3 & 457.3 corresponds to sodium & potassium salts of the compound respectively



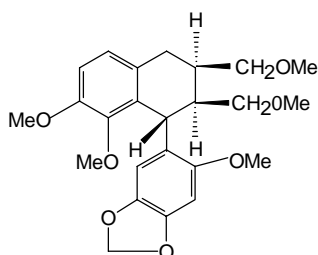
HPLC Profile of phyllanthin





ANALYTICAL SPECIFICATION OF HYPO-PHYLLANTHIN

r-1-(3,4-dimethoxyphenyl) -6- methoxy-t-2, c-3-bimethoxymethyl-7-8-methylene dioxy-1234 tetra hydro naphthalene.
Hypo-Phyllanthin is an lignan isolated from the whole plant of *Phyllanthus amarus* Schum & Thonn.



Hypophyllanthin

TESTS

RESULTS

Description

White Crystalline powder

Solubility

Soluble in chloroform, methanol, ether,
Sparingly soluble in petroleum ether & insoluble in water

Identification (by Spectroscopy & Chromatography)

UV absorption

Exhibits maxima at 278nm

TLC & HPTLC

Gives a single spot

FTIR

Characteristic of hypophyllanthin

^1H NMR

Characteristic of hypophyllanthin

^{13}C NMR

Characteristic of hypophyllanthin

MASS SPECTRUM

The base peak of the isolated hypophyllanthin
corresponded to its mass number

HPLC

UV spectra of the principal peak matches with the
hypophyllanthin

Loss on drying at 105°C

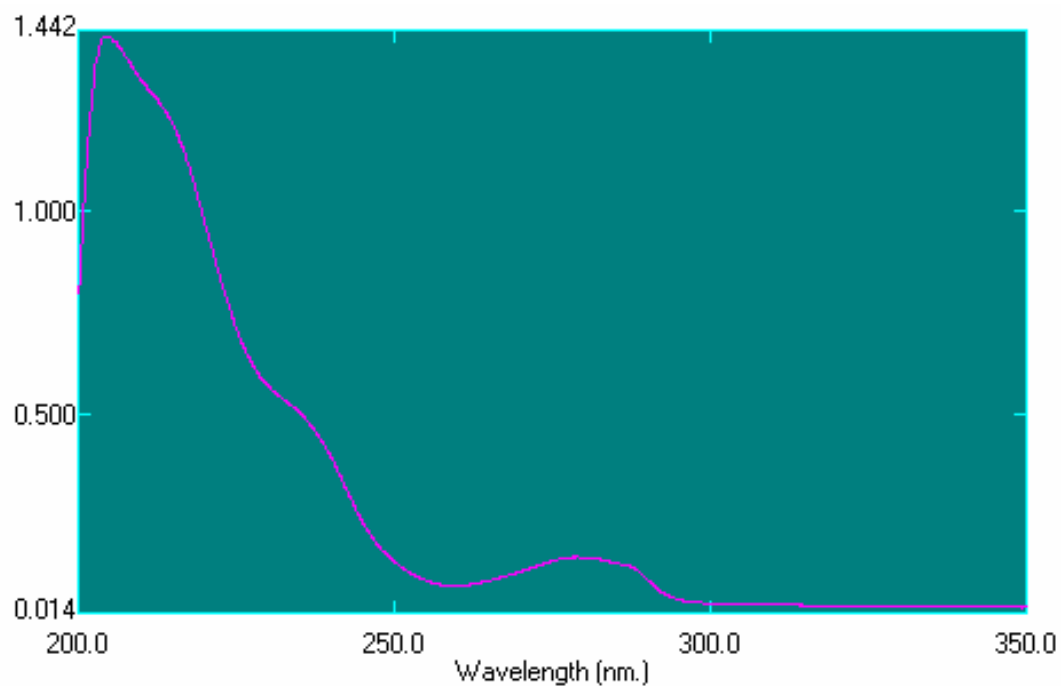
< 0.5% w/w

Purity (HPLC)

> 98% w/w



UV absorption spectrum of Hypo-phyllanthin



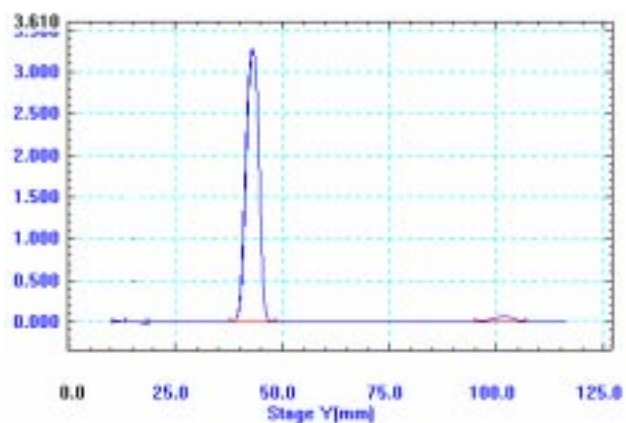
UV - Absorption of 0.0184% w/v solution in methanol

| No | wave length (nm) | Abs |
|----|---------------------|-------|
| 1 | 278 | 0.226 |
| 2 | 210 | 2.360 |



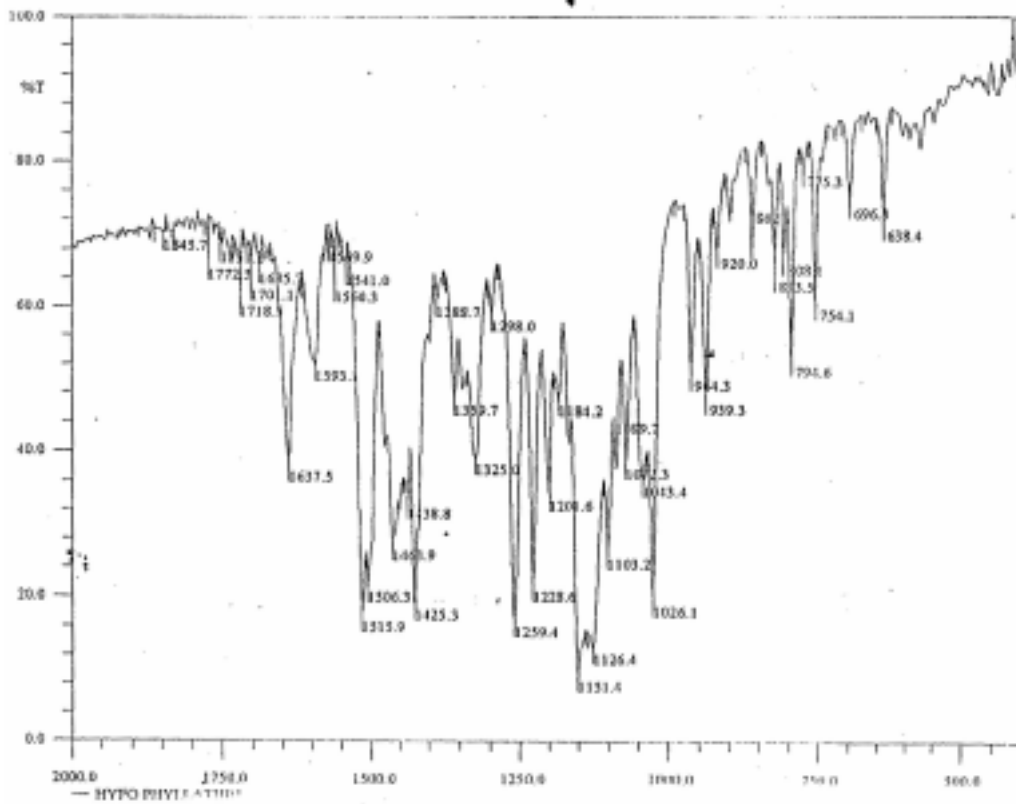
TLC PROFILE

| | | |
|-------------------------------|---|--|
| Sample detail | : | Hypophyllanthin |
| Adsorbant | : | Precoated silica gel 60F ₂₅₄ |
| Solvent system | : | n-Hexene : Ethyl Acetate 2 : 1 |
| Sample preparation | : | 10 mg of hypophyllanthin dissolved in 1ml of methanol 10 µ l applied on TLC Plate. |
| Solvent front run upto | : | 9 cms |
| Detection | : | 10% sulphuric acid in methanol (fig.2) |
| Scanning | : | 205 nm (fig.3) |
| Application | : | Camag Linomat IV |





FTIR in KBr dispersion



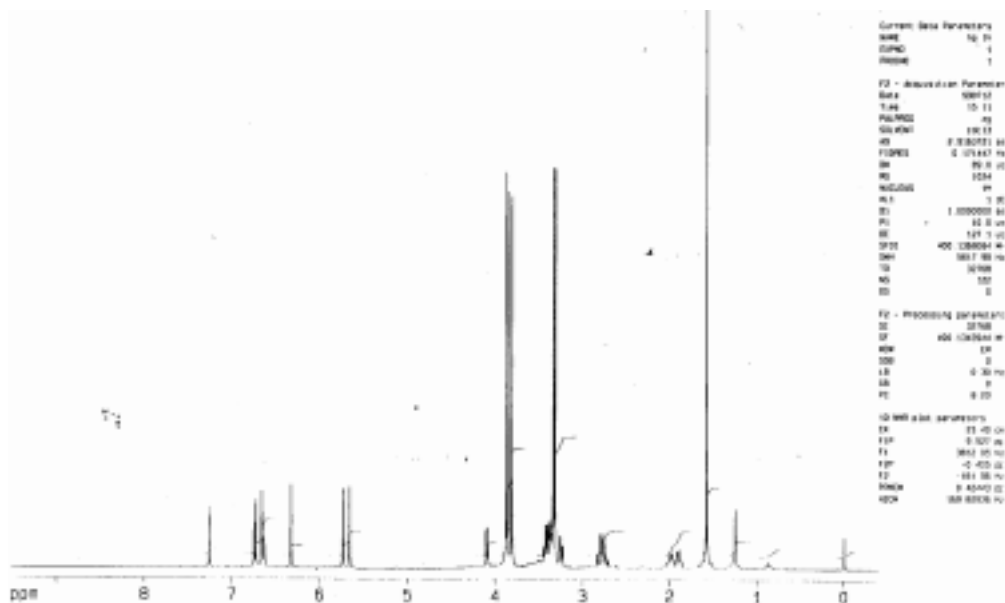
Sample

Instrument : Fourier Transmission Infrared Spectra

Make : SHIMADZU



¹H NMR SPECTRUM in CDCl₃



Sample

Instrument : AMX - 400MHz High Resolution multi nuclear H¹NMR Spectrometer

Solvent : CDCl₃

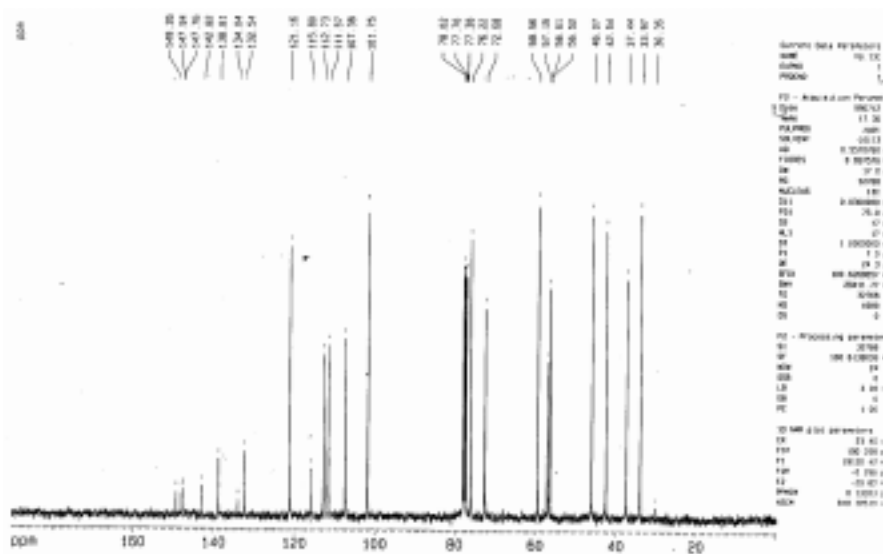
Nucleus : H¹

Reported Values :

Values of isolated :



¹³C NMR SPECTRUM in CDCl₃



Instrument : ¹³CNMR Spectrometer

Solvent : CDCl₃

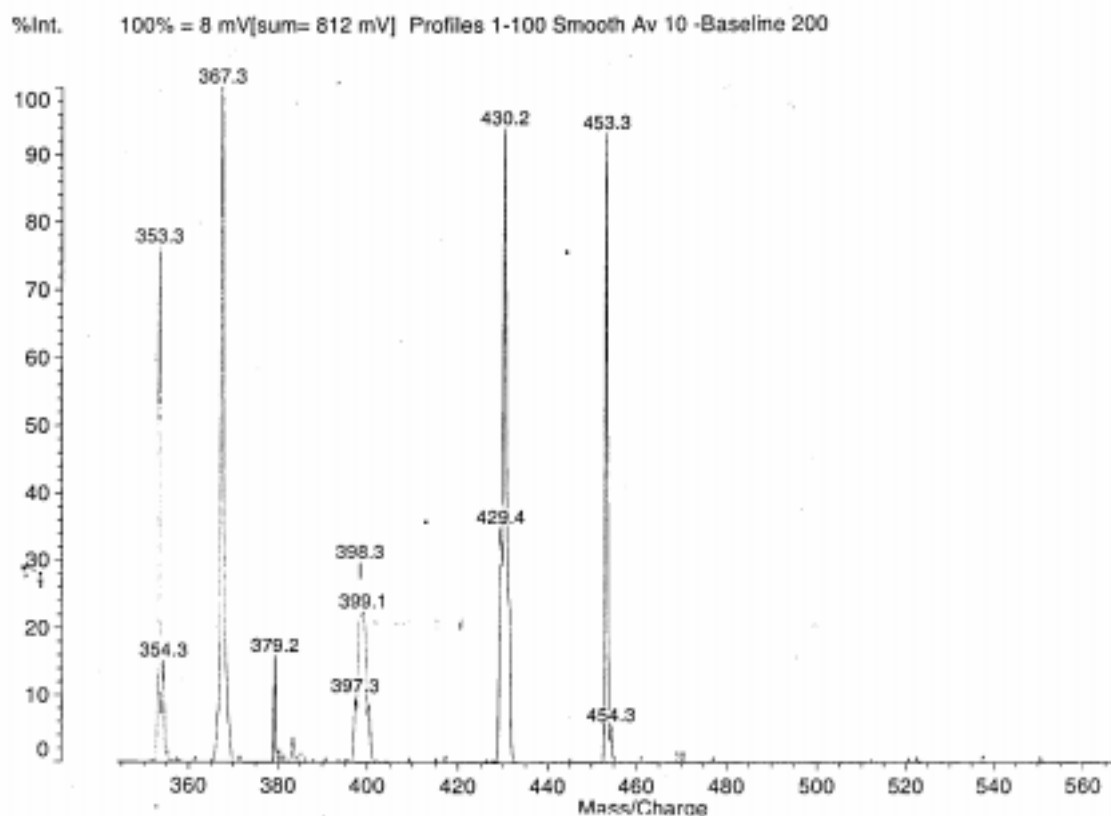
Nucleus : ¹³C

| Reported Values | Values of isolated : |
|-----------------|----------------------|
| : | 42.54 |
| 41.9 | 46.07 |
| 45.4 | 72.68 |
| 71.8 | 37.44 |
| 35.9 | 76.22 |
| 75.5 | 33.97 |
| 33.3 | 132.54 |
| 131.8 | 107.38 |
| 106.5 | 142.82 |
| 142.1 | 134.04 |
| 133.3 | 147.76 |
| 147.0 | 115.80 |
| 115.1 | 138.81 |
| 138.1 | 112.73 |
| 111.9 | 149.35 |
| 148.6 | 147.94 |
| 147.1 | 111.57 |
| 110.7, | 121.16 |
| 120.4 | 59.56 |
| 58.9 | 57.15 |
| 55.9 | 56.61 |
| 56.52 | 101.75 |
| 101.1 | |



MASS SPECTRUM

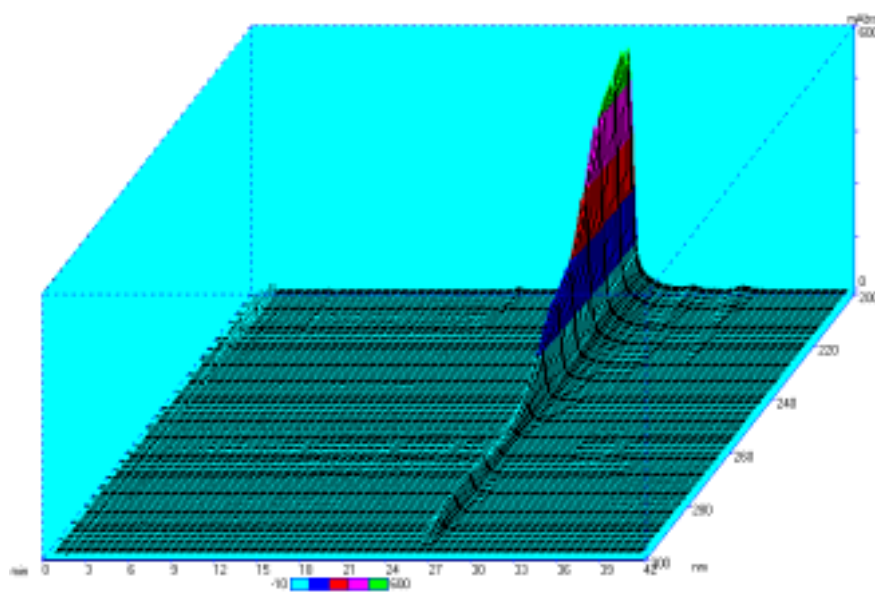
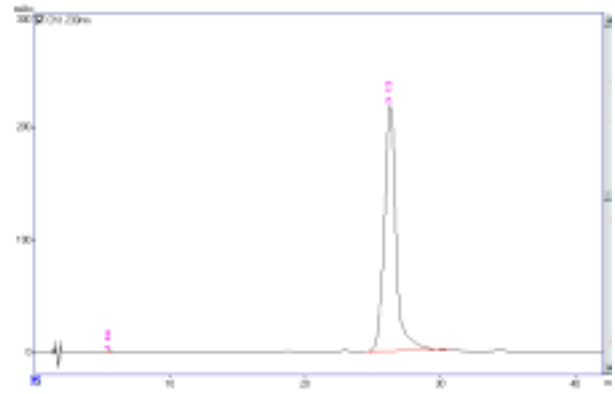
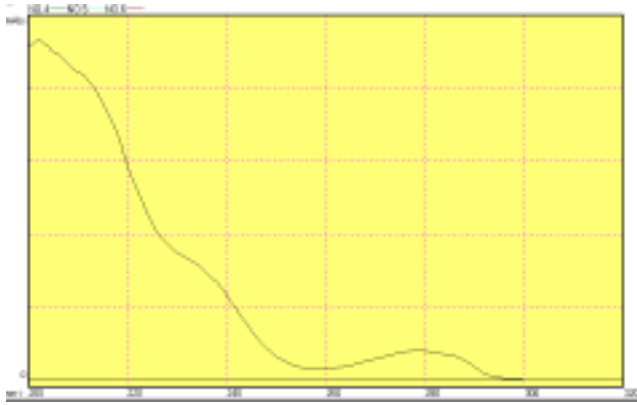
Data: hypo-phyllanthin 13 Jul 99 14:54 Cal: S4 11 Jan 98 12:34
Kratos PC-Kompact MALDI 4 V1.0.3: + Linear High Power: 80, P.Ext. @ 500 (bin 83)



Isolated compound gave peak at 430.2
Other Reported values :- 398 367 & 222
Values of isolated :- 398.3 & 353.3



HPLC Profile of Hypo-phyllanthin





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Botanical Description

1. Bagchi G.D, Srivastava G.N , Singh S.C, Distinguishing features of medicinal herbaceous species of *Phyllanthus* occurring in Lucknow District (U.P) India, Int. J . Pharmacognosy, 1992, 30(3), 161-168

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8. Ott M, Thyagarajan S.P, Gupta S, *Phyllanthus amarus* suppresses Hepatitis B virus by interrupting interaction between HBV enhancer I and cellular transcription factors, Eur. J. Clin. Invest., 1997, 27(11), 908-915.



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10. Moshi M.J, Uiso F.C , Mahunnah R.L. et al , A study of the effect of *Phyllanthus amarus* extract on blood glucose in rabbits, Int J. Pharmacog., 1997, 35(3), 167 - 173.
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