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Microscopic and chemical profiling of aerial parts of *Phyllanthus reticulatus* Poir: A wound healing herb

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Abstract
About: *Phyllanthus reticulatus* Poir of the family Phyllanthaceae *a* widely growing shrub with wide medicinal uses. Leaves and stem parts are used as best wound healing agents whereas edible berries are used as blood purifiers and stem pieces as tooth-brushing sticks.
Materials and Methods: The matured plant is selected leafy twigs along with fruits are collected, macro-microscopic and phytochemical. Physicochemical study and HPTLC were conducted as per standard methodology.
Results: Oval leaf, glabrescent pink flower and woody stem are macroscopic features. The histology of the leaf shows the presence of palisade and spongy parenchyma with three layers of collenchyma in between. Stem microscopy shows trichomes at the epidermal region in between collenchyma. Anther, trichomes, pollen grains, fibers in bundles, oleoresin cells and cork cells are features of powder microscopy. Steroids, carbohydrates, tannins, and phenols found as secondary metabolites. Physicochemical standards and HPTLC recorded represent purity and prime chemicals of test drug.
Conclusion: Standard illustrative Pharmacognostic monograph prepared on this drug mark as its quality standard.

Keywords: *Phyllanthus reticulatus*, Macro-microscopic, HPTLC, collenchyma, phytochemical

Introduction
Phyllanthus reticulatus Poir (*Kirganelia reticulata*) belonging to the family Phyllanthaceae is a large glabrous, pubescent sub-scandent shrub, with smooth, lenticel late branches [1]. Leaves elliptic to oblong or obovate, with unisexual axillary flowers. Fruits are purplish black berry, globose, smooth, and shining, with trigonous seeds [2]. The plant is common on low moist ground along river banks, irrigation channels, and waste places sometimes it is found climbing among bushes [3].
Leaves of the plant are eaten in times of scarcity at few places [4]. Leaf powder is applied to sores, burns, suppurations, and chafing of the skin [5].
Fruits are astringent and useful in inflammations and in diseases of the blood. An ink is prepared from ripe fruits whereas roots are used in red dye [6]. The leaves are recommended to use in the form of decoction for wound washing whereas powder application mentioned in burn wounds [7]. Stem pieces are advised in tooth brushing as they possess astringent taste [8]. The leaf decoction is also suggested in epidemics like measles.
Beta–sitosterol, *Glochidion*, Betulinic acid and Friedelinare are principle chemical constituents of leaf whereas betulin, *Glochidion*, Friedelin, Octacosanol, Taraxeryl and taraxeryl acetate are from the root [9]. The whole plant is also said to be spasmolytic, hypotensive, and antiviral [10]. In texts of Ayurveda, it is said to be the source of Kambhoji a wound healing agent, leaves in the form of decoction are recommended in wound washing [11]. Plant is said to be styptic and advised in diarrhea. It is also termed as Bhudhatri (Small gooseberry) plant and indicated in jaundice [12]. Medicinal plant with multiple benefits needs its standard monograph related to morphology and chemical constituents. Hence an attempt is made in this paper to illustrate a monograph on this shrub.

Materials and Methodology
Materials
Matured plant is selected from its local habitat, leafy twigs along with fruits and stem pieces are collected, few pieces kept in FAA solution for microscopic study. Rest shade dried powder is prepared and used for further study [13].

Methodology

Macroscopy

The external features of the test samples were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication [14].

Microscopy

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5 ml + Acetic acid-5 ml + 70% Ethyl alcohol-90 ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse sections using a sharp blade and the sections were stained with saffranine. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached to Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale bars [15].

Powder microscopy

A pinch of the sample was mounted on a microscopic slide with a drop of glycerin water. Characters were observed using Zeiss AXIO trinocular microscope attached to Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the pre-calibrated scale-bars using Zeiss Axio Vision software [16].

Physicochemical standards [17]

Loss on drying at 105 °C

10 g of sample was placed in a tarred evaporating dish. It was dried at 105 °C for 5 hours in a hot air oven and weighed. The drying was continued until the difference between the two successive weights was not more than 0.01 after cooling in a desiccator. The percentage of moisture was calculated with reference to the weight of the sample.

Total Ash

2 g of sample was incinerated in a tarred platinum crucible at a temperature not exceeding 450 °C until carbon-free ash is obtained. The percentage of ash was calculated with reference to the weight of the sample.

Acid insoluble Ash

To the crucible containing total ash, 25 ml of dilute HCL was added and boiled. Insoluble matter on ashless filter paper (Whatmann 41) was collected and washed with hot water until the filtrate become neutral. Filter paper containing the insoluble matter was transferred to the original crucible, dried on a hotplate, and ignited to constant weight. Allowed the residue to cool in a suitable desiccator for 30 mins and weighed without delay. The content of acid-insoluble ash was calculated with reference to the air-dried drug.

Water soluble ash

Ash boiled for 5 min with 25 ml of water; insoluble matter collected on an ashless filter paper, washed with hot water, and ignited for 15 min at a temperature not exceeding 450 °C. The weight of the insoluble matter is subtracted from the weight of the ash; the difference in weight represents the water-soluble ash with reference to the air-dried sample.

Alcohol soluble extractive

4 g of the sample weighed in a glass Stoppard flask. 100 ml of distilled Alcohol (approximately 95%) added and stirred occasionally for 6 hours and allowed to stand for 18 hours. Then Filtered rapidly taking care not to lose any solvent. 25 ml of the filtrate pipette out in a pre-weighed 100 ml beaker. Evaporated to dryness on a water bath. Kept it in an air oven at 105 °C for 6 hours, cooled in desiccator for 30 minutes and weighed. Calculated percentage of Alcohol extractable matter of the sample. Repeated the experiment twice, and took the average value.

Water soluble extractive

Weighed accurately 4 g of the sample in a glass Stoppard flask. Added 100 ml of distilled water, stirred occasionally for 6 hours, and allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent. 25 ml of the filtrate was pipette out in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. Kept it in an air oven at 105 °C for 6 hours then cooled it in a desiccator and weighed. The experiment was repeated twice and the average value was taken.

Preliminary phytochemical tests [18]

Plant powder was tested for the presence of alkaloids, carbohydrates, steroids, saponins, tannins, flavonoids, phenol, coumarins, triterpenoids, carboxylic acid, resin, and quinone as per standard guidelines.

HPTLC [19]

1.0 g of dried plant powder was taken in 10.0 ml of methanol. The methanol extract obtained was dried. 3, 6, and 9µl of the above samples were applied on a pre-coated silica gel F_{254} on aluminum plates to a bandwidth of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl Acetate (8.0:4.0). The developed plates were visualized in short UV, and long UV and then derivatized with Vanillin sulphuric acid (VSA) reagent and scanned under UV 254nm, 366nm, 540nm and 620nm. RF, the color of the spots, and the densitometric scan were recorded.

Results

Macroscopy

Leaves are oval to oblong, rounded at both ends. Flowers are glabrescent, pink in color, borne in clusters, and are unisexual. Stem woody, cylindrical, bearing leaves, flowers, and fruits. Fruits are berry-like, reddish brown when unripe Ned, and turn black on ripening.

Leaf microscopy

TS leaf part was taken at midrib region. The upper epidermis was continuous at the laminar region and trichomes were visible in the midrib region. The Laminar region consists of palisade and spongy parenchyma with intracellular spaces. Palisade cells were elongated and continuous in nature whereas spongy parenchyma has space in between for gaseous exchange. Three layers of collenchyma are found between the epidermal regions. Xylem was centrally located and the phloem was arranged at the periphery. Ground tissue was parenchymatous in nature.

Stem microscopy

TS of the stem when observed under a microscope showed epidermis as continuous and holds numerous trichomes. Below epidermis, there were 5-6 layers of cortical parenchymal cells which were closely associated with collenchyma cells. Phloem fibers crown the phloem region in the pericycle which were lignified and this region found comparatively thin. Xylem entails of xylem vessels, xylem

rays and xylem fibers. The central pith holds parenchymal cells which were rich in starch and aleurone grains.

Powder microscopy

Powder microscopic characters showed mesophyll, anther, trichomes, pollen grains, fibers in bundles, oleo resin cells and cork cells.

Physiochemical standards

Loss on drying was 16.63% denotes its moisture content. Total ash was 6.53% showing the presence of organic and inorganic matter. Acid insoluble ash was 0.69% representing inorganic matter present in the plant material. Water soluble ash was 2.28%. Alcohol soluble extractive value was 9.06% whereas water soluble extractive value was 33.29% indicative of more polar components of test substance.

Phytochemicals

Preliminary phytochemical tests show the presence of constituents such as steroids, carbohydrates, tannins and phenols.

HPTLC

Methanolic extract of aerial parts of the test drug sample was applied 3, 6, and 9µl using the solvent system toluene ethyl acetate. The chromatogram scanned at 254 nm and 366 nm showed 9 peaks, whereas at 540 nm and 620nm showed 8 peaks. The peak at HPTLC indicates a measure of the concentration of the compound it represents. Each peak value stands for the retardation factor. RF 0.43±.02 (2.28) was evident at 366 nm and RF 0.43±.02 (2.32%) at 620 nm. RF 0.43 indicates a sterol which is beta-sitosterol.

Fig 1: Macroscopy of *Phyllanthus reticulatus* Poir

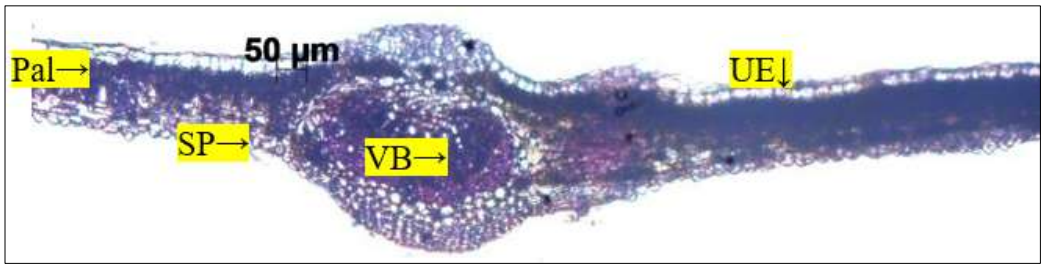


Fig 2 a: TS of leaf

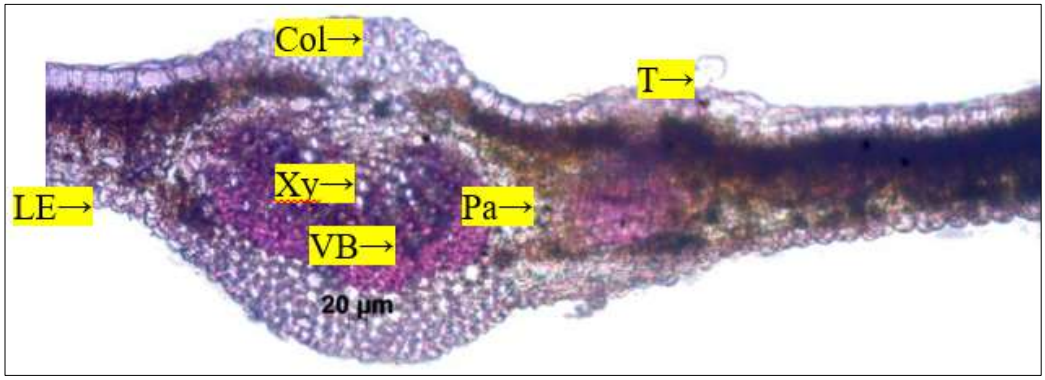
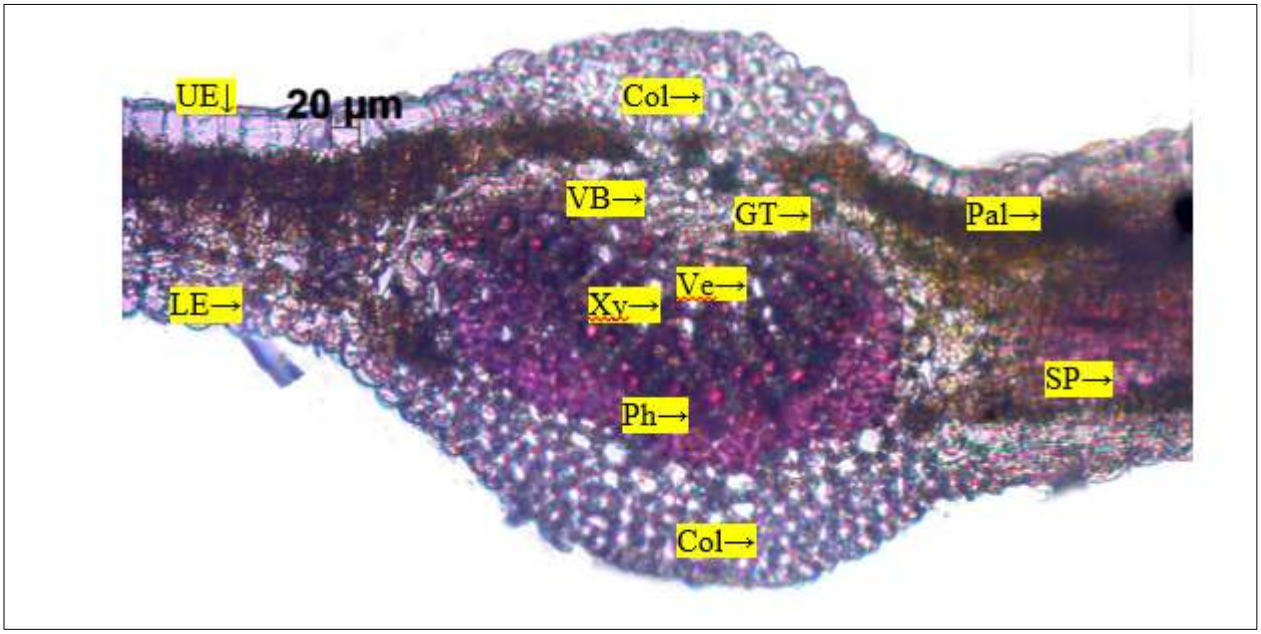


Fig 2b: T.S enlarged



Col – collenchyma; GT – glandular trichomes; LE – lower epidermis; Pa – parenchyma; Pal – palisade; RC-rosette crystals; SP–spongy parenchyma; T–trichomes; UE–upperepidermis; VB – vascular bundle; Ve – vessel; Xy – xylem

Fig 2c: Midrib enlarged

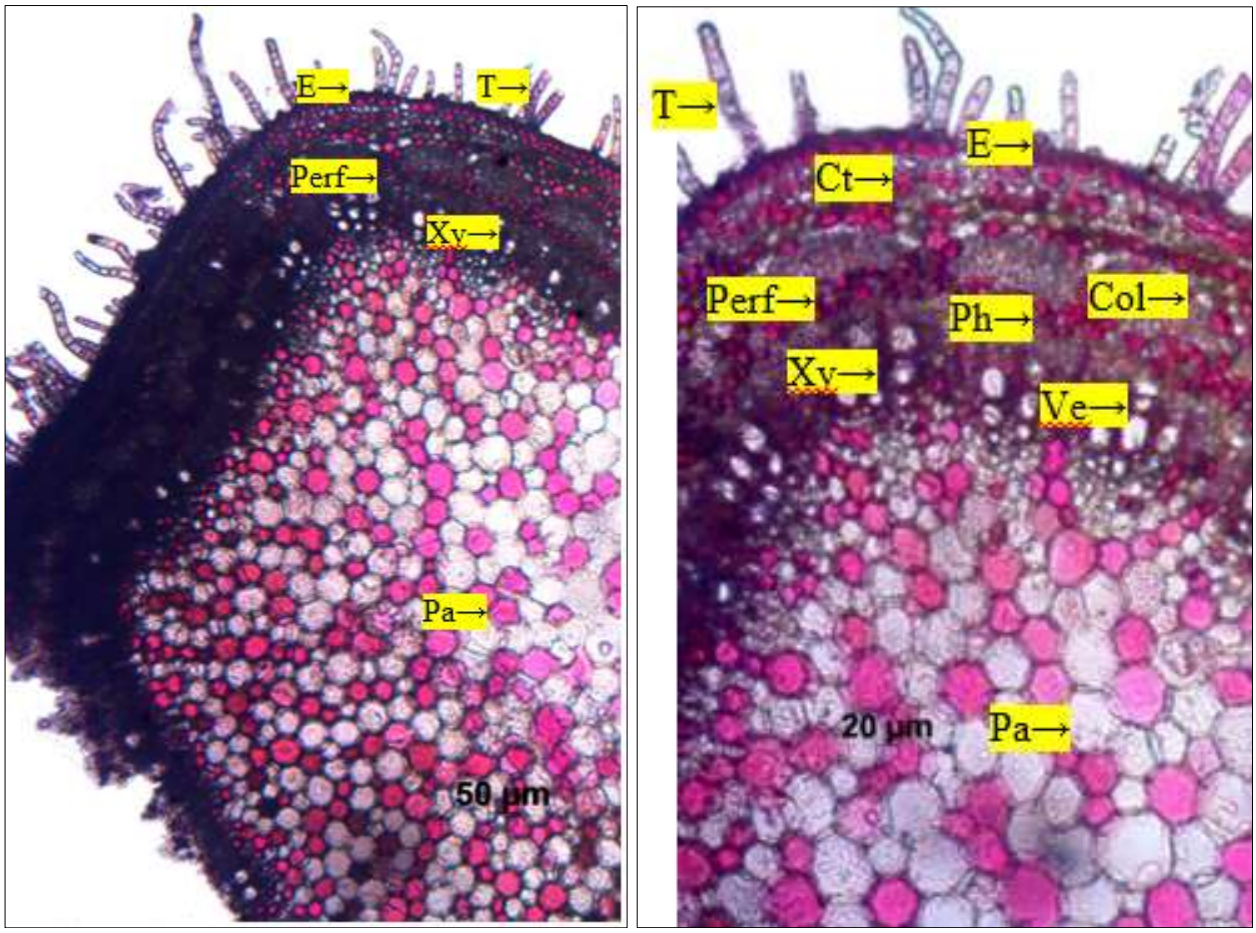


Fig 3a: TS of stem	Col–collenchymas; Ct–cortex; E–epidermis; Pa–parenchyma; Perf–pericyclic fibres; Ph–phloem; Ve–vessels; Xy–xylem. T-Trichomes
	Fig 3b: Cortex and Vascular region

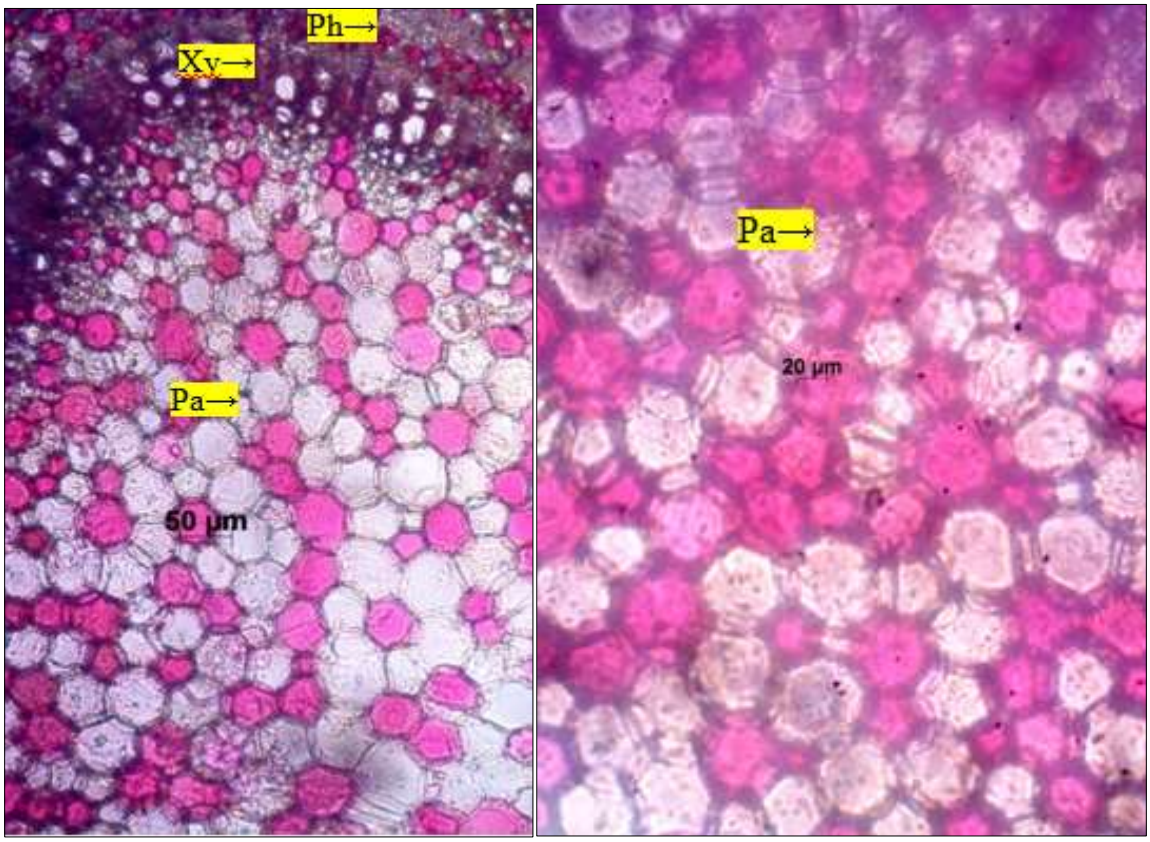


Fig 3c: Xylem and Phloem and central region	Col–collenchymas; Ct–cortex; E–epidermis; Pa–parenchyma; Perf–pericyclic fibres; Ph–phloem; Ve–vessels; Xy–xylem. T-Trichomes **Fig 3d:** Parenchyma
Fig 3: Microscopy of *Phyllanthus reticulatus* Poir stem	

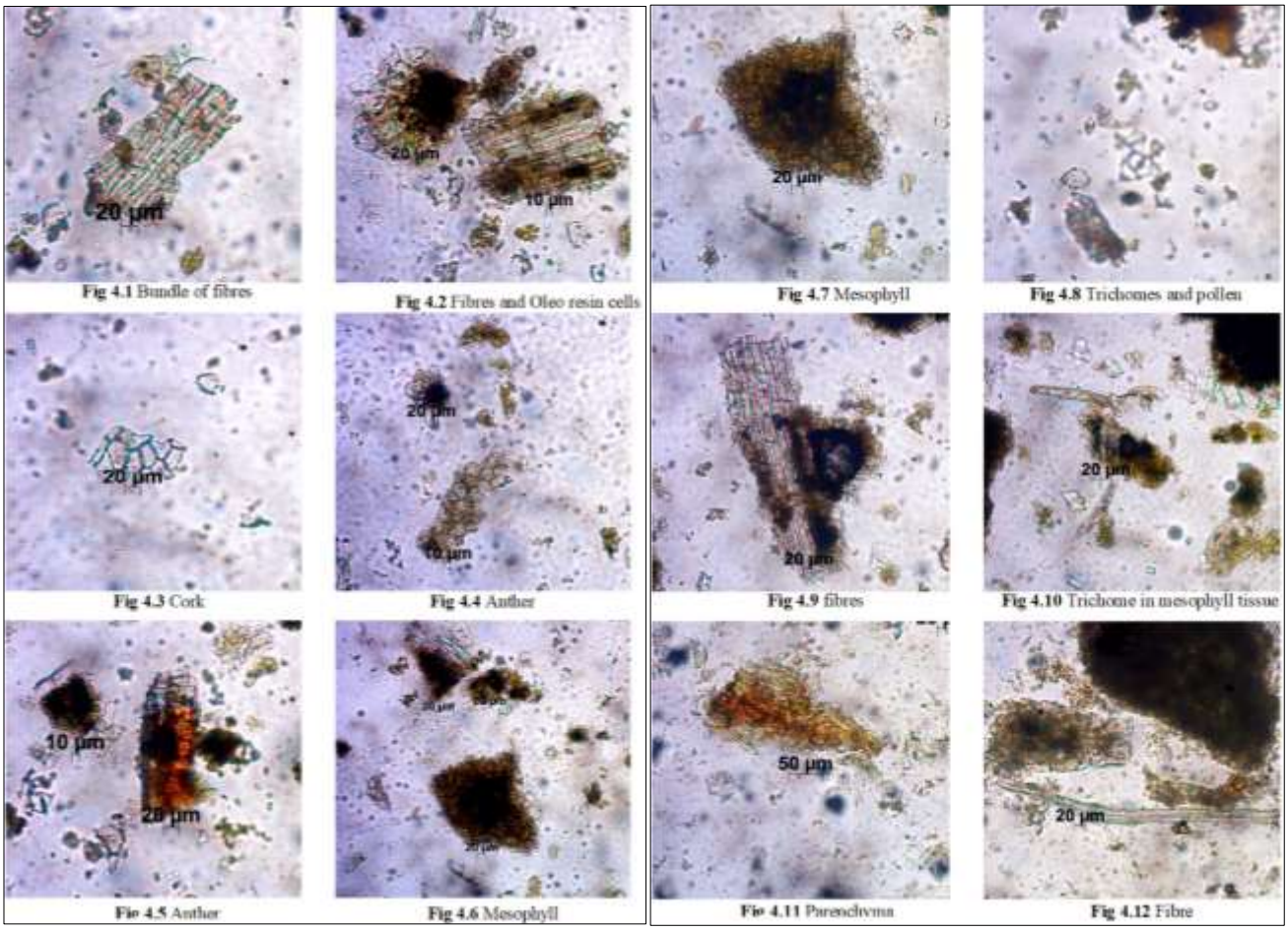


Fig 4: Powder microscopy of *Phyllanthus reticulatus* Poir upper aerial part

Table 1: Physicochemical standards of *Phyllanthus reticulatus* Poir

Parameter	Results n = 3% w/w Avg ± SD
Loss on drying	16.63±0.00
Total Ash	6.53±0.04
Acid Insoluble Ash	0.69±0.01
Water soluble Ash	2.28±0.01
Alcohol soluble extractive value	9.06±0.00
Water soluble extractive value	33.29±0.01

Table 2: Phytochemical screening of *Phyllanthus reticulatus Poir*

Test	Inference
Alkaloid	-
Steroid	+
Carbohydrate	+
Tannin	+
Flavanoids	-
Saponins	-
Terpenoid	-
Coumarins	-
Phenols	+
Carboxylic acid	-
Amino acids	-
Resin	-
Quinone	-

(+) - present; (-) – negative

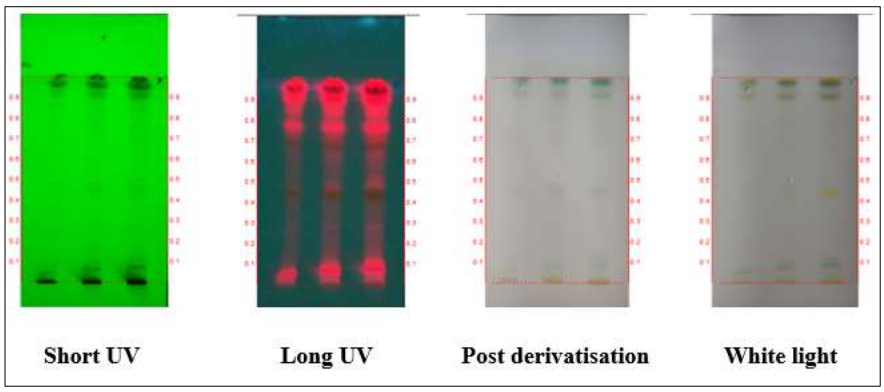


Fig 5: HPTLC photo documentation of methanol extract of *Phyllanthus reticulatus* Poir

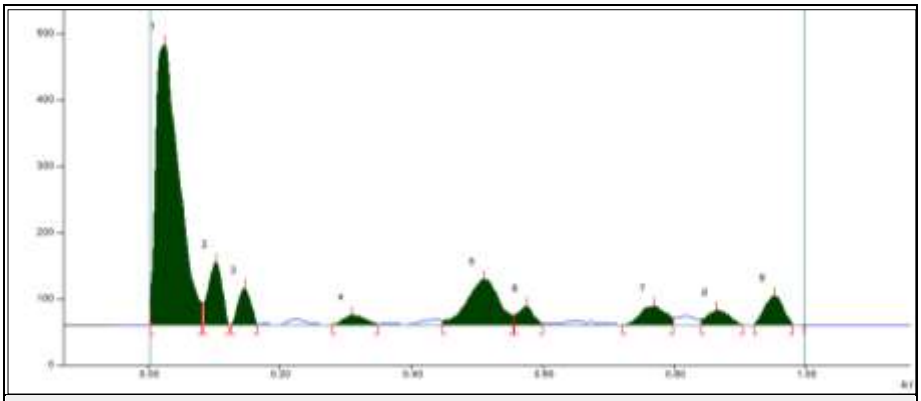
Track 1 – Test sample – 3µl
Track 2 -Test sample– 6µl
Track 3 – Test sample – 9µl

Solvent system – Toluene: Ethyl Acetate (8.0: 4.0)

Table 3: R_f values *Phyllanthus reticulatus* Poir

Short UV	Long UV	Post derivatization	White light
-	0.06 (F. red)	-	-
0.08 (Green)	-	0.08 (Green)	0.08 (Green)
0.12 (Green)	-	0.12 (Green)	0.12 (Green)
-	0.28 (F. red)	-	-
-	0.43 (F. red)	-	-
0.45 (Green)	-	0.45 (Green)	0.45 (Yellow)
-	0.48 (F. red)	-	-
-	-	0.51 (Green)	0.51 (Green)
-	0.64 (F. red)	-	-
0.70 (Green)	0.71 (F. red)	0.69 (Green)	-
0.77 (Green)	0.76 (F. red)	0.76 (Green)	-
-	-	0.79 (Green)	0.78 (Green)
0.85 (Green)	0.84 (F. red)	-	-
0.92 (Green)	0.92 (F. red)	0.92 (Green)	0.92 (Green)

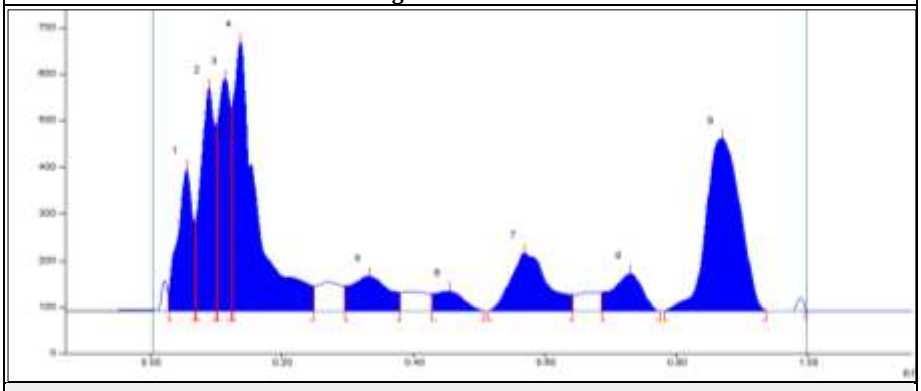
*F – Fluorescent; L –Light; D – Dark



Track 3, ID: Phyllanthus reticulatus upper aerial part of leaf

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	24.2 AU	0.02 Rf	421.9 AU	54.50 %	0.08 Rf	33.3 AU	10576.1 AU	58.64 %
2	0.08 Rf	33.3 AU	0.10 Rf	94.6 AU	12.22 %	0.12 Rf	1.2 AU	1403.8 AU	7.78 %
3	0.13 Rf	1.8 AU	0.15 Rf	55.1 AU	7.12 %	0.17 Rf	0.5 AU	735.7 AU	4.08 %
4	0.28 Rf	0.7 AU	0.31 Rf	14.7 AU	1.89 %	0.35 Rf	0.6 AU	336.9 AU	1.87 %
5	0.45 Rf	6.1 AU	0.51 Rf	68.4 AU	8.84 %	0.56 Rf	14.4 AU	2366.5 AU	13.12 %
6	0.56 Rf	15.1 AU	0.58 Rf	27.2 AU	3.51 %	0.60 Rf	1.4 AU	458.8 AU	2.54 %
7	0.72 Rf	0.0 AU	0.77 Rf	27.7 AU	3.57 %	0.80 Rf	10.1 AU	772.5 AU	4.28 %
8	0.84 Rf	7.8 AU	0.87 Rf	21.3 AU	2.75 %	0.91 Rf	0.4 AU	513.2 AU	2.85 %
9	0.92 Rf	0.4 AU	0.95 Rf	43.3 AU	5.59 %	0.98 Rf	0.7 AU	871.7 AU	4.83 %

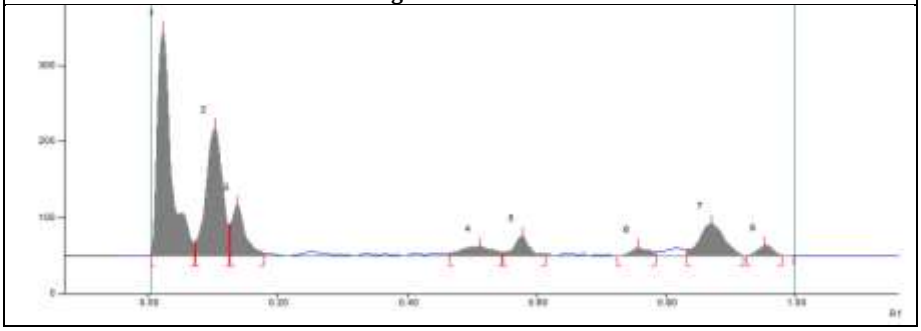
Fig 6a: At 254 nm



Track 3, ID: Phyllanthus reticulatus upper aerial part of leaf

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	38.4 AU	0.05 Rf	305.2 AU	11.96 %	0.07 Rf	90.5 AU	4890.8 AU	8.23 %
2	0.07 Rf	195.5 AU	0.09 Rf	479.0 AU	18.78 %	0.10 Rf	94.8 AU	7322.2 AU	12.33 %
3	0.10 Rf	399.5 AU	0.11 Rf	498.4 AU	19.53 %	0.12 Rf	32.0 AU	6846.3 AU	11.52 %
4	0.13 Rf	440.1 AU	0.14 Rf	576.5 AU	22.60 %	0.25 Rf	53.6 AU	14585.1 AU	24.55 %
5	0.30 Rf	53.0 AU	0.33 Rf	74.4 AU	2.91 %	0.38 Rf	40.0 AU	3081.1 AU	5.19 %
6	0.43 Rf	37.4 AU	0.45 Rf	42.4 AU	1.66 %	0.51 Rf	0.0 AU	1354.0 AU	2.28 %
7	0.51 Rf	0.2 AU	0.57 Rf	124.9 AU	4.89 %	0.64 Rf	34.7 AU	5013.7 AU	8.44 %
8	0.69 Rf	40.1 AU	0.73 Rf	79.9 AU	3.13 %	0.78 Rf	0.0 AU	2623.8 AU	4.42 %
9	0.78 Rf	0.2 AU	0.87 Rf	370.6 AU	14.53 %	0.94 Rf	0.3 AU	13691.3 AU	23.05 %

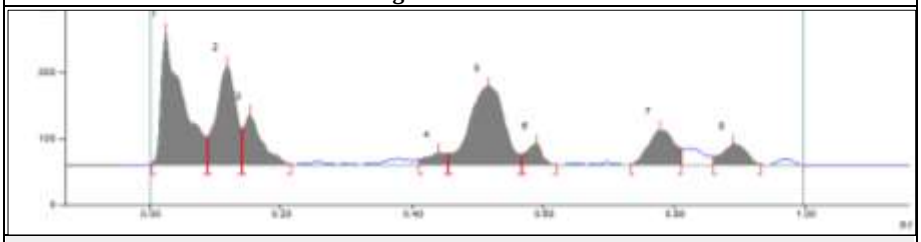
Fig 6b: At 366 nm



Track 3, ID: Phyllanthus reticulatus upper aerial part of leaf

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	5.0 AU	0.02 Rf	295.1 AU	46.42 %	0.07 Rf	17.5 AU	4602.1 AU	41.37 %
2	0.07 Rf	18.2 AU	0.10 Rf	168.3 AU	26.47 %	0.13 Rf	39.6 AU	3050.6 AU	27.42 %
3	0.13 Rf	41.8 AU	0.14 Rf	67.5 AU	10.62 %	0.18 Rf	3.4 AU	983.4 AU	8.84 %
4	0.47 Rf	4.1 AU	0.51 Rf	12.6 AU	1.98 %	0.55 Rf	4.2 AU	430.9 AU	3.87 %
5	0.55 Rf	4.4 AU	0.58 Rf	26.0 AU	4.09 %	0.61 Rf	2.6 AU	441.5 AU	3.97 %
6	0.73 Rf	0.2 AU	0.76 Rf	10.8 AU	1.69 %	0.79 Rf	4.5 AU	215.4 AU	1.94 %
7	0.83 Rf	8.3 AU	0.87 Rf	41.9 AU	6.59 %	0.92 Rf	0.2 AU	1159.2 AU	10.42 %
8	0.93 Rf	0.1 AU	0.95 Rf	13.5 AU	2.13 %	0.98 Rf	0.2 AU	240.7 AU	2.16 %

Fig 6c: At 540 nm



Track 3, ID: Phyllanthus reticulatus upper aerial part of leaf

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	2.8 AU	0.02 Rf	200.5 AU	29.43 %	0.09 Rf	42.3 AU	4769.8 AU	27.57 %
2	0.09 Rf	43.2 AU	0.12 Rf	150.1 AU	22.02 %	0.14 Rf	55.0 AU	3154.6 AU	18.23 %
3	0.14 Rf	55.2 AU	0.15 Rf	76.3 AU	11.20 %	0.22 Rf	1.1 AU	1526.1 AU	8.82 %
4	0.41 Rf	8.5 AU	0.44 Rf	18.6 AU	2.72 %	0.45 Rf	17.2 AU	401.9 AU	2.32 %
5	0.46 Rf	17.0 AU	0.52 Rf	119.3 AU	17.51 %	0.57 Rf	16.1 AU	4510.8 AU	26.07 %
6	0.57 Rf	16.5 AU	0.59 Rf	32.3 AU	4.74 %	0.62 Rf	0.8 AU	558.8 AU	3.23 %
7	0.73 Rf	1.2 AU	0.78 Rf	52.8 AU	7.75 %	0.81 Rf	24.6 AU	1522.0 AU	8.80 %
8	0.86 Rf	12.6 AU	0.89 Rf	31.5 AU	4.63 %	0.93 Rf	1.4 AU	858.0 AU	4.96 %

Fig 6d: At 620 nm

Fig 6: Densitometric scan of *Phyllanthus reticulatus* Poir

Discussion

Phyllanthus reticulatus Poir **(***Kirganelia reticulata*) belonging to family Phyllanthaceae *a* widely growing shrub identified as source of Kamboji as per few lexicons of Ayurveda. Aerial parts of this plant along with fruits are used in medicine worldwide. Leaves and stem parts are used as best wound healing agent, in the form of decoction for washing, application etc. Edible purplish ripened berries used as blood purifier whereas stem pieces as tooth brushing sticks.

Pharmacognostic standards are essential to record anatomical, histological, chemical characters of plant material which in future prevent admixture or substitute [20].

On naked eye examination leaf was oval to oblong, flowers glabrescent pink with woody stem having brown black berries. Histology of leaf show the presence of palisade and spongy parenchyma at laminar region with three layers of collenchyma in between. Xylem found located centrally whereas phloem at periphery. Stem microscopy show trichomes at epidermal region. Below epidermis cortical parenchymal cells found attached along with collenchyma. Thin phloem in the pericycle with centrally phased xylem along with parenchymal pith containing starch and aleurone grains are characteristic findings. Aerial parts of dried plant powder comprise mesophyll, anther, trichomes, pollen grains, fibers in bundles, oleo resin cells and cork cells.

Physiochemical standards are representative of organic and inorganic content of plant. Loss on drying was 16.63% which denote its moisture content. Total ash was 6.53% showing the presence of organic and inorganic matter. Acid insoluble ash was 0.69% representing inorganic matter present in the plant material. Water soluble ash was 2.28%. Alcohol soluble extractive value was 9.06% whereas water soluble extractive value was 33.29% indicative of more polar components of test substance [21].

Steroids, carbohydrates, tannin, and phenols were prime phytochemicals detected as per standard testing.

Methanolic extract of aerial parts of test drug sample was applied 3, 6 and 9µl using the solvent system toluene: Ethyl acetate. The chromatogram scanned at 254 nm and 366 nm showed 9 peaks, whereas at 540 nm and 620nm showed 8 peaks. Each peak value stands for retardation factor [22]. Rf 0.43±.02(2.28) was evident at 366 nm and Rf 0.43±.02(2.32%) at 620 nm. Rf 0.43 indicates a sterol which is beta sitosterol.

Conclusion

Phyllanthus reticulatus Poir of family Phyllanthaceae large glabrous shrub with diverse medicinal benefits. Standard monographs prepared on this drug beneficial in future researches as well as future clinical applications.

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