



PHARMACOGNOSTIC STUDIES ON *PHYLLANTHUS* *RETICULATUS* POIR – A FOLK UNANI MEDICINAL PLANT

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ABSTRACT:

This paper deals with the detailed pharmacognostical evaluation of *Phyllanthus reticulatus* Poir (Euphorbiaceae); a lesser known medicinal plant. It is synonymously also called as *Kirganelia reticulata* (Poir) Baill. A survey of folk Unani herbal system of Amravati District (Maharashtra) shows that the leaves are used by local hakims to treat menstrual disorders and sexually transmitted diseases in males. Pharmacognostic studies were carried out including morphoanatomy of the entire plant and micromorphology and phytochemistry of leaves to establish the authenticity of the *P. reticulatus* and differentiate the drug from its other species. The interview and discussion with the traditional healers proved the medicinal value of *Phyllanthus reticulatus*. Hence suitable measures should be taken for ensuring need based use of this valuable species and to preserve the same in its natural habitat by making use of the traditional knowledge from the unani hakims.

KEYWORDS: Folk Unani system, Euphorbiaceae, micromorphology, pharmacognosy, phytochemistry, *Phyllanthus reticulatus*.

INTRODUCTION:

A survey of local Unani health system was carried out for Amravati District during 2001-2003. As a result 78 plant species were recorded; used to treat 84 ailments by local Hakims. Of these 19 plant species were found to be less known. One of the less known species is

Phyllanthus reticulatus Poir. found to grow abundant in and around Achalpur city. It is locally known as 'Kala Mehmooda' by Hakims and is used to regulate the menstrual cycle, to reduce body heat and in the treatment of venereal diseases of males.

In Sanskrit the plant is known as 'Krishna Kambhoji'. The plant is astringent, sweet, cooling, diuretic and constipating. It is useful in vitiated conditions of pitta, burning sensation, ophthalmodynia, diarrhea, skin eruption and obesity (Warrier et.al.,2003). This plant is a multipotential which has the capacity to cure many ailments. Medicinal properties of this plant include curing heatstroke, helminthiasis, oral infections, and oral lesions in the mouth, tooth and tongue (Md.Ariful et. al, 2010). Leaves are recorded to have diuretic and cooling property; also used to treat the bleeding gums and infant diarrhea. The juice of leaves is used medicinally in the Konkan. It is made into a pill with camphor and cubebs, which is allowed to dissolve in the mouth as a remedy for bleeding gums (Kirtikar and Basu., 1991). Leaves have antidarrheal properties and roots are used in asthma. Fruits of the plant are used in inflammation. *Phyllanthus reticulatus* has been proved to show antidiabetic, antiviral, anticancer, antiparasmodial, hepatoprotective, antibacterial and anti-inflammatory activities. The plant contains tannic acid, terpenoids, flavonoids, phenolic compounds and steroids as main chemical constituents. (Sharma et al 2013). Decoction of bark is used as astringent, diuretic and alternative (Nadkarni., 1976), bark is alterative and attenuant (Chopra et al., Rpr.1996). In Indo-China, the whole plant is used in the treatment of small pox and syphilis. The fruit is said to be eaten in times of scarcity in E. Africa. An ink is prepared from ripe fruits in the Philippines. The roots are used in Madras as a red dye (The wealth of India, 1969). Chewing of the leaves of the plant healed mouth ulcer. Chewing of fresh leaves as well as the twigs act as remedy for bleeding gums. Intake of juice prepared from fresh leaves and fruits reduced body tremors. External application of either fresh leaf paste or paste prepared from dry leaf powder cured long lasting diabetic foot ulcer. Root extract of the plant improved the menstrual problems in females. Soup prepared from the leaves reduced the apin and improved the movement of hands in case of carpal tunnel syndrome. Oral intake of decoction prepared from the leaves, fruits and stem of the plant was able to improve liver disorder. Chewing of fresh leaves frequently healed cracks and dryness in the tongue (V Manjula and T. S.J.Norman, 2017). Phytochemicals are chemical constituents of plants and are used for as therapeutic agents. Very little information is available regarding phytochemistry. Chatterjee and Pakrashi (1997) have recorded occurrence of octacosanol, taraxeryl acetate, friedelin, epifriedelinol, taraxerone, betulin, b-sitosterol, glochidonol and 21-hydroxyfriedelin-3-one isolated from roots.

MATERIALS AND METHODS:

Plant species was identified with the help of flora (Hooker Rpr. 1999 and Singh et al., 2001). For morphological and anatomical characterization fresh plants were used. Single stained hand sections were used for anatomical studies and camera lucida sketches were drawn. For phytochemical studies leaves were shade dried, made into fine powder and was stored in zip-lock bags at 30-32⁰ C. Detection of bioactive compounds was done following Gibbs 1974, Harborne 1973, Paech and Tracey 1979 and Evans 1996. Amino acid profile was studied by two dimensional chromatography comparing with standard amino acids. Estimation of ash value and detection of ash elements was done as per Johansen 1940 & Kokate et al., 1998. Quantitative estimation of potassium, calcium and phosphorus was done with the help of flame photometer. TLC profile of drug powder was also studied (Anonymous 1987).

RESULTS AND DISCUSSION:

Nomenclature - *Phyllanthus reticulatus* Poir. in Lam. Encycl. 5: 298.1804.; syn. *Kirganelia reticulata* (Poir.) Baill Fam. Euphorbiaceae

Large, glabrous or pubescent, straggling shrubs. Branches terete, smooth. Leaves oblong-elliptic or almost round, 10-28 x 8-18 mm. acute or subcordate at base, entire, obtuse or acute, thin, glabrous, dark green above, paler beneath; petioles 1.5 – 3 mm. long; stipules ovate, long, often bristle pointed. Flowers axillary, the males in fascicles, the female's solitary; pedicels slender. Perianth segments alternating with disk glands. Stamens 5, the inner three with their filaments connate into a column, outer two free, shorter; ovary 5 - 10 locular; styles 3, minute. Fruits fleshy, depressed globose, dark purple or black, shining berries. Seeds 8 - 10, 3 - gonous, 1 - 1.5 mm. long, granulate. (Figure 1 – 3)

Micromorphology

Petiole vascular strand single, crescent shaped surrounded by a sheath of somewhat thick walled cells. Ground tissue parenchymatous, cells containing tannin. (figure 4)

Lamina dorsiventral, amphistomatous; cells of both epidermis bluntly angular; stomata paracytic as well as anomocytic; frequently more in the lower epidermis. Mesophyll differentiated into palisade and spongy tissue. Palisade two layered, cells of lower layer much shorter, becoming almost isodiametric or irregular above lateral vein. Spongy tissue 3-5 layered, enclosing large intercellular spaces; chloroplasts few. (figure 5-7).

Midrib slightly elevated on abaxial side; vascular bundle single. Hypodermis collenchymatous on both sides, 2-4 layered; ground tissue parenchymatous, tanniniferous

(figure 8). Trichomes eglandular, uniseriate, unbranched as well as branched with abundant chloroplasts, appearing like algal filaments (Figure 9(a) and figure 9(b))

Phytochemistry

The material was screened for the presence of 14 bioactive compounds out of which 11 were found to be present in the drug plant (Table 1).

Amino Acids: - In all 9 amino acids were found to be present. They are Aspartic acid, Glutamic acid, DL - α - amino - n- butyric acid, Valine, Methionine, Tyrosine, Iso-leucine, DL - Alanine, Unknown. (Ph.W.- 0.57, BAW-0.15, Purple).

Ash analysis: Qualitative studies showed the presence of Sulphur, Calcium, Magnesium, Iron, Chlorine, Phosphorus and Sodium.

Quantitative analysis shows that the drug plant is rich in potassium and calcium. (Table 2).

TLC Fingerprinting: Extracts in four different solvents were used for fingerprinting. Best characterization was found to be produced by acetone extract when developed with H₂SO₄. (Table 3).

Anatomically it is characteristic in having trichomes that look like algal filaments. Phytochemical studies carried out here propose the species as promising candidate to be used in the health care system. Simple phenols present in the form of tannic and gallic acid can serve as antioxidant, antitumor, diuretic and antimicrobial. They are also known to possess antiallergic and vasoprotective properties. Plant contains tannin distributed in the tissue of root, stem and petiole. It tested positively both for true tannins and pseudo tannins. They impart antidiarrhoeal property to the drug. They are also useful as detoxicants in heavy metal poisoning. Anthraquinones are laxative in action and are known to occur in Euphorbiaceae (Evans 1996). Presence of alkaloids is also common for Euphorbiaceae. They are known to have various pharmaceutical activities. The way in which drug plant is used suggests the presence of alkaloids that act like hormones. Presence of iridoids is surprising, since they are supposed to be absent from Euphorbiales (Dahlgren 1981).

CONCLUSION:

The present study on pharmacognostical evaluation of *Phyllanthus reticulatus* leaves will provide useful information for its identification. Macro, Micro and physiochemical standards discussed here can be considered as identifying parameters to substantiate and authenticate the drug.

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Table 1: Phytochemical profile of *P. reticulatus* leaves

S.No.	Phytochemical Compounds	Presence
1.	Acubins	+
2.	Alkaloids	
	a) Mayers reagent	++
	b) Dragendorffs reagent	+
	c) Wagners reagent	++
3.	Anthraquinones glycosides	-
4.	Free anthraquinone derivatives	+++
5.	Anthraquinone having free carboxyl group	-
6.	Cardenolide	+++
7.	Flavonoids (Shinoda test)	+++
8.	Flavonol	-
9.	Flavone and flavanone	+
10.	Leucoanthocyanin	+
11.	Tannic acid	+
12.	Gallic acid	+
13.	True tannins	+
14.	Pseudo tannins	+++
Legend: +, ++ and +++ denotes low, moderate and high concentration of respective compounds).		

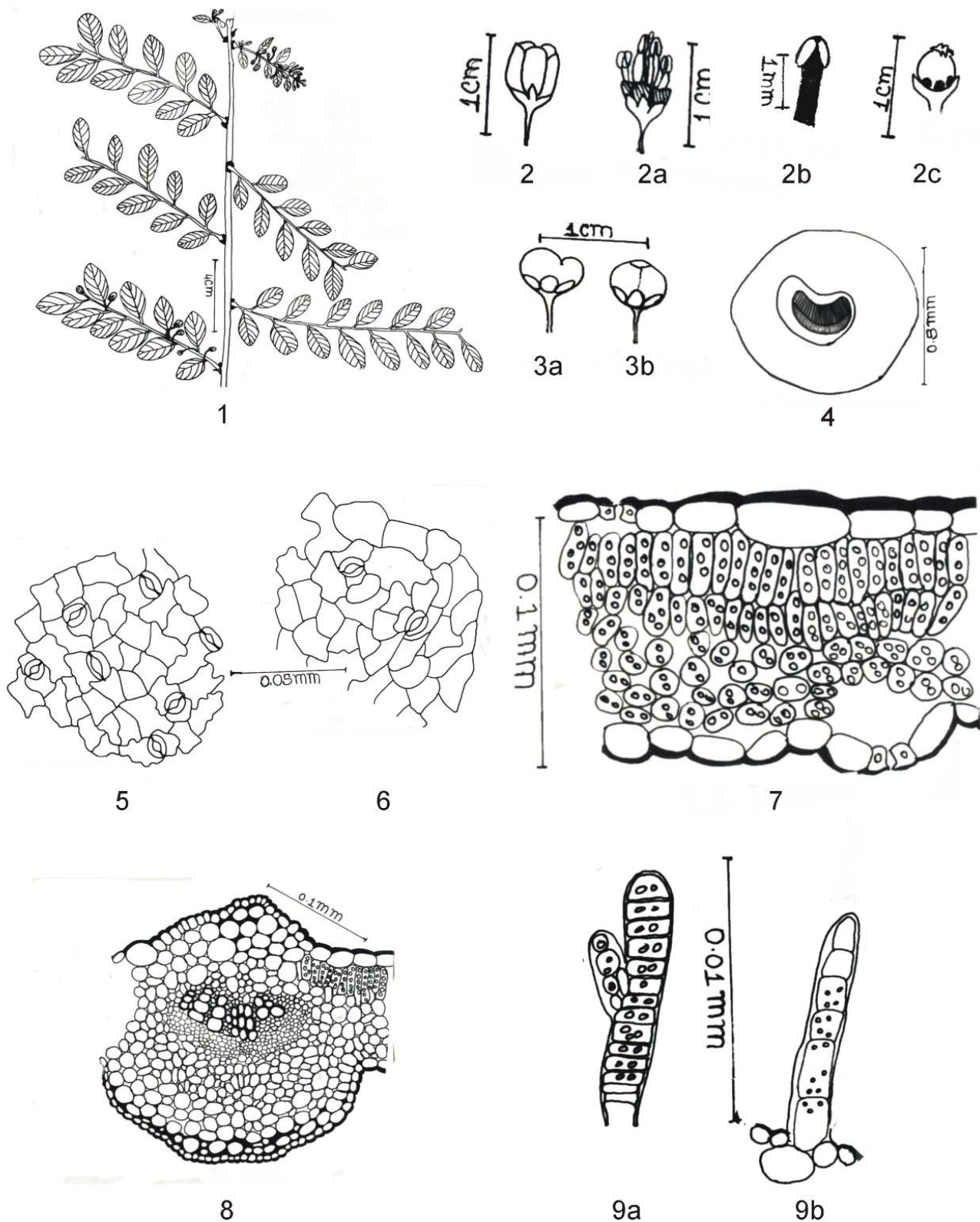
Table 2: Quantitative Analysis

Ash value	With Hcl.	With HNO ₃	Potassium	Sodium	Calcium
903 mg/gm dry weight	Soluble ash: 506 mg/gm Insoluble ash: 494 mg/gm	Soluble ash: 682 mg/gm Insoluble ash: 318 mg/gm	303.03 mg/gm	20 mg/gm	180 mg/gm

Table 3: TLC Fingerprinting

S. No.	Extract	Solvent	Spray	No. of spots	Rf values and colour
1.	Alcohol	Chloroform: Benzene	H ₂ SO ₄	8	0.14 Br, 0.2G, 0.25Pk, 0.32 L.G., 0.39 L.G, 0.54 Bl.Pl, 0.71 Pk.Pl., 0.98Y.
			Iodine	3	0.05 Br., 0.09 Y, 0.98 Br
2.	Acetone	Chloroform: Benzene	H ₂ SO ₄	10	0.08 Br, 0.25 G, 0.39 G, 0.47 Br, 0.57 Pk with Bl.outline, 0.71 Pk, 0.74 L.Bl, 0.79 L.Bl, 0.90 Br, 0.90 Pk.Pl.
			Iodine	3	0.07 Br., 0.19 Br. 0.57 Br.
3.	Chloroform	Chloroform: Benzene	H ₂ SO ₄	6	0.07 Br.,0.32 Pk.,0.32 Pl.,0.57 Br.,0.90 Y.Br.,0.96 Rd.Br.
			Iodine	1	0.98 Br
4.	Petroleum ether	Chloroform: Benzene	H ₂ SO ₄	5	0.07 G., 0.09 Br.,0.16 Bl.Br., 0.26 Br., 0.90 Pl. Br.
			Iodine	3	0.1 Br., 0.23 Br. Y., 0.74 Y.

Colour key: Br. – Brown, G. – Green, Pk. – Pink, Bl. Pl. – Blue purple, Y. – Yellow, Bl. – Blue, Y.Br. - Yellowish brown, Br. Y. – Brownish yellow, Pl. Br. – Purplish brown, Pk. pl.- Pink purple, Rd. Br. – Reddish brown, Bl. Br.- Blue brown. L. – denotes light colour.



Legends for Figures

Figure 1: Twig
 Figure 2: Flower
 Figure 2(a): Androecium
 Figure 2(b): Stamen
 Figure 2(c): Gynoecium
 Figure 3(a, b): Fruit
 Figure 4: Petiole
 Figure 5: Lower Epidermis

Figure 6: Upper Epidermis
 Figure 7: T.S. Lamina
 Figure 8: T.S. Midrib
 Figure 9 a): Branched Trichome
 Figure 9 b): Unbranched Trichome