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Pharmacognostical standardization of leaves of *Ixora coccinea*, linn.

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Abstract
Ixora coccinea Linn. is traditionally found to be useful for many ailments like hepatoprotective, Chemoprotective , antimicrobial, anti oxidant , antinociceptive and anti – inflammatory activities. The present study highlights the botanical as well as phytochemical studies including parameters such as macroscopic, microscopic characters, physiochemical evaluation and preliminary phytochemical studies of the leaves. These observations will help in the botanical identification and standardization of the drug in the crude form and also to distinguish the drug from its adulterants

Introduction:

Ixora coccinea Linn., (Rubiaceae) is Known as ‘ Jungle of Geranium (or) Flame of the woods or vetchi ‘ in Ayurvedha . It is a common flowering shrub native to Asia. It is name derived from an Indian deity. Although there are some 400 species in the genus Ixora , only a handful are commonly cultivated. And the common name , *Ixora* is usally used for *Ixora coccinea*, is a dense, multi – branched ever green shrub commonly 4-6 ft (1-2-2m) in height, but capable of reaching up to 12ft (3.6m) hight. It is traditionally used as hepatoprotective, Chemoprotective , antimicrobial, anti oxidant , antinociceptive, anti – mitotic and anti – inflammatory activities. Decoction of roots used for nausea, hiccups and anorexia.Powered roots used for sores and chronic ulcers In indo – china , root decoction used to clarify the urine, poultice fresh leaves and stems for sprains, eczema, boils and contusions[1,2] .

Eventhough the plant is rich in bio-active richness active constituents and potential therapeutic activities there is a lacuna in the Pharmacognostical standardization on the leaves of *Ixora coccinea* Linn. So the present study is undertaken to produce some Pharmacognostical standards for the leaves.

Materials and Methods:

The fresh healthy plant material of *Ixora coccinea* Linn., was collected from various domestic places in Chennai and authenticated by Prof. P. Jayaraman, Ph.D, Botanist, Director , Plant Anatomy Research Centre, Thambaram. A voucher specimen is deposited in the department of Pharmacognosy, Madras Medical College, Chennai-03 for future reference. The fresh leaves were collected and fixed immediately using FAA (formalin: Acetic acid: ethyl alcohol) as fixative agent for anatomical studies. The materials were cut into small pieces before fixing.

Macroscopic and microscopic analysis

The macroscopic characters such as colour , odour, taste, nature, texture were studied for morphological investigation. For anatomical studies customary technique of microtmy was followed[3]. Paraffin sections of 10µm thick were stained with safranin – fast green. Photomicrography were taken with Nikon lab photo – microscopic unit .The quantitative microscopy was studies as per the procedure given by Wallis[4] and P.K.Lala[5]. The powder analysis has been carried out according to the method of Brain and Turner[6].

Physico – chemical studies

The ash values, extractive values and loss on drying were performed according to the officinal methods prescribed in Indian pharmacopeia[7] and the WHO[8] guidelines on quality control methods for medicinal plants materials. Fluorescence analysis was carried out according to the method of Chase and Pratt[9] and Kokoski [10] .

Preliminary phytochemical screening

The dried leaves were extracted with petroleum ether, chloroform, ethylacetate and methanol. The behavior of powder with various chemical reagent and preliminary

chemical tests for various extracts were also carried out according to the standard procedures described by Kokate[11] and Horborne[12].

Results:

Macroscopy

The plant is a dense, multi – branched evergreen shrub, commonly 4-6 ft (1-2-2m) in height , but capable of reaching up to 12ft (3.6 m). Leaves are oblong are about 10cm long , with entire margins and are carried in opposite pairs or whorled on the stem. They are sessile to short – petiolate, blades elliptic, oblong or obovate, usually leathery, base cordate to rounded, apex rounded , mueronate or shortly tapering ; stipules basally sheathing , lobes Triangular and strongly acon – tipped. Flowers sessile; calyx lobes short, triangular, persistent , corolla tube usually 1-1.5 inches long , lobes lanceolate to ovate, less than 0.25 inches long , acute or sometimes obtuse fruit thinly fleshy , reddish black.

Microscopic features of the leaves

Microscopical studies are useful to establish the botanical identity for the valuable herbal drugs, which forms the basis for the identification and determination of adulterants.

The leaf is dorsiventral, hypostomatic and mesomorphic. It has thick midrib projecting both adaxially and abaxially (fig 1).The midrib has adaxial broadly conical hump and wide semicircular abaxial past. The midrib is 1.1 mm thick. The adaxial past is 400µm wide. The abaxial part is 900 µm thick.

The epidermal layer of thin midrib consists of small, squarish, thick walled cells with prominent cuticle, the cells are with prominent cuticle and the cells are 22 µm thick. Beneath the adaxial hump is a small patch of angular, compact thick walled cells, the palisade layer of the lamina extend up to the lateral part of the hump (fig.2)

The lower semicircular midrib has parenchymatous ground tissue. The cells are wide thin walled, angular and compact. Calcium oxalate are occasionally seen in some of the parenchyma cells (fig.4)

The vascular system of the midrib consists of an adaxially flattened closed cylinder of xylem and phloem ; with in the cylinder are two small rectangular segments of vascular bundles. The outer cylinder has a thin layer of xylem fibres and short radial files of narrow, thick walled angular xylem elements and outer continuous zone of phloem.

Hedullary accessory bundles are collateral with xylem elements facing the adaxial side and phloem elements placed toward the centre. The vascular cyclinder is 170 µm thick. The xylem elements are 20µm wide.

Lamina (Fig 5) : The lamina has wide , radially oblong thick walled adaxial epidermis with prominent cuticle. The adaxial epidermis is 40 µm thick. The abaxial epidermis has comparatively small cells which are squarish in shape, the cuticle is thicker; stomata are present on the lower epidermis.

These are two layers of palisade cells along the upper part. The cells are wide, cyclindrical and the palisade zone in 60µm in height. The spongy parenchyma cells are in 4 (or) 5 rows. They are large thin traveled, spherical lobed and form wide air – chambers (fig – 6)

The vascular strands of the lateral veins are circular with thick cylinder of fibers and small central case of xylem and phloem.

Quantitative microscopy

Quantitative microscopy

Quantitative microscopic data are found to be constant for a species. These values are especially useful for identifying the different species of genus and also helpful in the determination of the authenticity of the plant. The study of the leaf constants showed that the average stomatal number is

Fig 1. Shrub *Ixora Coccinea* Linn.,

Fig 2. Leaves of *Ixora Coccinea* Linn.,

Table – 1
Data showing the values of quantitative microscopy

S.NO.	METHOD	RANGE	AVERAGE
1.	Stomatal number	23 – 25mm^2	24mm^2
2.	Stomatal index	11 – 13mm^2	12.2mm^2
3.	Vein islet number	13 – 15mm^2	13.5mm^2
4.	Vein termination	26 – 28mm^2	13.5mm^{2}26.3mm^2
5.	Palisade ratio	9 - 11	9.3
6.	Linear measurement Length Width	44-65µm 20 -24µm	51.2 µm 22 µm

24mm^2, the stomatal index is 12.2mm^2. The vein islets number and vein termination numbers are 13.5mm and 26.3mm/sqmm respectively. The palisade ratio is 9.3. The microscopic linear easurement of the trichomes showed that the length of the trichome is 44-65µm and the width is around 20 -24µm (Table no1).

Powder characters

The powder characters of a drug are mainly used in the identification of the drug in the powder from. The leaf powder was light green in colour with strong and characteristic in taste on microscopical examination the powder showed thick cylinder of fibers, anisocytic stomata, unicellular covering trichomes.

Physico-chemical constants

The physico-chemical parameters are mainly used in judging the purity and quality of the drug. Ash values of a drug give an idea of the earthy matter or inorganic composition or other impurities present along with the drug. The ash values of the powdered leaves revealed a high percentage of sulphated ash. Extractive values give an idea about the chemical constituents present in the drug as well as useful in the determination of

TABLE NO: 2
Data showing the physico – chemical standard values of the leaf of *Ixora coccinea*

S.No	Total Ash%	Water Soluble Ash%	Acid Insoluble ash %	Sulphated ash%	Water soluble Extractive	Alcohol soluble Extractive	Loss on Drying
1	10.01	4.66	3.33	5.29	6.78	6.00	0.3
2	9.90	3.93	4.01	5.13	7.93	5.98	0.2
3	10.05	4.62	4.08	4.93	6.85	6.05	0.5
4	10.08	4.53	3.48	5.18	7.98	6.09	0.3
5	9.98	3.58	3.93	5.23	7.89	5.93	0.2
MIN	9.90	3.58	3.33	4.93	6.78	5.93	0.2
AVE	10.01	4.26	3.766	5.15	8.84	6.01	0.3
MAX	10.08	4.66	4.48	5.29	7.98	6.09	0.5

TABLE-3
Fluorescence analysis of various extract of leaves of *Ixora coccinea* Linn

S.No.	Extracts	Day light	UV light
1.	Petroleum ether	Yellowish brown	Dark brown
2.	Chloroform	Dark green	Blackish green
3.	Ethyl Acetate	Light Brown	Green
4.	Methanol	Yellowish orange	Dark green

exhausted or adulterated drugs. The results suggest that the powdered leaves have high water soluble extractive value. The loss on drying reveals the percentage of moisture present in the drug are also studied and presented in (Table no 2).

Fluorescence analysis of drug powder and extracts:

The fluorescence analysis of powdered leaves was studied in both UV and day light. The powder showed green fluorescence with methanol in UV light at 254nm, which indicates the presence of chormophore in the drug (Table No. 3 & 4)

Extraction of phytoconstituents

The dried leaves are extracted with petroleum ether, choloroform, ethylaceate and methanol. The extractive values are given in table no . the percentage yield of methanolic extract is more when compared to other extracts (Table no.5).

TABLE - 4
Fluorescence analysis of drug powder of leaves of *Ixora coccinea* Linn

S.No.	Treatment	Day light	UV light
1.	Powder	Greenish brown	Pale green
2.	Powder + water	Yellowish green	Dark green
3.	Powder + IN HCl	Yellowish green	Green
4.	Powder + IN HNO_3	Light brown	Green
5.	Powder + IN H_2SO_4	Pale brown	Pale Green
6.	Powder + IN NaOH	Yellowish brown	Blackish green
7.	Powder + Alc. NaOH	Dark brown	Dark green
8.	Powder + IN KOH	Yellowish brown	Blakish green
9.	Powder + AIc. KOH	Light brown	Green
10.	Powder + Ammonia	Yellowish brown	Dark green

Preliminary phytochemical test for extracts

All the extracts namely hexane, chloroform, ethanol and aqueous were tested with various reagents and results are presented in the (Table no.6). the various extracts showed the presence of alkaloids, carbohydrates, glycosides, flavanois and phytostrols.

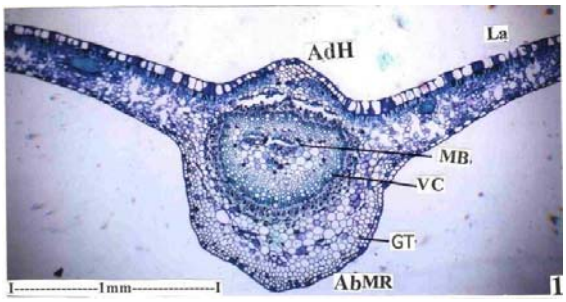

Fig .3. T.S. of Leaf through Midrib with Lamina

Fig .4. T.S. of midrib - Enlarged

Abe – Abaxial Epidermis
Abmr – Abaxial midrib
Adh –Adaxial hump
Gp – Ground Parenchyma
La – Lamina
MB – Medullary accessory bundle
Ph – Phloem
VC – Vascular Cylinder
X – Xylem
Ade - – Adaxial epidermis

TABLE - 5
Data showing the extractive values of the leaves of *Ixora coccinea* Linn.

Plant Name	Part Used	Method of Extraction	Petroleum Ether	Chloroform	Ethyl Acetate	Methanol
Ixora Coccinea Linn	Leaves	Continuous percolation using soxhlet apparatus	3.24% w/v	4.87 % w/v	6.37 % w/v	10.54 % w/v

TABLE NO. 6
Data showing preliminary phytochemical screening of the leaf extracts of *Ixora coccinea* Linn.

S. No.	Constituents	Petroleum ether	Chloroform	Ethyl Acetate	Methanol
1.	Alkaloids	-	+	+	+
2.	Carbohydrates	-	-	+	+
3.	Glycosides	-	-	+	+
4.	Phytosterol	+	+	-	-
5.	Fixed oil	-	-	-	-
6.	Saponins	-	-	-	-
7.	Tannins	-	-	-	+
8.	Protein and Amino acids	-	-	-	+
9.	Gum & Mucilage	-	-	-	-
10.	Flavanoids	-	-	-	+
11.	Lignin	-	-	-	-

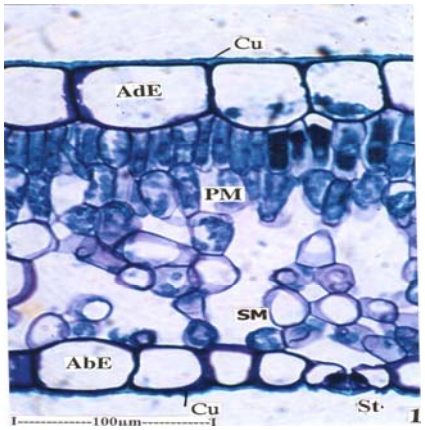

Fig .5. T.S. of Lamina

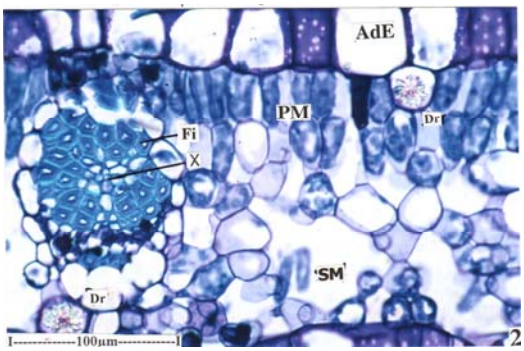

Fig .6. T.S. of Lamina through Lateral Vein

Abe – Abaxial epidermis, Ade – Adaxial epidermis, Cu – cuticle, Dr – Druses, Fi - Fibres, Pm – palisade mesophyll, Sm – Spongy mesophyll, St – stoma.

Conclusion:

Establishing standars is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacoparia, these standars must be established. The majority of the information on the identity, puring and quality of the plant material can be obtained from its macroscopy, microscopy and physio – chemical parameters . As there is no record on pharmacognostical work on leaves of Ixora coccinea Linn, The present work is undertaken to produce some pharmacognostical standards . The above studies provide information in respect of their identification , chemical constituents & physicochemical characters which may be useful for pharmacognostical study and standardization of herbal drugs of folk medicinal practice of present era and enrichment of ayurvedic pharmacopoeia . It will also determine therapeutic diagnostic tools for the scientistics who are keen and sincere to evaluate the herbal medicine of indigenous resources.

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