Research article

Rapports De Pharmacie Vol.1 (3), 2015, 152-160
ISSN: 2455-0507

PHARMACOGNOSTIC, PHYSICOCHEMICAL AND PHYTOCHEMICAL INVESTIGATION OF *ATALANTIA MONOPHYLLA* (L.) CORREA LEAVES

P. Pandian

Department of Pharmacy, Annamalai University, Annamalai Nagar-608002, Tamilnadu, India.

ABSTRACT

Objective*:* The aim of the present was to investigate the microscopic characteristics of *Atalantia Monophylla* (L.) correa leaf and to determine the physicochemical and phytochemical parameters. **Methods***:* Microscopic characters of Transverse section of leaf were studied under microscope. The physicochemical properties such as Total ash value, Water soluble ash value, acid insoluble ash value, extractive values and solubility of *Atalantia Monophylla* (L.) correa leaf were carried out. The presence of phytochemical constituents was studied. **Results**: The leaf shows abundant sphaeraphides and rhomboidal calcium oxalate crystals. The leaf shows no trichomes. leaf shows a continuous network of veins. Micromorphological studies conducted on the leaf gave value of stomatal index to be 11.48, Vein islet number 15, vein termination number 31and palisade ratio to be 4.94. Phytochemical tests performed indicate the presence of steroids, triterpenoids, carbohydrates, coumarins, flavonoids, phenolics, and alkaloids. **Conclusion***:* The results of this study could be useful in setting some diagnostic indices for identification, authentication and for future investigators in their pharmacological analysis of this species.

KEYWORDS:

Atalantia monophylla, Indian atalantia, Rutaceae, pharmacognostic, physicochemical, phytochemical

INTRODUCTION

Atalantia monophylla (L) correa., is commonly known as Indian atalantia. It is a species in the genus Atalantia, belonging to the family Rutaceae. Atalantia is a genus with 62 species of flowering plants. The genus Atalantia contains small trees somewhat resembling Citrus in general aspect, bearing fragrant white flowers and globose fruits with the appearance of diminutive greenish-yellow oranges. The pulp-vesicles are, however, different from those of Citrus in being sessile instead of stalked. The leaves of Atalantia, although almost always unifoliolate like those of Citrus, are very different in having much more prominent, more numerous lateral veins, and veinlets forming reticulations between the lateral veins. The nomenclature of this species is in a confused state. *Limonia monophylla* of Linnaeus has been assumed by almost all taxonomists to be this species. However, in the later stage *Limonia monophylla* was identified as a synonym of *Atalantia ceylanica*[1, 2].

The Indian atalantia is a small tree which reaches a height of about 25 feet (7.8 meters) and a spread of 12-15 feet (3.7- 4.7 meters) at maturity. The tree grows relatively rapidly, forming a dense, much-branched canopy with a broadly columnar shape. The trunk is smooth at first, but becomes deeply fluted as the tree matures. The leaves are alternate, elliptical, with obtusely rounded, emarginate apices. They are 2.8-5.5 inch (7-14 centimeters) long and 1.2-2.2 inch (3-5.5 centimeters) wide. The leaves are very dark green and glossy on the upper surface and pale green on the lower surface. The tree is evergreen and individual leaves persist for around 2 years. Flowers seen small in axillary racemes. Fruits small, round berries contain small seeds[3, 4].

This plant is widely distributed in India (except in Himalayan region and in Bombay State), Ceylon, Burma, Thailand, Cambodia, Laos, North Vietnam, and South Vietnam. In folk medicine, this plant is used for several medicinal purposes such as anti-rheumatic, anti-spasmodic, stimulant, in hemiplegia and for the treatment of paralysis. The essential oil from the leaves has been reported for antimicrobial and strong inhibitory activities against some pathogenic fungi, whereas decoction of the leaves is used for itching and other skin complaints [5, 6].

Botanical description

Scientific name	:	*Atalantia monophylla (*L.*)* Corr. Serr.
Botanical name	:	*Atlantia monophylla* Linn.
Kingdom	:	Plantae
Phylum	:	Magnoliophyta
Class	:	Magnoliatae

Order	:	Sapindales
Family	:	Rutaceae
Genus	:	Atalantia
subfamily	:	Aurantioideae
Tribe	:	Aurantieae
Subtribe	:	Citrinae.
Plant name in different languages		
English	:	Wild lime tree, Wild lemon
Hindi	:	Banjamir nimbu, Bannimbu, Jungli nimbu
Sanskrit	:	Aranyanimbuka, Aranyajambira, Atavi-Jambira
Malayalam	:	Kattunarakam, Kattunaragam, Kattunarenga
Tamil	:	Kattanarangam, kattelumicchai
Telugu	:	Adavi-Nimma
Kannada	:	Kadu Nimbu
Marati	:	Makad Limbu
Oriya	:	Kata narunga, Narguni
Trade name	:	Wild lime
Folk	:	Jungli nimbu

Authentication and standardization are prerequisite steps especially for herbal drugs and their formulations in traditional systems of medicine. Hence, the present investigation of *Atalantia monophylla* (L) correa leaves was taken up to establish pharmacognostic profile which will help in crude drug identification as well as standardization of the quality and purity of the drug in crude form. The present study comprises the microscopical, physicochemical and phytochemical analysis of the leaves of *Atalantia monophylla* (L) correa, since no proper report is available on the pharmacognosy and anatomy of the leaf of this plant.

MATERIALS AND METHOD

The plant specimens for the study were collected in the month of February-2013 from Nilgiri hills, Tamil Nadu, India at an altitude of 1800 m and authenticated as *Atalantia monophylla* (L) correa by Dr.V.Chelladurai, Ex. Professor (Botany), Siddha, Government of India. A voucher specimen (M) has been deposited at the Museum of the Department of Pharmacy, Annamalai University, Annamali Nagar, Tamilnadu, India. The shade dried leaves were powdered for physicochemical and phytochemical analysis. Fresh leaves were used for micromorphological and anatomical studies.

Pharmacognostic analysis

Anatomy

Sections of fresh leaf were subjected to double staining using Safranine (0.5% in water) and Fast green (0.25%). The slides were then mounted and sealed using DPX. The slides were then observed under the microscope at different magnifications, depending upon the anatomical details to be brought out. The sections were photographed under a Leitz Meopta research microscope using Leica asahi pentax 35mm slr spotmatic 11 camera and Konica color film (SR100ASA).

Micromorphology

Fresh leaves were washed and small fragments of leaves were taken from the middle region of the lamina of mature leaves. For anatomical studies, sections of 10-12 µm thick were prepared by double staining using Safranine (0.5% in water) and fast green (0.25%) and then mounted in 50% xylol. All the slides after staining with safranine were dehydrated by employing graded series of ethyl alcohol (30%,50%,70%,90% and absolute alcohol) and stained with fast green in clove oil and xylol-alcohol(50-50) and passed through xylol. Clearing of leaf was done by using 5% sodium hydroxide along with chlorinated soda solution supplemented with gentle heat. The margins of the cover slips were sealed with DPX, and the slides were observed under the microscope. Stomatal index, Stomatal number, vein islet number, vein termination number and palisade ratio were then calculated using standard procedures [7, 8].

Physicochemical Analysis

Physicochemical parameters such as ash values, extractive values and solubility were performed as per the official standard procedures [9, 10].

Fluorescence analysis

Fluorescence analysis of dried and powdered leaf and extract were carried out according to the procedure described elsewhere by using the reagents and viewed in day light and ultraviolet

radiations. The colours and fluorescence (if any) observed by application of different reagents in different radiations were recorded [11, 12].

Preliminary photochemical screening

The crude powder of *Atalatia Monophylla* leaves was subjected to qualitative phytochemical analysis [13].

Thin layer chromatography

Thin-layer chromatography (TLC) was employed in the qualitative analysis of organic extracts of the powdered leaves. The plant material was extracted successively with hexane and Chloroform between intervals of 24 hours. The extracts were spotted on activated silica gel plates. Two solvent systems of Hex-Benzene(1:1) and Chloroform-Benzene (1:1) were used for development of the plates. Spots were detected on TLC plates by spraying with sulphuric acid, followed by charring at 110°C for 10 minutes in an oven. The retention factor (Rf) for each spot was calculated using the formula:

Rf = Distance moved by solute /Distance moved by solvent

RESULTS

Pharmacognostic analysis

Anatomy

T.S. of the Petiole

Transverse section of the petiole is truncated elliptic in outline. The adaxial surface is flat and the abaxial surface is convex in shape. Epidermis is two layered and the outer one is covered by a thick cuticle. The cortex is differentiated into outer 3 to 4 rows of collenchymas and inner 10 to 12 layers of closely arranged parenchyma cells. In the central region, it exhibits a closed cylinder of xylem and phloem. The vessels occur in radial rows of 3 to 6. This sibhonostele is encircled by a discontinuous ring of per cyclic fibers. Schizogenous/lysigenous cavities are seen in the outer cortical region.Some of the parenchyma cells contain prismatic crystals (Figure 1A, 1B, 1C, 1F).

T.S. of the Lamina

The lamina in transverse section reveals the dorsiventral construction. The adaxial epidermis is composed of layer cells. The palisade is two layered made up of columnar closely arranged cells of which the upper layer cells are longer than the lower one. The spongy mesophyll is 4 to 6 layered, made up of closely arranged round cells (Figure-1H, 1G).

T.S. of the Midrib

Transverse section of midrib projects as a hemispherical protrusion on the ad axial side and show a convexity on the abaxial side. 2 to 3 rows of collenchymas cells are seen below the upper epidermis. In the centre a large vascular bundle is seen which the sclerenchyma fibers surround. The rest of the portion is filled with parenchymatous cells. Some of these cells contain prismatic calcium oxalate crystals (Figure-1D, 1E).

The Epidermis in surface view

The ad axial foliar epidermis is composed of penta-hexagonal cells with straight walls (Figure-2J, 2K). Stomata are totally absent. The ad axial foliar epidermal cells have wavy margins. It is profusely perforated by diacytic stomata (Figure-2L, 2M).

Micromorphology

As a part of quantitative microscopy stomatal index, stomatal number, palisade ratio, vein islet number and vein termination number were determined by using fresh leaves of the plant. Mean value, were calculated and recorded (Table No. 1-5)

Physicochemical Parameters

Physicochemical characterization of powder of *Atalantia Monophylla* leaf is shown in Table 6. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The ash value was determined by three different forms viz., total ash, water soluble ash and acid insoluble ash. The total ash was found to be around 7.96%, while water soluble ash and acid insoluble ash was 0.73% and 1.70% respectively. The extractive value in hexane is 2.42% and in chloroform it is 0.90%. The solubility values in alcohol and water are 6.37% and 20.49% respectively.

Fluorescence analysis

The dried leaf powder and extract were examined in visible light and ultraviolet light to detect the fluorescent compounds by the reported method. The observations are given in Table 7 & 8.

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of steroids, triterpenoids, carbohydrates, Coumarins, flavonoids, phenolics, and alkaloids (Table 9).

Thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) was employed in the qualitative analysis of organic extracts of the powdered leaves. The spots obtained from the extract were examined under day light and ultra violet light (Figure 3). The resolution factor was calculated by using the formula Rf = distance

travelled by solute/distance travelled by solvent (table 10).

Table 1: Stomatal index - Lower epidermis

Field No	No of Stomata per sq.mm(s)	No of epidermal cellsper sq,mm.(E)	Stomatal Index $I = \frac{S}{E} + S \times 100$
1	90	420	17.6
2	60	540	10
3	50	590	7.8
4	60	510	10.5
5	65	500	11.5

Average stomatal index = 11.48

Table 2: Stomatal number - Lower epidermis

Field No	No of stomata in one field(0.25mm)	No of stomata in one square mm.
1	22	88
2	15	60
3	13	52
4	15	60

Average stomatal number = 65

Table 3: Palisade ratio

Field No	No of epidermal cells(E)	No of palisade cells(P)	Palisade ratio P/E
1	4	22	5.5
2	4	19	4.7
3	4	18	4.5
4	4	22	5.5
5	4	18	4.5

Average palisade ratio = 4.94

Table 4: Vein islet number

Field No	No of vein-islet in 0.5mm area(A)	Vein-islet No. Per Sq.mm.(B),B = A×2
1	6	12
2	9	18
3	5	10
4	10	20

Average vein-islet No = 15

Table 5: Vein termination number

Field No	No of vein termination in 0.5mm area(A)	Vein termination No in 1mm(A×2)
1	13	26
2	16	32
3	17	34
4	16	32

Average vein termination No = 31

Table 6: Physicochemical constituents

S.No	Analysis	Values
Ash values		
1	Ash values	7.96%
2	Water soluble ash	0.73%
3	Alkalinity of water soluble ash	2.05%
4	Acid insoluble ash	1.70%
Extractive values		
1	Hexane	2.42%
2	Chloroform	0.90%
Solubility		
1	Alcohol	6.37%
2	Water	20.49%

Table 7: Fluorescence analysis of drug powder

S.No	Treatment With Powder	Colour	UV Light
1	Drug powder	Ash green	greenish
2	Drug powder & 1N NaoH(Aq)	Yellowish brown	Fluorescent green
3	Drug powder & 1 N NaoH(Alc)	Fluorescent yellow	Fluorescent green
4	Drug powder & 1 N Hcl	Yellowish brown	Fluorescent green
5	Drug powder & 50%H_2SO_4	Dark greenish black	Dark greenish black

Table 8: Fluorescence analysis of drug extract

S.No	Solvent	Colour	UV Light
1	Hexane	Yellowish green	Fluorescent green
2	Benzene	Greenish yellow	Pale green
3	Chloroform	Dark green	brown
4	Alcohol	Fluorescent green	Bright green
5	Water	Pale yellowish brown	Pale green
6	Acetone	Fluorescent green	Fluorescent green

Table 9: Phytochemical screening

S.No	Test	Observation	Inference
1	Libermann-burchard test	Bluish green colour	Presence of steriods
2	Noller's test	Pink colour	Presence of triterpenoids
3	Test for sugar	Dark green colour	Presence of sugar
4	Test for coumarin	Yellow colour	Presence of coumarin
5	Shimoda test	Red or pink colour	Presence of flavanoids
6	Test for phenols	Green or purple blue colour	Presence of phenols
7	Test for alkaloid Mayer's reagent Dragandroff's reagent	White precipitate Yellow precipitate	Presence of alkaloids

Table 10: HRF values of TLC spots of hexane soluble portion and chloroform soluble portion

SOLVENT SYSTEM	HRF VALUES	
	HEXANE SOLUBLE PORTION	CHLOROFORM SOLUBLE PORTION
HEXANE-BENZENE-1:1	12,71,93	-
CHLOROFORM-BENZENE-1:1	-	15,26,50,73,92

HRF=Rf × 100

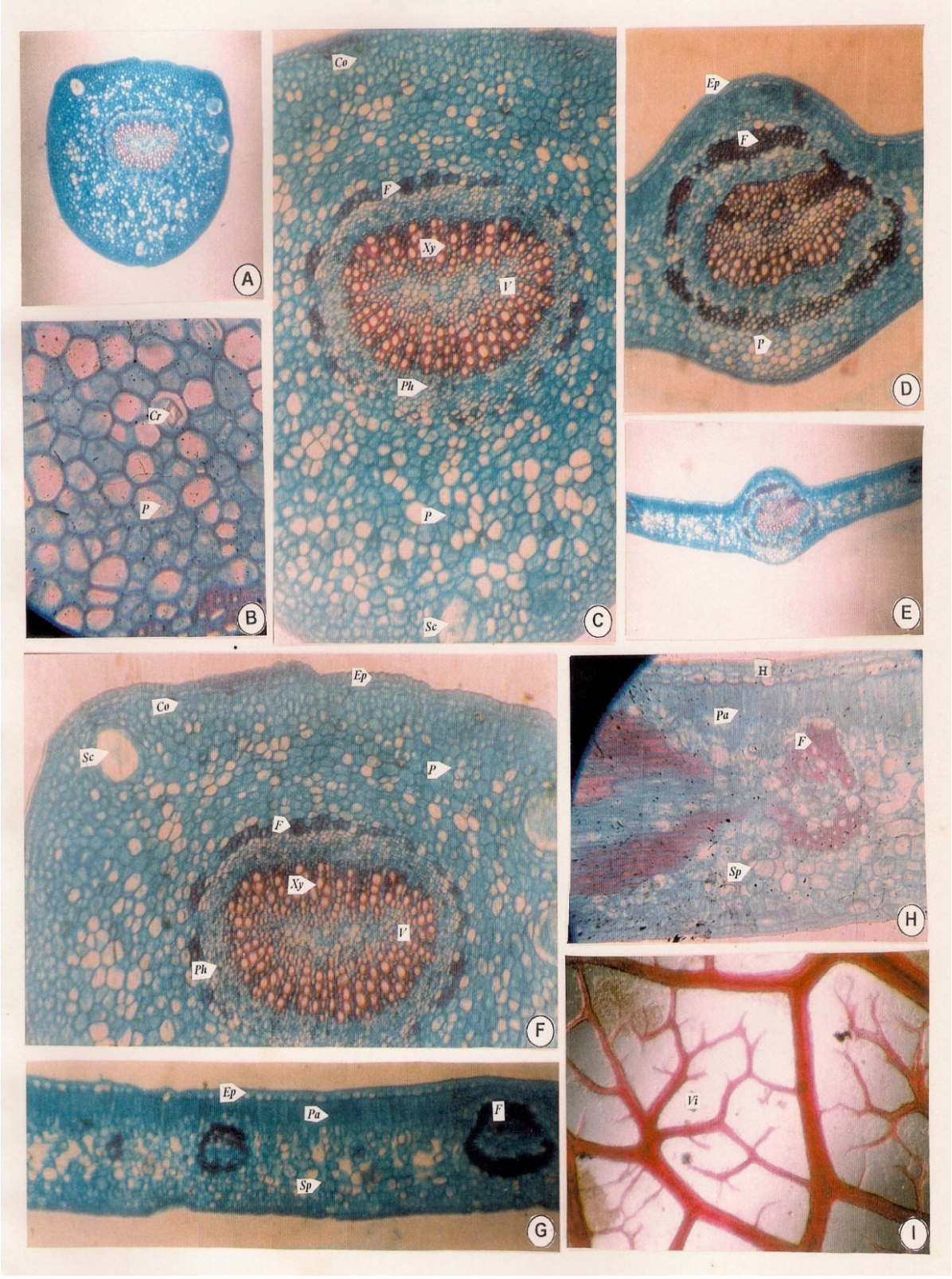

Figure 1

A-T.S. of petiol (scale-Q) **B-**T.S. of petiole showing prismatic crystal (scale-O**)**

C- T.S. of petiole- apportion enlarged (scale-P) **D-**T.S. of midrib (scale-P)

E- T.S. of leaf (scale-Q) **F-**T.S. of petiole- a portion enlarged (scale-P)

G-T.S. of lamina (scale-P) **H-**T.S. of lamina (scale-O)

I-vein islet (scale-Q**)**

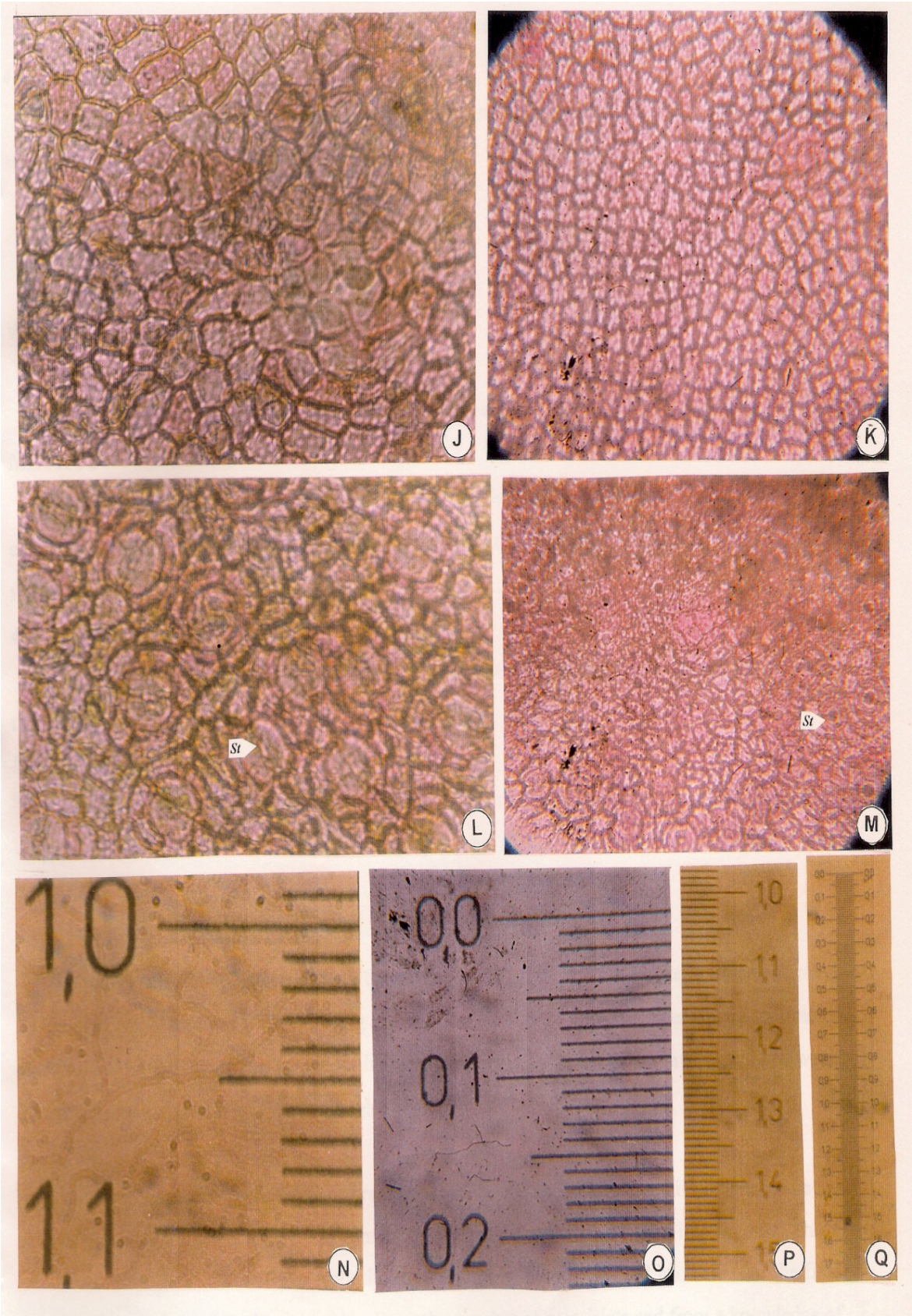

Figure 2
J- Adaxial epidermis (scale-N)
K-adaxial epidermis (scale-O)
L-abaxial epidermis (scale-N)
M-abaxial epidermis (scale-O)
N,O,P,Q-scale applicable to photomicrographs

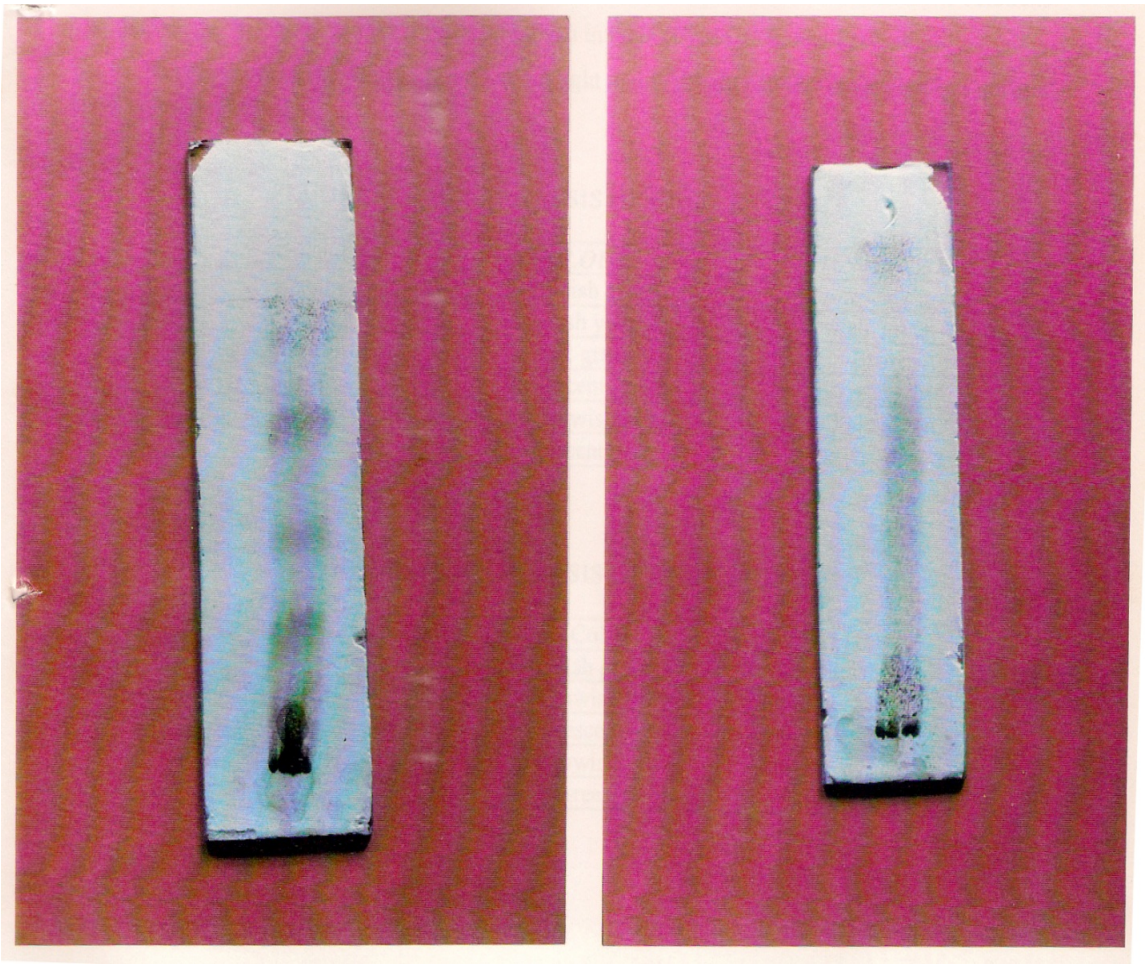

Figure 3: TLC chromatography

DISCUSSION

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopeia, these standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its microscopy and physicochemical parameters. The present work is undertaken to produce some pharmacognostical standards for Atalanta Monophylla. The above studies provide information in respect of their identification, chemical constituents and physicochemical characters which may be useful for pharmacognostical study and standardization for the plant.

The histology of petiole shows a double layered epidermis and the outer layer is covered by a thick cuticle. The cortex is differentiated into outer 3 to 4 rows of collenchymas followed by parenchyma and presence of prismatic crystals in parenchyma is a characteristic feature. The T.S of lamina shows that it is dorsi ventral in structure. The palisade is represented key two layered columnar closely arranged cells. The spongy mesoPhyll is 4-6 layered and is made up of closely arranged rotund cells. The T.S of midrib shows that it is hemispherical structure 2-3 rows of collenchymas cells are seen below the upper epidermis. A vascular bundle is seen in the centre which is surrounded by sclerenchyma prismatic crystals are seen in parenchymatous cells. The surface view of epidermis is ad axial foliar epidermis, this is composed of pentahexogonal cells with straight walls, and Stomata are absent. The abaxial foliar epidermis cells have wavy margins, dicyotic stomata is present.

The quantitative microscopy of stomata index of average lower epidermis is 11.48/$mm^{2,}$ the average stomata number of lower epidermis is 65, the average palisade ratio is 4.94, and the average vein termination number is 31. Ash values are used to determine quality and purity of crude drug. It indicates the presence of impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compounds present in crude drugs. The acid insoluble ash measures the amount of silica present, especially sand. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also useful to estimate the specific constituents

soluble in particular solvent [14]. The physicochemical constituents for total ash value is 7.96%,the water soluble ash is 0.73%,the alkalinity of water soluble ash is 2.05%,the acid in soluble ash is 1.70%. Less amount of these parameters indicate that the drug containing fewer amounts of inorganic matter and silica.

Fluorescence studies of powder with various reagents revealed the presence of green fluorescence with conc. HCl and sodium hydroxide, under UV light. Drug extracts in Hexane and acetone also revealed the presence of green fluorescence. Phytoconstituents of the leaves have potential of phytosterols, terpenoids, flavonoids, phenolic compounds, tannins and reducing sugars which has to found possesses antioxidant, can act against allergies, ulcers, tumors, platelet aggregation, and controlling hypertension and immunomodulatory effects. The constituents of this plant have tremendous impact on the health care system and may provide medical health benefits including the prevention and or treatment of diseases.

REFERENCES

1. Swingle WT, Reece PC. The botany of Citrus and its wild relatives. In: Reuther W, editor. The citrus industry. Vol. I. University of California: Division of agriculture sciences.316-318 (1967)
2. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New York, NY: Springer (2007).
3. Pullaiah T. Encyclopaedia of World Medicinal Plants, Volume 1.New Delhi: Regency; (2006).
4. Carl WC. Indian Atalantia, An ornamental tree for landscaping in southern florida. Proc Fla State Hort Soc .92:215. (1979)
5. Panda H. Handbook On Medicinal Herbs With Uses. India: Asia Pacific Business Press Inc. (2004).
6. Radhakrishnan M, Ankur J, Baldev G, Shvetank B, Dilip P. Evaluation of anti-diabetic activity of methanolic extract from the bark of *Atalantia monophylla* (Linn.) in alloxan-induced diabetic rats. *Int J Green Pharm* .6(2):133-137 (2012).
7. Wallis TE. Textbook of Pharmacognosy. 5th ed. London: J. and A. Churchill. (1957).
8. Johansen DA. Plant Microtechnique. New York: McGraw-Hill Book Company; (1940).
9. GOI. The Ayurvedic Pharmacopoeia of India. 1st ed. New Delhi: NISCAIR; (2004).
10. WHO. Quality Control Methods for Medicinal Plant Materials. (An authorized publication of World Health Organisation, Geneva). New Delhi: A.I.T.B.S. Publishers & Distributors; (2002).
11. Pratt RJ, Chase CR. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J Am Pharm Assoc*. 38:324-333. (1949)
12. Kokoshi J, Kokoski R, Slama FJ. Fluorescence analysis of powered vegetable drugs under ultraviolet radiation. *J Am Pharm Assoc*. 47 : 75-77 (1958)
13. Harborne JB. Phytochemical methods.A guide to modern techniques of plant analysis. 3rd ed. New York: Chapman and Hall Int. Ed. (1998).
14. Kalpna R, Sumitra C. Pharmacognostic, Physicochemical and Phytochemical Investigation of *Mangifera indica* L. var. Kesar leaf. *Asian Pac J Trop Biomed.* 2(1): 680-684 (2012)