Appendix

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1. Chemotaxonomic studies on some members of the Lythraceae. *BioSci. Reptr. 3(2) 2005-194-196*

2. Chemical and Pharmacognostic characters of *Lagerstroemia flos-reginae* and *Woodfordia floribunda in Herbal Technology: Recent Trends and Progress* (M. Daniel Ed.) Scientific Publishers, Jodhpur (2007) pp. *93- 100*

3. Studies on the biomarkers of *Ammania baccifera* Linn. and *Lawsonia inermis* Linn. In *Recent Trends and Progress* (M. Daniel Ed.) Scientific Publishers, Jodhpur (2007) pp. *129- 138*

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CHEMOTAXONOMIC STUDIES ON SOME MEMBERS OF THE LYTHRACEAE

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ABSTRACT

Seven members of the family Lythraceae have been screened for their stem and leaf chemical constituents. The family is found to be rich in flavonoids, tannins and quinones. The two tribes Lythreae and Ammanieae are chemically distinct in that the former contains flavones, *p*-hydroxy benzoic acid and *p*-coumaric acid which are absent in the latter. Though morphologically Ammanieae is advanced due to their herbaceous habit, Lythreae with their flavones seems to be chemically advanced, bringing out a clear example of Heterochrony common in many Angiosperms

INTRODUCTION

Classification of plants was generally based on morphological concepts. The application of anatomical characters in plant classification dates back to Bureau (1864) who for the first time used anatomical characters for classification of Bignoniaceae. Later onwards data from palynology, embryology, phytochemistry etc were added to make Taxonomy a "Synthetic Science". Now even molecular data are used in classifying and deducing phylogeny of plants. Chemical characters have been used earlier for the classification of Algae and microorganisms. They have been used for Angiosperms only in the early seventies with rapid advancement of Chromatography and Spectrophotometry, the techniques of isolating and identifying compounds in a micro scale. Now they are accepted equal to other characters in assessing relationships and phylogeny.

The Lythraceae are a very small family of dicots. According to the 'Flora of British India' there are 7 genera, classified into two tribes Ammanieae & Lythreae. In Gujarat this family is represented by 4 genera and a number of species. Members of this family are of great importance economically especially due to their medicinal properties. For the present work eleven plants belonging to this family have been screened for their leaf constituents and the data on the distribution of these compounds have been used in assessing the taxonomic relationships among the taxa screened

MATERIALS AND METHODS

All the plants screened, are collected from the various localities of Baroda. Voucher specimens of these plants are deposited in The Herbarium of Department of Botany, M.S.University, Baroda (BARO). Standard procedures are followed for the extraction, isolation and identification of the various plant products such as Flavonoids, Phenolic acids, Alkaloids and terpenoids (Mabry et al., 1970; Harborne, 1984; Markham, 1984).

Table 1 The distribution of various phytochemicals in the leaves/stem of some members of the Lythraceae

Sr. No.	Name of the plant	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1.	Tribe Ammaniae																					
	Ammania tenuis C.B. Clarke	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-	+	+
2.	Ammania baccifera Linn.(Leaf)	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+	-	-	+
	Ammania baccifera Linn. (Stem)	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+	-	+	+
3.	Tribe Lythreae																					
	Lagerstroemia flos-reginae Retz. (Leaf).	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-
	Lagerstroemia flos-reginae Retz. Stem	+	+	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	-	-
4.	L .indica Linn. (Leaf)	+	-	+	-	+	+	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-
	L .indica Linn. (Stem)	+	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	+	-	-
5.	L. parviflora Roxb. (Leaf)	-	-	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	-	+	-	+
	L. parviflora Roxb. Stem	-	-	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	-	+	-	-
6.	Lawsonia inermis Linn. (Leaf)	-	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	+	+	-	-	-
	Lawsonia inermis Linn. (Stem)	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+	-	+	+	+	-
7.	Woodfordia floribunda Salisb. (Leaf)	+	+	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	-	-	-
	Woodfordia floribunda Salisb. (Stem)	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	-	-	-	-

(1) Quercetin, (2) 3'-OMe quercetin, (3) Kaempferol, (4) 4'-OMe Kaempferol, (5) Acacetin, (6) 7'-OMe Acacetin, (7) Luteolin, (8) 3'-OMe Luteolin, (9) 3',4'-diOMe Luteolin, (10) Glycoflavones, (11) Quinones, (12) Steroids, (13) Alkaloids, (14) Tannins, (15) Vanillic acid, (16) syringic acid, (17) *p*-OH benzoic acid, (18) Gallic acid, (19) *p*-Coumaric acid, (20) Ferulic acid, (21) Melilotic acid

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CHEMICAL AND PHARMACOGNOSTIC CHARACTERS OF *LAGERSTROEMIA FLOS-REGINAE* AND *WOODFORDIA FLORIBUNDA*

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ABSTRACT

Woodfordia floribunda and *Lagerstroemia flos-reginae,* two important medicinal plants of India are studied for their pharmacognostic and phytochemical characters. *Woodfordia* is characterized by the unicellular, conical trichomes and spherical glandular hairs in leaves and patches of stone cells, intraxylary phloem and sphaeraphides in stem. The leaves are found to contain flavonoids such as quercetin and 3' OMe quercetin and phenolic acids like syringic, vanillic, gallic and *p*- hydroxy benzoic acids. The stem of this plant contained only acids such as syringic, vanillic and *p*-hydroxybenzoic acids. In *Lagerstroemia,* leaves possessed two layers of palisade and sphaeraphides along the veins while the stem contained mucilage cells, tannin cells, stone cells in phloem and intraxylary phloem. Chemically this plant contained quercetin and 4'OMe quercetin as well as syringic, gallic, vanillic, *p*-coumaric and *p*-hydroxybenzoic acids in both leaves and stem.

INTRODUCTION

The family Lythraceae comprises of 11 genera classified under two tribes Ammanieae and Lythreae. In Gujarat this family is represented by only four genera *Woodfordia*, *Lawsonia*, *Lagerstroemia* and *Ammania*. Members of this family are of great medicinal importance.

comprising of stomatal index, trichome index, type of stomata and epidermal cells etc. were based on the average of 20 readings.

(b) Phytochemical studies

The leaves and stems of both these plants have been analysed for their phytochemicals such as alkaloids, flavonoids, phenolics, saponins, tannins etc. using standard procedures (Harborne, 1984; Daniel, 1991).

RESULTS AND DISCUSSION

Micro morphological features

Leaves of both *Woodfordia* and *Lagerstroemia* are hypostomatic with stomata present only on the lower/abaxial surface of the leaf. Epidermal cells are mostly polygonal and isodiametric. There is no definite pattern of arrangement of epidermal cells. The epidermal cell walls are straight to curved or sinuate. The surfaces of the cells are smooth without any striations.

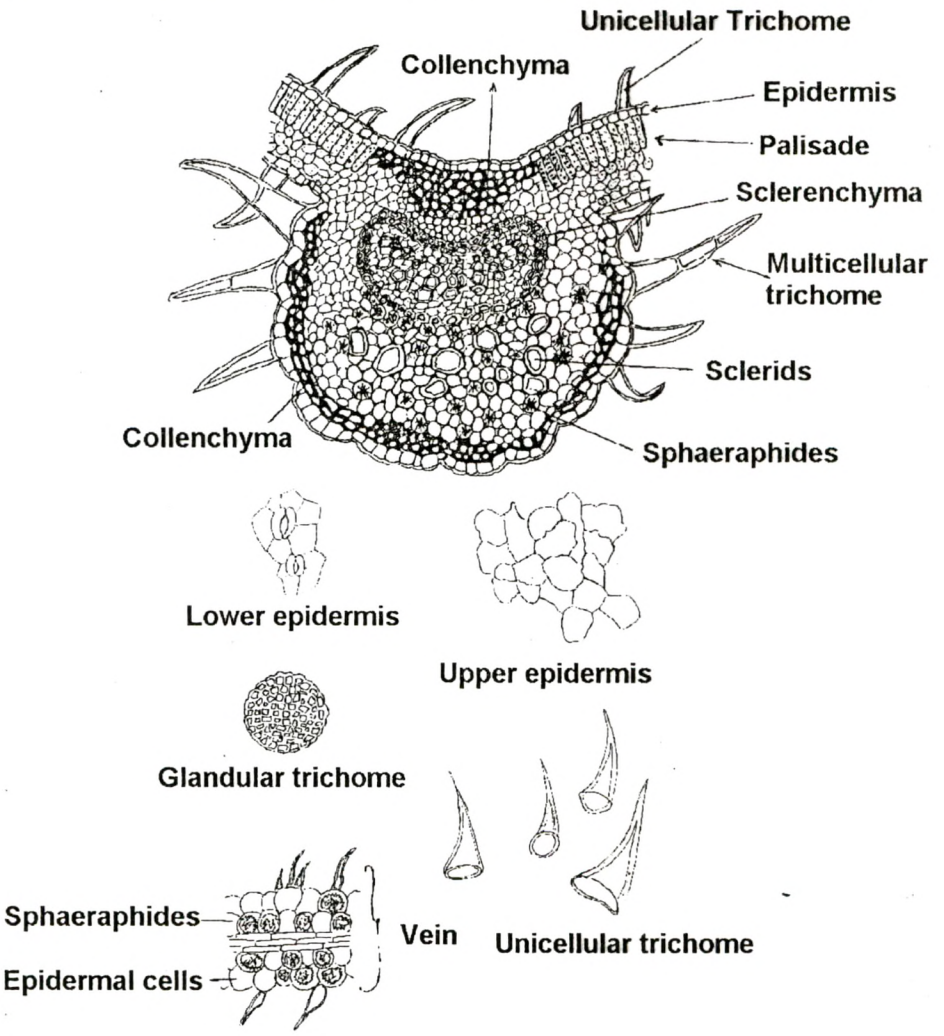

Fig. 1. T.S. and micromorphological features of the leaf of *Woodfordia floribunda*

curved trichomes. Glandular spherical/spheroidal trichomes appear sunken in pits filled with secretion which makes the lower surface sticky. Each glandular trichome consist of a one celled stalk and and a glandular head of 20-40 or more radially elongated cells more or less separated. Length of the trichome is 63.84 μm. The trichome index is 48-50 (Figs. 1 and 2).

Anatomical attributes of leaves

Leaves in *Woodfordia* and *Lagerstroemia* are dorsiventral with a distinct differentiation of the mesophyll tissue into palisade and spongy layer protected on either side by upper and lower epidermis respectively. In *Woodfordia* palisade tissue is made up of a single layer of short palisade cells which are compactly arranged, while in *Lagerstroemia* palisade layer constitutes of two layers of elongated palisade cells which are loosely arranged. Both layers of palisade cells are of the same height. Spongy layer is composed of rounded or elongated cells with large prominent intercellular spaces.

In *Woodfordia* the midrib is continuous with few layers of collenchyma, within which compactly arranged thin walled polygonal cells of parenchyma are present embedded in which a semicircle fibro vascular bundle is present. A large number of sclereids and sphaeraphides are evenly distributed lining the vascular bundle. In *Lagerstroemia* the midrib contains two large crescentiform collateral vascular bundles placed opposite to each other and are separated by thick walled pitted parenchyma cells. Parenchymatous cells surrounding the vasculature show sphaeraphides. Cortical region of midrib is heterogenous consisting of few layers of collenchyma followed by parenchyma (Figs. 1 & 2).

The results obtained from this investigation revealed a number of interesting features which may be useful in classifying the two plants. Variability in epidermal cells architecture among different groups of plants is an attribute that makes cuticular feature a reliable tool in taxonomic analysis and diagnosis (Olowokudejo, 1990, Metcalfe and Chalk, 1950). Leaf of *Woodfordia* is distinctly differentiated by the presence of trichomes. The upper and lower leaf surface is also apparently different with hooked covering trichomes and multicellular glandular trichomes on the abaxial surface and only conical covering trichome on the upper / adaxial surface.

Anatomical attributes of stem

Epidermis in the stem of *Woodfordia* and *Lagerstroemia flos-reginae* are single layered made up of barrel shaped cells which appears to be crushed due to the enlarging and addition of internal tissues by the secondary growth. In *Woodfordia* the epidermis is followed by few layers of hypodermis comprising of collenchyma. Cortex is composed of sclerenchyma cells interspersed with mucilage cells followed by large parenchyma cells intermingled with islands of stone cells. In *Lagerstroemia flos-reginae,*it is made up of 8-10 layers of large polygonal parenchyma cells. Vascular region comprises of solid cylinder of xylem with phloem on both sides in *Woodfordia* while in *Lagerstroemia flos-reginae* phloem is present only towards outside.

Woodfordia floribunda

The results of screening this plant is also presented in Table 1. Stem was found to be devoid of flavonoids while the leaves were found to possess quercetin and 3'-OMe quercetin. Except for gallic acid, both stem and leaves contain the same phenolic acids such as vanillic, syringic and *p*-hydroxybenzoic acid. Lawsone and steroids were seen in both the parts. Alkaloids were absent.

Table 1. Distribution of phytochemicals in the leaves and stems of *Lagerstroemia flos-reginae* and *Woodfordia floribunda*

S. No	Name of the plant	1	2	3	4	5	6	7	8	9	10	11	12
1.	*Lagerstroemia flos-reginae* (leaf)	+	-	+	+	+	+	+	+	-	-	+	+
	(stem)	+	-	+	+	+	+	+	+	-	-	+	+
2.	*Woodfordia floribunda* (leaf)	+	+	-	+	+	+	-	+	-	-	+	+
	(stem)	-	-	-	+	+	-	-	+	-	-	+	+

(1) Quercetin, (2) 3'OMe quercetin, (3) 4'OMe quercetin, (4) Syringic acid, (5) Vanillic acid, (6) Gallic acid, (7) *p*-Coumaric acid, (8) *p*-OH Benzoic acid, (9) Glycoflavones, (10) Alkaloids, (11) Quinones, (12) Steroids.

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STUDIES ON THE BIOMARKERS OF *AMMANIA BACCIFERA* LINN. AND *LAWSONIA INERMIS* LINN.

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ABSTRACT

Pharmacognostic and phytochemical studies on *Ammania baccifera* Linn. and *Lawsonia inermis* Linn. two medicinal plants of the family Lythraceae have been carried out. The leaves of *Ammania* are characterised by bicollateral vascular bundle, a characteristic pattern of ridges and network on the cuticle, gallic acid and 3',4'-diOMe quercetin while its stem contained outer ring of larger xylem tracheids and inner ring of smaller tracheids, 3'-OMe quercetin and 3'-OMe kaempferol. Leaves of *Lawsonia* contain stone cells, lawsone, gallic acid and 3'-OMe luteolin whereas the stem contains stone cells surrounded by cells containing brown deposits, lawsone, gallic acid and 3', 4'-diOMe luteolin.

INTRODUCTION

Ammania baccifera Linn. and *Lawsonia inermis* Linn. are two medicinal plants belonging to the Lythraceae. The former plant is included in the tribe Ammaniae and the latter in the tribe Lythreae. *A. baccifera* is a glabrous, erect branching herb, found as a weed in rice fields and marshy localities throughout India. Leaves of this plant are acrid and used in treatment of rheumatic pains and fever. It is prescribed as stomachic and laxative. Fresh bruised leaves are used as rubefacient and external remedy for ringworm and other skin affections. Leaves are mixed with oil and applied to cure herpetic

eruptions. The herb is rich in vitamin C and is reported to possess anti-typhoid and antitubercular properties (Anon., 1960). Leaves are used to treat rheumatism and fever (Pandey, 2005). *Lawsonia inermis* (Henna, Mehandi) is a glabrous, much branched shrub or a small tree with grayish brown bark. Henna leaves are used as a prophylactic against skin diseases and have astringent properties. They are used externally in the form of a paste or decoction against boils, burns, bruises and skin inflammations. Decoction is used as gargle for relaxed sore throat. Alcoholic extracts of leaves show mild antibacterial activity against *Micrococcus pyogenes* var. *aureus* and *E. coli*. Mehandi oil is used in perfumery. Leaves are also used to treat chest and lung diseases while stem is used for jaundice, growth of hair, headache, jaundice, leprosy, leucoderma, ulcers, lumbago, ophthalmia, skin diseases, stomachic etc. (Pandey, 2005). Triterpenoids have been isolated from *L. inermis* (Siddiqui, 2001). A naphthaquinone from stem bark has been isolated and 24β-Ethylcholest – 4- en-3β-ol from, the roots (Gupta, 1992) is the other compound reported.

Only the leaves of *Lawsonia* had been studied for their pharmacognostic characters. The pharmacognostic data on stem of this plant as well as the leaves and stem of *Ammania* are not available. The chemistry of *Ammania* also is not at all studied. Therefore, the present paper contains a detailed phytochemical and pharmacognostic studies on the leaves and stem of both these medicinal plants. It is hoped that the data presented here will be of great help in locating the biomarkers needed for the quality control programmes of these plants.

MATERIALS AND METHODS

Both plants were collected from the various localities of Baroda. Voucher specimens of these plants are deposited in the Herbarium of Department of Botany, M.S. University, Baroda (BARO). Standard procedures are followed for the extraction, isolation and identification of the various plant products such as flavonoids, phenolic acids, quinones, steroids etc. (Harborne, 1984; Daniel, 1991). For anatomical studies, sections were taken, stained with saffranin and mounted in glycerin. For micromorphological studies, epidermal peels were taken by direct peel or by heating in 10% KOH. The peels were then stained with saffranin and mounted on a clean microscopic slide. The quantitative and qualitative data comprising of stomatal index, trichome index, type of stomata, epidermal cells etc. were based on the average of 20 readings (Wallis, 1953).

RESULTS

(a) Pharmacognosy

(1) *Ammania baccifera*

Leaf of this plant contains mesophyll differentiated into single layer of palisade and closely packed spongy cells. In the midrib region, 3-4 layers of

chlorenchyma were there on upper side of vascular bundle. There were two patches of phloem on both upper and lower sides of xylem. 6-7 Layers of parenchyma were seen with sphaeraphides on the lower side followed by a layer of collenchyma. Cuticle exhibited a pattern of net-like ridges and meshes. Leaf was found to be amphistomatic with anomocytic stomata. Stomatal index of upper epidermis and lower epidermis was 13-14 and 14-15 respectively (Fig. 1).

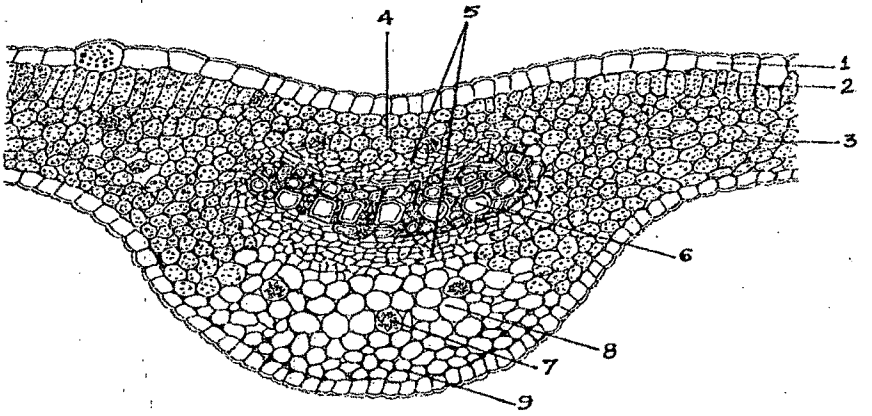

Fig. 1a. *Ammania baccifera* (leaf): 1. Epidermis, 2. Palisade, 3. Spongy, 4. Chlorenchyma, 5. Phloem, 6. Xylem, 7. Sphaeraphides, 8. Parenchyma, 9. Collenchyma

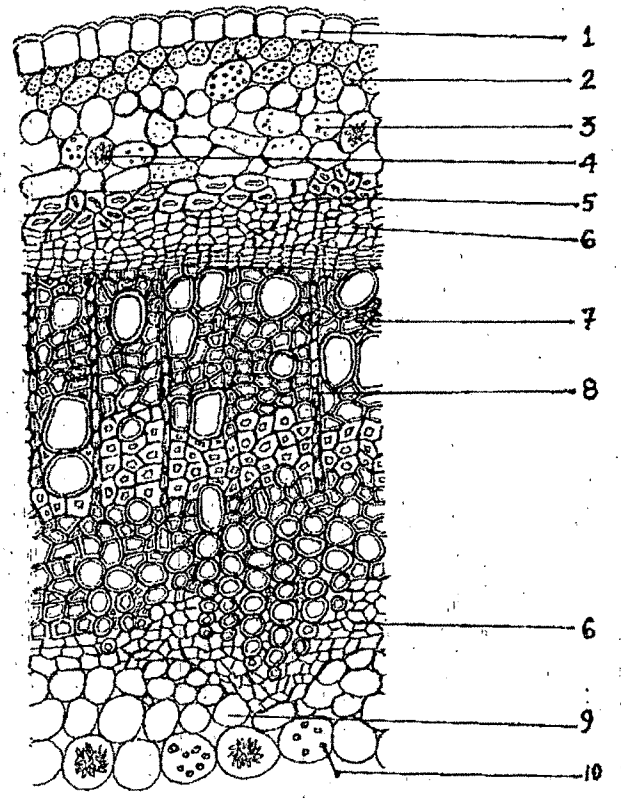

Fig. 1b. *Ammania baccifera* (stem)
1. Epidermis, 2. Chlorenchyma, 3. Parenchyma, 4. Sphaeraphides, 5. Sclereids, 6. Phloem, 7. Xylem, 8. Medullary rays, 9. Pith, 10. Starch grains

Stem of this plant has epidermis made of square shaped cells covered with cuticle having net like ridges and furrows. Cortex consisted of 5-6 layers of chlorenchyma where first 2-3 layers were full of chloroplasts as compared to the rest of the layers. Patches of stone cells encircled the upper phloem. Here also, inner phloem was seen. Xylem tracheids of outer and inner regions were with a large lumen while those of middle region were having narrow lumen giving an appearance of annual rings. Pith consisted of parenchymatous cells with sphaeraphides and very few starch grains (Fig. 2).

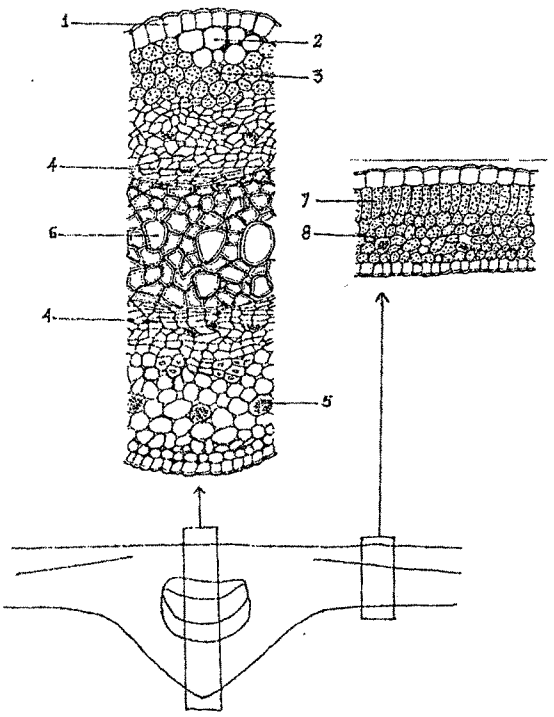

Fig. 2(a) *Lawsonia inermis* (leaf)

1. Epidermis, 2. Collenchyma, 3. Chlorenchyma, 4. Phloem, 5. Sphaerahides, 6. Xylem, 7. Palisade, 8. Spongy

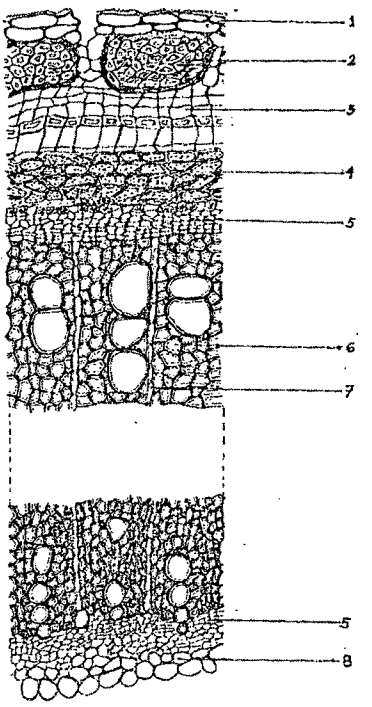

Fig. 2(b) *Lawsonia inermis* (stem)

1. Epidermis, 2. Sclereids, 3. Collenchyma with chloroplasts, 5. Phloem, 6. Xylem, 7. Medullary rays, 8. Pith

Powdered drug of the leaf consisted of fragments of anomocytic stomata, palisade, epidermal cells, sphaeraphides, vessel with double spiral thickening, cuticle with ridges and furrows, phloem fibres and crystal while that of stem consisted of sclereids, vessels with bordered pits alongwith medullary rays and tracheids, pieces of phloem, vessels with spiral and helical thickenings, cells containing chloroplasts and sphaeraphides and cork cells.

(2) *Lawsonia*

In **leaf,** the mesophyll was differentiated into one layered palisade and closely packed spongy cells containing sphaeraphides. Midrib on the upper side contained a patch of collenchyma surrounded by chlorenchymatous cells. Here also, there were two patches of phloem on both upper and lower sides of

xylem. Lower phloem elements were less in number than upper phloem. Patches of stone cells were seen below the lower phloem. On the lower side of midrib, parenchymatous cells of 5-6 layers followed by collenchyma cells are seen. Upper epidermis was found larger than the lower epidermis. Leaves were amphistomatic containing ranunculaceous stomata. Stomatal index of upper epidermis was 14-16 while that of lower epidermis was 21-23 (Fig. 3).

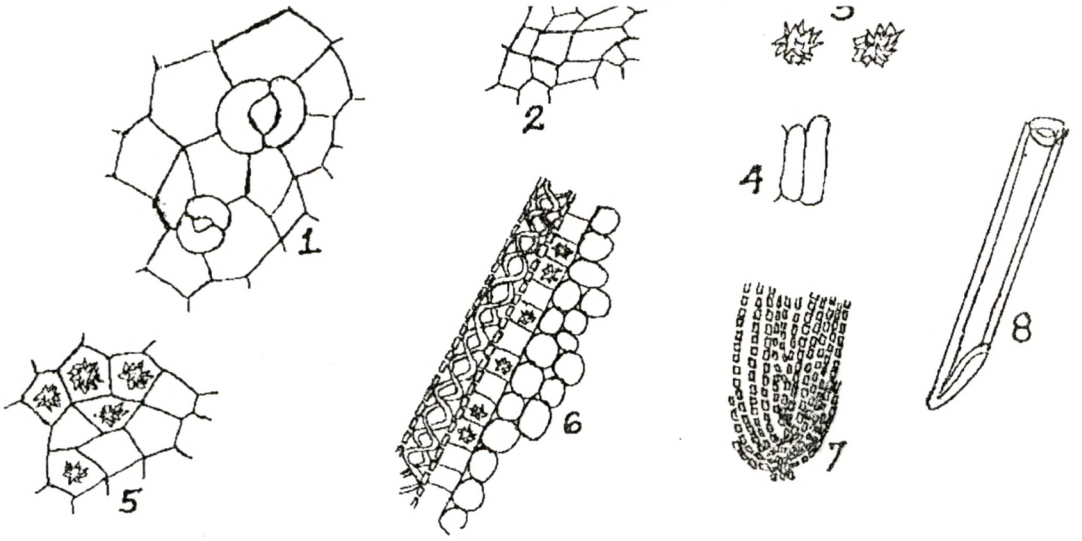

Fig. 3a. *Ammania baccifera* (leaf):
1. Anomocytic stomata, 2. Epidermal cells, 3. Sphaeraphides, 4. Palisade, 5. Cells containing sphaeraphides, 6. Vessel with double spiral thickening, 7. Cuticle with ridges and furrows, 8. Phloem fibre, 9. Crystal.

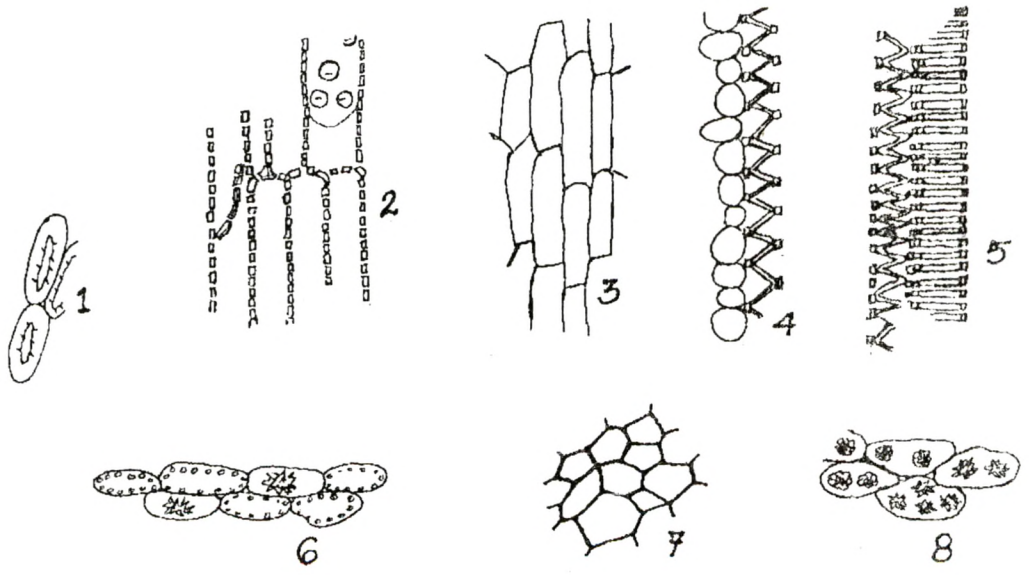

Fig. 3b. *Ammania baccifera* (stem)
1. Sclereids, 2. Vessel with bordered pits alongwith medullary rays and tracheids, 3. Phloem, 4. Vessel with spiral thickening, 5. Vessels with spiral and helical thickenings, 6. Cells containing chloroplasts and sphaeraphides, 7. Cork cells, 8. Cells with sphaeraphides

Stem – The outermost layer was cork followed by patches of stone cells surrounded by brown colored cells. Secondary cortex were found to be of 5-6 layers made up of colorless square to polygonal shaped cells. Primary cortex consisted of collenchyma cells with chloroplasts in it. Phloem was seen on the inner side of xylem also. The outermost layer of upper phloem showed presence of sphaeraphides. Medullary rays were also present. The inner phloem was followed by pith made of parenchymatous cells (Fig. 4).

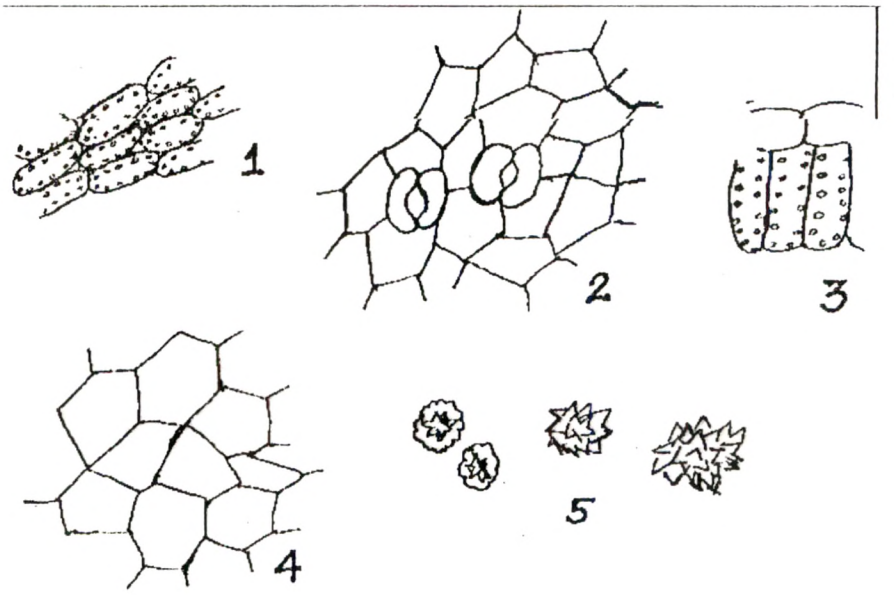

Fig. 4a. *Lawsonia inermis* (leaf)
1. Chlorenchyma, 2. Anomocytic stomata, 3. Palisade, 4. Epidermal cells, 5. Sphaeraphides

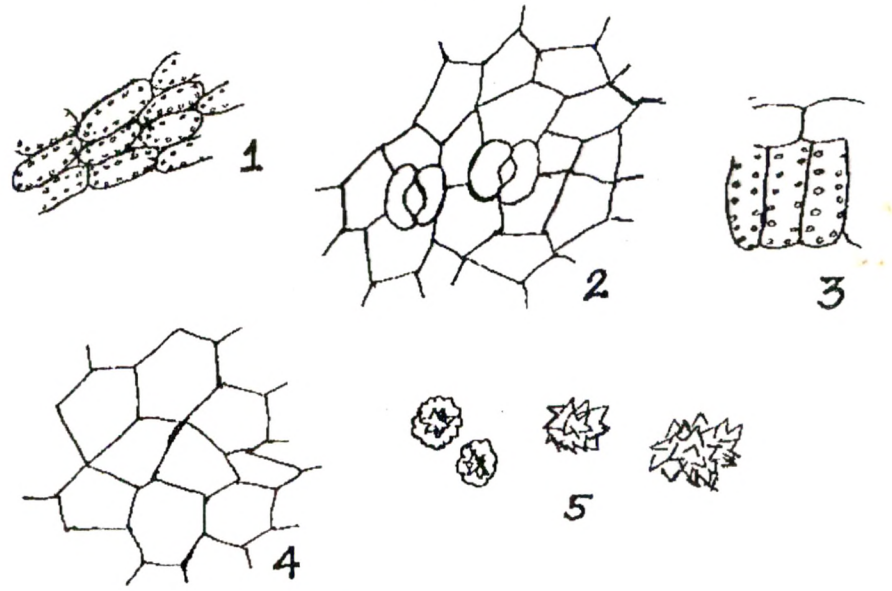

Fig. 4b. *Lawsonia inermis* (stem)
1. Cork cells, 2. Phloem cells with sphaeraphides, 3. Chlorenchyma cells, 4. Phloem fibre, 5. Sclereids, 6. Vessel with bordered pits alongwith medullary rays and tracheids

Powder of the leaf consisted of fragments of chlorenchyma, anomocytic stomata, palisade, epidermal cells and sphaeraphides and that of stem consisted of cork cells, phloem cells with sphaeraphides, chlorenchyma, phloem fibres, sclereids and vessel with bordered pits alongwith medullary rays and tracheids.

(b) Phytochemistry

The results of screening of leaves and stem of the two plants for their phytochemicals are presented in **Table 1**. Leaves of *A. baccifera* were found to contain 3', 4'-diOMe quercetin and phenolic acids like syringic, vanillic, gallic and melilotic acids. Quinones (other than lawsone, the compounds are yet to be identified) were present while steroids and glycoflavones were absent. Stem contained 3'-OMe quercetin and 3'-OMe kaempferol and phenolic acids like melilotic, gallic, syringic and vanillic acids.

Table 1. Distribution of phytochemicals in the leaves and stems of *Ammania baccifera* and *Lawsonia inermis*

S.N.	Name of Plant		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Ammania baccifera*	(leaf)	+	-	-	-	-	-	+	+	+	+	-	-	-	-	+	-	-
		(stem)	-	+	+	-	-	-	+	+	+	+	+	-	-	-	+	+	-
2	*Lawsonia inermis*	(leaf)	-	-	-	+	+	-	-	+	+	-	-	+	-	-	+	+	-
		(stem)	-	-	-	-	-	+	+	+	+	-	+	-	+	+	+	+	-

(1) 3', 4'-diOMe Quercetin, (2) 3'-OMe Quercetin, (3) 3'OMe Kaempferol, (4) Luteolin, (5) 3'-OMe Luteolin, (6) 3', 4'-diOMe Luteolin, (7) Syringic acid, (8) Vanillic acid, (9) Gallic acid, (10) Melilotic acid, (11) cis- & trans-ferulic acid, (12) *p*-OH benzoic acid, (13) *p*-coumaric acid, (14) Gentisic acid, (15) Quinones, (16) Steroids, (17) Glycoflavones

Leaves of *L. inermis* contained luteolin and 3'-OMe luteolin and vanillic, gallic and p-OH benzoic acids. Stem contained 3',4'-diOMe luteolin and phenolic acids such as syringic, vanillic, ferulic, gallic, *p*-coumaric and gentisic acids. Quinone (lawsone) and steroids were present and glycoflavones were absent in both leaves and stem.

DISCUSSION

The present study brings out the biomarkers of both these plants clearly. *Ammania* has peculiar mesh like thickenings on the cuticle, quinones, gallic acid and flavonols. *Lawsonia* possesses stone cells, lawsone, gallic acid and 3'-OMe luteolin in the leaves and stone cells surrounded by cells containing brown deposits, lawsone, gallic acid and 3', 4'-diOMe luteolin in the stem.

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