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Research Article

COMPARATIVE PHARMACOGNOSTICAL AND PHYSICO-PHYTOCHEMICAL STANDARDIZATION ON LEAF OF *IXORA COCCINEA* LINN. AND *IXORA ARBOREA* ROXB.

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ABSTRACT

The knowledge of the medicinal plants used by tribal people has great values. Presentation of old concepts and folk claims needs extensive experimental research work. So, To avoid the errors due to other affecting factors and to test the genuinity of folklore claim with respect to their pharmacognostical and phytochemical study. *I. coccinea* leaves are used to treat various disorders like cough, asthma, catarrhal bronchitis. Their Anti-inflammatory, Anti-tussive, Anti-asthmatic, Anti-meiotic activity is well established. *I. arbore* shows antiviral, hypotensive and spasmolytic activity. The present study was aimed to compare the pharmacognostical and phytochemical aspect of *I. coccinea* and *I. arbore* leaves, including their morphology, microscopic characteristics, physico-phytochemical parameters, and HPTLC fingerprint. It revealed that they had basically similar pharmacognostical characteristics but with certain Morphological differences. The present study underwrites to the standardization and verification of these medicinal plants.

Keywords: Ayurveda, HPTLC, *Ixora arborea*, *Ixora coccinea*, Pharmacognosy, Quantitative Microscopy

INTRODUCTION

The traditional systems of medicines viz. Ayurveda, Siddha, Unani, Western Herbal Medicine, Traditional Chinese Medicine and Homeopathy have roots in medicinal herbs. The use of the medicinal herbs for curing diseases has been documented in the history of all civilizations[1]. There are around 500 species in the genus *Ixora*, only handfuls are commonly cultivated. People have been using *Ixora* for generations, not only for ornamental purposes but more importantly because of their medicinal values.[2] *Ixora coccinea* and *Ixora arborea* are species of flowering plant in the Rubiaceae family. *I. coccinea* is a dense, multi-branched evergreen 60-200 cm tall shrub, commonly 2-4 ft in height. The leaves are glossy, leathery, oblong with entire margins, and are carried in opposite pairs or whorled.[3] The leaves of *I. coccinea* were found to have anti-inflammatory, antidiarrheal, anti-asthmatic, antiulcer and anti-tussive activity, also used to pacify vitiated pitta, skin diseases, colic, flatulence, diarrhea, indigestion, ulcers, wounds, and used as antiseptic.[4] *I. coccinea* leaves contain lupeol, ursolic and oleanolic acid, sitosterol, rutin, quercetin, lecocyanadin, anthocyanins, proanthocyanidins.[5] *I. arborea* is a small much branched evergreen tree or shrub. Leaves are sub sessile, coriaceous, elliptic or oblong, obtuse or shortly acuminate, opposite or whorled. [6,7] Both the plants are used as antiseptic in scabies and other skin diseases.[8,9] *I. arborea* leaves show antioxidant, cytotoxic and antimicrobial activities. Leaves contain Ixoral and β-sitosterol, betulin, erythrodiol, lupeol, and stigmasterol.[5]

MATERIALS AND METHODS

Collection & certification of raw drug

Selected plants were collected from natural habit which was free from pollution, botanical garden of Jamnagar in month of November-December 2014. Pharmacognostical identification and authentification was done in Pharmacognosy lab, IPGT & RA. Fresh samples were used for various Pharmacognostical evaluations. Healthy uninfected samples were made into herbarium Phm 6105/2015 and 6106/2015 *Ixora coccinea* Linn. and *Ixora arborea* Roxb. Respectively and kept in the Lab for further reference. Leaves were separated, dried in shed, powdered at 80 # for further powder microscopy and analytical studies.

Macroscopic or Morphology Study

The collected samples were identified and authentified by studying their different characters as per the methods described in the textbooks of pharmacogosy. The specimens were observed as such with necked eyes and compare with local and other different floras. [3,10,11,12]

Microscopic Study

Fresh samples were taken for detailed microscopic study. Free hand sections were taken, cleared with chloral hydrate and observed under microscope for the presence of any crystal. Then, the sections were stained with phloroglucinol and

hydrochloric acid and observed for lignified elements like fibres, vessel etc. Microphotographs were taken by using Carl Zeiss Trinocular microscope attached with camera. Same procedure was followed for detailed powder microscopy. [12-15]

Quantitative Microscopy

Quantitative leaf microscopy was carried to determine palisade ratio, stomata number and stomatal index as per the standard methods. The leaf epidermal studies were carried out on fresh specimens. Fresh leaf (both the leaf) was placed on glass slide and tissues were scrapped off with sharp edge of blade carefully. Water was slowly added and scrapping was done until transparent epidermis was obtained.[12,14,15]

Organoleptic characters of the powder

Organoleptic characters i.e. colour, odor, taste and feel of drug to touch by sensory observations were noted done.[14]

Powder microscopy

For powder microscopy, slides were prepared by using water, chloral hydrate as a clearing agent. Leaves powder slides were also prepared for histochemical and micrometric evaluation.[12,13]

Histochemical evaluation

Sample thick sections subjected to histochemical tests to find starch, tannin, calcium etc. by treating various reagents.[12,14]

Physicochemical Evaluation

Physico-chemical Parameters like loss on drying, total ash, alcohol soluble extractive (90% methanol), water soluble extractive and pH values were determined in both the leaves sample as per the API guidelines.[16]

Phytochemical Evaluation

Phytochemical analysis of methanolic and water extract of both the leaves sample was carried out for Steroids, glycosides, tannins, proteins, flavonoids, alkaloids, saponins etc. according to standard procedure.[17]

HPTLC profile

High-Performance Thin Layer Chromatography (HPTLC) profile of the methanol extract of both the leaves was carried out to generate the Profile and spectral comparison between both the samples as per the Ayurvedic pharmacopoeia of India.[16]

OBSERVATION & RESULTS
Morphological characters

Both the plants are evergreen shrub, following Rubiaceae family and abundantly available all over the India. Morphological characters including its color, size, shape, touch etc. were studied by observing leaf and results were depicted in the table 1. (Figure 1)

Transverse section of petiole of *Ixora coccinea*

Transverse section of petiole shoes single layered epidermis with thick cuticle. Some of the epidermal cells bear unicellular covering trichomes. One or two layers of collenchymatous hypodermal cells present beneath the epidermis. Ground tissue occupy most part of the section, which are made up of parenchyma cells, loaded with cluster and rosette crystal of calcium oxalate, oil globules and simple and compound starch grains. Pith consist vascular bundle at the centre. Vascular bundle close and centrally arranged, di-arch, phloem toward outside, xylem towards pith. (Figure 1)

Transverse section of leaf through midrib of *Ixora coccinea*

Leaf is dorsi-ventral type & differentiated into upper palisade parenchyma and lower mesophyll tissue. Epidermis single layered both upper and lower epidermis interrupted by paracytic stomata. Transverse section through midrib shows large vascular bundle present at the centre.

Epidermis

Epidermis single layered. Upper epidermis consists of single layered barrel shaped compactly arranged cells. Some of the cells lead to form unicellular simple trichomes. Lower epidermis also consists of epidermal cells as in upper epidermis but some epidermal cells interrupted by stomata; stomata are mainly paracytic in nature. Both the epidermis is covered with thick cuticle.

Mesophyll

Mesophyll is differentiated into upper palisade & lower parenchymatous layers. Palisade consists of 3-4 layered of elongated compactly arranged cells, below the upper epidermis. Chloroplast, oil globules and cluster and rosette crystals of calcium oxalate are very common in palisade parenchyma. There are 5-7 layered loosely arranged rounded to oval shaped spongy parenchyma with many air chambers located above the lower epidermis. Oil globules and chlorophyll pigments and rosette crystals are very common in spongy parenchyma tissue.

Vascular bundle

Vascular bundle is situated at the centre through the midrib section and is closed type. Xylem circularly arranged forming central pith, pith also consists smaller vascular bundle. Xylem differentiated into protoxylem & metaxylem. Metaxylem is found towards outer surface & protoxylem towards inner surface. Xylem composed of its parenchyma and fibres. Phloem circularly covers the xylem, and composed of some sieve elements. Inner vascular bundle xylem towards upper epidermis and phloem towards lower epidermis. Vascular bundle is surrounded by 2-3 celled lignified pericyclic fibres rest of the section made-up of parenchymatous cells & covering the bundle sheath. 3-5 layered collenchymas cells beneath the both surfaces lead to parenchymatous bundle sheath. (Figure 1)

Transverse section of petiole of *Ixora arborea*

Transverse section of petiole shows single layered epidermis with thick cuticle. Some of the epidermal cells bear unicellular covering trichomes. One or two layers of collenchymatous hypodermal cells present beneath the epidermis. Ground tissue occupy most part of the section, made up of parenchyma cells, loaded with, cluster and rosette crystal of calcium oxalate, oil globules and Simple and compound starch grains. Vascular bundle close and centrally arranged, phloem toward outside, xylem towards pith consist xylem parenchyma and its fibres. Centrally located pith made up of parenchyma cells with some oil globules and cluster crystals.

Table 1: Morphological characters of both the leaves

Sr. No.	Characters	*I. coccinea* Linn.	*I. arborea* Roxb.
1.	Type	Simple, opposite, petiolate, stipules intra petiolar	Simple, opposite, petiolate, stipules intra petiolar
2.	Color (Fresh)	Upper- Dark green Lower- Light green	Upper- Parrot green Lower- yellowish green
3.	Color (Dry)	Upper- olive brown Lower- Light brown	Upper- Greenish brown Lower- Light brown
4.	Shape	oblong-elliptic or obovate, base oblique	Elliptic-oblong or ovate- oblong
5.	Margin	Entire	Entire
6.	Apex	Obtuse or shortly acute	Obtuse
7.	Size (Blade)	10–14 cm X 3-7 cm (l x b)	15–17 cm X 5-8 cm (l x b)
8.	Main nerve	15 - 20 pairs	8-11 pairs
9.	Touch	Coriouseous more or less pubescent	Glabrous, Smooth

Table 2: Comparative micrometric values of both the plant species

Sr. No.	Characters	*I.c* u.e.	*I.c* l.e	*I.a* u.e	*I.a* l.e
01	Stomata length	0.9mm	0.8mm	0.8mm	0.8mm
02	Stomata breadth	0.7mm	0.6mm	0.3mm	0.3mm
03	Stomatal No.	75	100	102	119
04	Stomatal Index	33.33	33.33	33.33	33.33

I. a- Ixora arborea; *I. c- Ixora coccinea*; u. e- upper epidermis; l. e- lower epidermis

Table 3: Organoleptic characters of both the leaves powder

Sr. no.	Characters	*Ixora coccinea*	*Ixora arborea*
1.	Color	Olive green	Dark green
2.	Odor	Characteristic	Characteristic
3.	Taste	Astringent	Astringent
4.	Nature of powder	Coarse	Slightly Coarse

Table 4: Comparative powder microscopy of both the powder

Sr. no.	Characters	*Ixora coccinea*	*Ixora arborea*
1.	Fragment of palisade cell	+	+
2.	Simple Starch grain	+	+
3.	Tannin content	+	+
4.	Cluster, Prismatic crystals	+	+
5.	Rosette crystals	-	+
6.	Fragments of fibres	+	+
7.	Fragment of spongy parenchyma	+	+
8.	Oil globules	+	+
9.	Annular, Spiral vessels	+	+
10	Pitted, Border pitted vessels	+	-
11	Fragment of stomata	+	+
12	Trichomes	+	+

++ = Present; -- = Absent

Table 5: Histochemical tests of both the leaves

Sr. no	Reagent	Observation	Characteristics	Result	
				I.c	*I.a*
1.	Phloroglucinol+Conc. HCl	Red	Lignified cells	++	++
2.	Iodine	Blue	Starch grains	++	++
3.	Phloroglucinol + Conc. HCl	Dissolved	Ca Ox - crystals	++	++
4.	$Fecl_3$ solution	Dark blue	Tannin	++	++
5.	Ruthenium red	Red	Mucilage	--	--
6.	Sudan III	Red	Oil globule	++	++
7.	HCl	Effervesces	$Ca\ Co_3$	--	--
8.	H_2so_4	Dissolved	Silica	--	--
9.	Iodine	Reflecting	Aluerone grains	--	--

++ = Present; -- = Absent

Table 6: Physico-chemical parameters of *Ixora coccinea* and *Ixora arborea* leaf powder

Sr. no.	**Parameters (%w/w)**	***I. coccinea***	***I. arborea***
1	Loss on drying	6.65	6.53
2	Ash value	8.12	10.18
3	Water soluble extractive	15.92	12.39
4	Alcohol soluble extractive	19.02	20.61
5	pH	6.5	6.2

Table 7: Preliminary phytochemical test of *Ixora coccinea* and *Ixora arborea* leaves powder

Sr. no.	Name of the test	*I. coccinea*	*I. arborea*
1	Carbohydrates	++	++
2	Starch	++	++
3	Mucilage	--	--
4	Protein	--	--
5	Amino acid	--	--
6	Steroid	++	--
7	Saponin glycoside	--	--
8	Flavonoid	--	--
9	Tannin	++	++
10	Alkaloid	++	--

++ = Present; -- = Absent

Table 8: HPTLC studies of methanolic extracts of both the sample at 254nm and 366nm

Sample	**254 nm**		**366 nm**	
	No. of Spots	**Rf Value**	**No .of Spots**	**Rf Value**
Track – 1	**12**	0.18, 0.24, 0.32, 0.40, **0.48**, 0.52, 0.58, **0.66, 0.72**, 0.84,0.88, 0.93	**10**	0.20, 0.26, **0.48**, 0.53, 0.61, 0.71, 0.76, **0.83**, 0.87, 0.97
Track – 2	**8**	0.06, 0.36, 0.56, **0.66, 0.72**, **0.78, 0.83,**0.91	**12**	0.06, 0.36, 0.45, 0.50, 0.57, 0.59, 0.67, 0.70, 0.73, **0.78**, **0.83**,0.94

FIGURE NO. 1

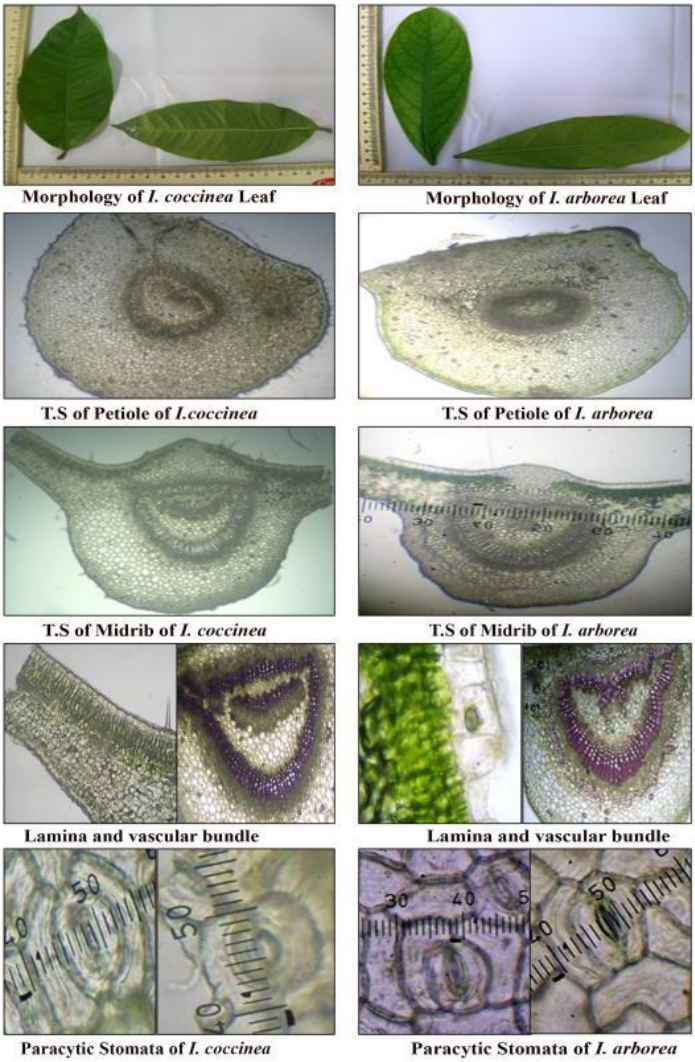
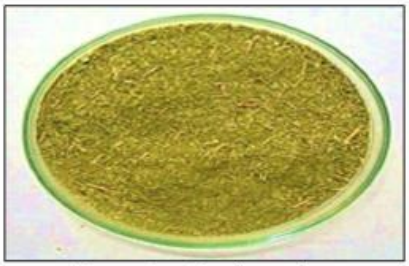
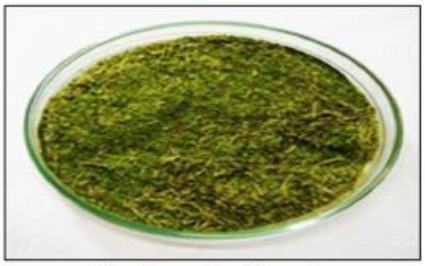


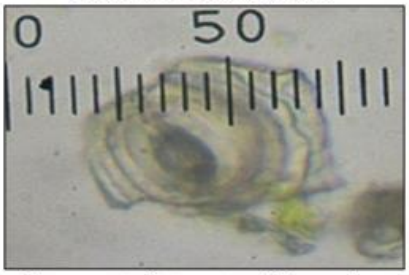
FIGURE NO. 2



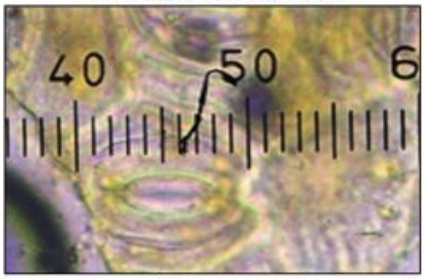
I. coccinea Leaf powder



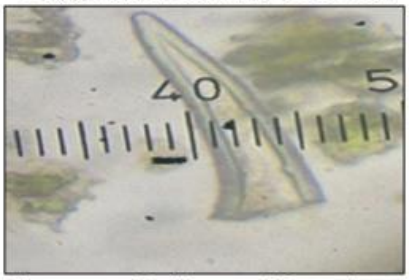
I. arborea Leaf powder



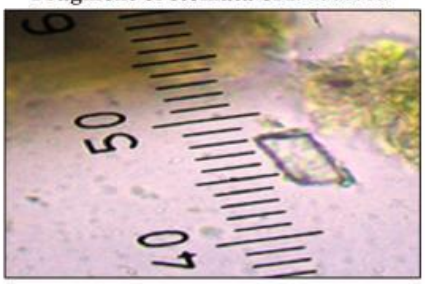
Fragment of stomata of *I. coccinea*



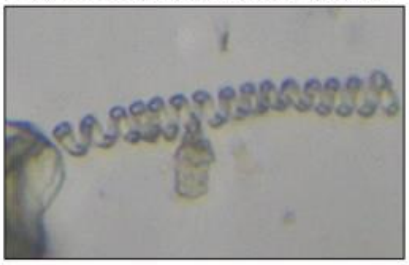
Fragment of stomata of *I. arborea*



Fragment of trichome of *I. coccinea*

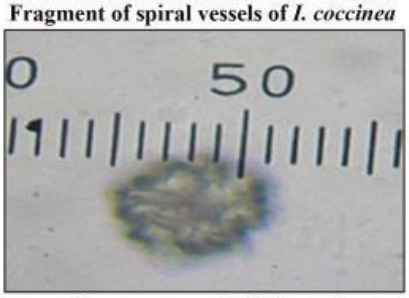


Prismatic crystal of *I. arborea*

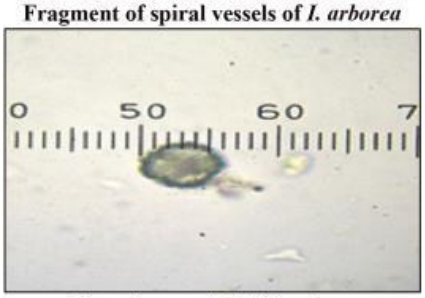


Fragment of spiral vessels of *I. coccinea*

Fragment of spiral vessels of *I. arborea*

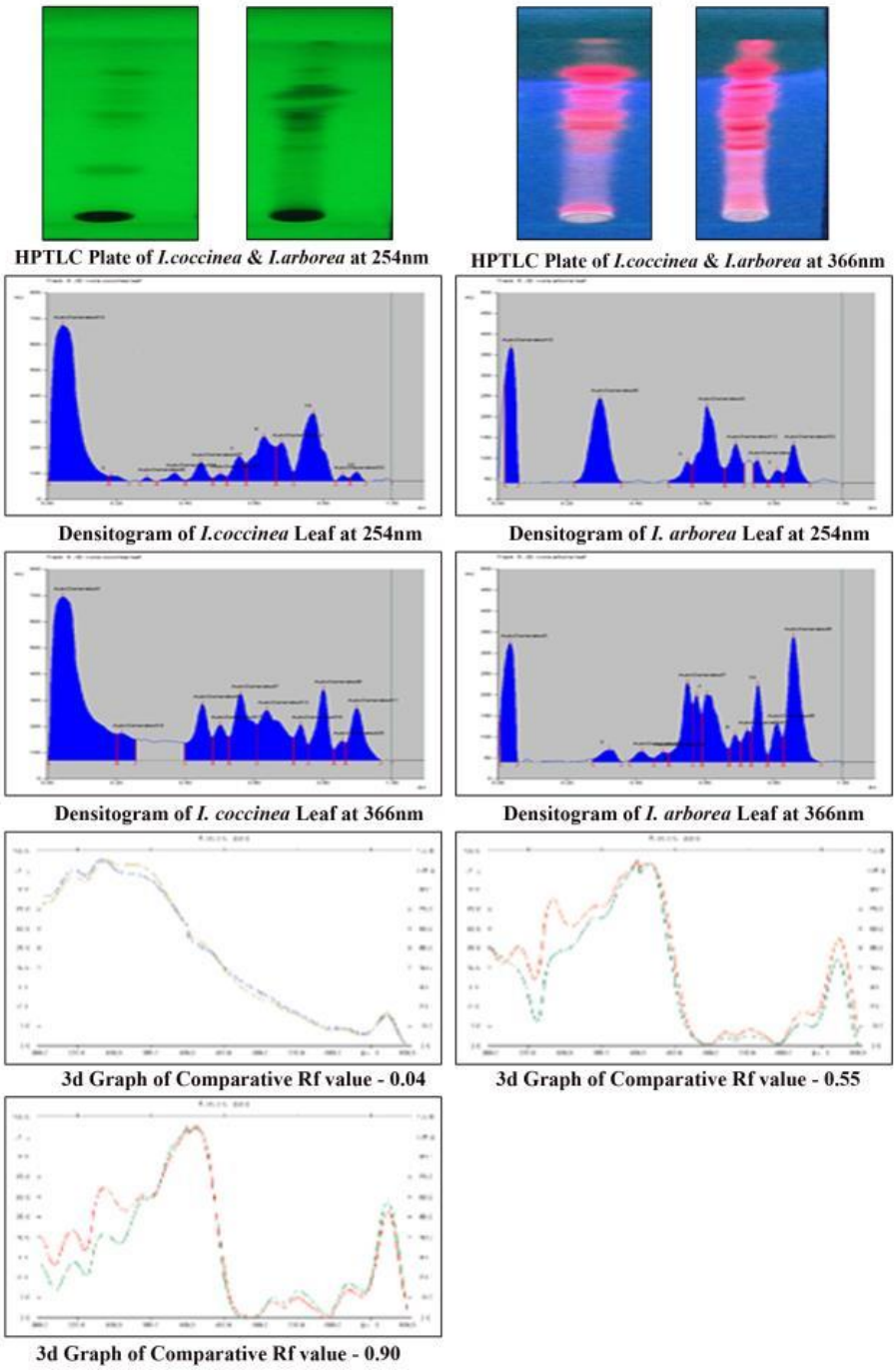


Rosette crystal of *I. coccinea*



Rosette crystal of *I. arborea*

FIGURE NO. 3



Transverse section of leaf through midrib of *Ixora arborea*

Leaf is dorsi-ventral type & differentiated into upper palisade parenchyma and lower mesophyll tissue. Epidermis single layered both upper and lower epidermis interrupted by paracytic stomata. Transverse section through midrib shows large vascular bundle present at the centre.

Epidermis

Epidermis single layered. Upper epidermis consists of single layered barrel shaped compactly arranged cells. Lower epidermis also consists of epidermal cells as in upper epidermis but some epidermal cells interrupted by stomata, stomata are mainly paracytic in nature. Both the epidermis is covered with thick cuticle.

Mesophyll

Mesophyll is differentiated into upper palisade & lower parenchymatous layers. Palisade consists of 2-3 layered of elongated compactly arranged cells, below the upper epidermis. Chloroplast, oil globules and cluster and rosette crystals of calcium oxalate are very common in palisade parenchyma. There are 5-7 layered loosely arranged rounded to oval shaped spongy parenchyma with many air chambers located above the lower epidermis. Oil globules and chlorophyll pigments and rosette crystals are very common in spongy parenchyma tissue.

Vascular bundle

Vascular bundle is situated at the centre through the midrib section of the leaf. Vascular bundle is closed type. Xylem differentiated into protoxylem & metaxylem. Metaxylem is found towards outer & protoxylem towards pith. Xylem composed of its parenchyma and fibres. Phloem situated outside the xylem, and composed of some sieve elements. Centrally located pith is made-up of loosely arranged parenchyma cells. Vascular bundle is surrounded by 2-3 celled lignified pericyclic fibres, Rest of the parenchymatous cells covering the bundle sheath. Below the both epidermis 3-5 layered collenchymas cells and leads to parenchymatous cells. (Figure 1)

Quantitative microscopy

Both the upper and lower surfaces of leaf was peeled out and observed under the microscope, the upper epidermis shows only epidermal cells, wavy parenchyma cells, oil globules and paracytic stomata, whereas lower epidermis shows paracytic stomata, epidermal cells, oil globules, rosette crystals. (Table 2) (Figure 1)

Organoleptic Characters

Organoleptic characters of *Ixora coccinea* and *Ixora arborea* leaf powder was carried out as per the sensory evaluation and the results are depicted in the Table 3. (Figure 2)

Powder Microscopy

Regarding comparative similar and dissimilar powder microscopic characters were scientifically observed and depleted in the table 4. (Figure 2)

Histochemical evaluation

Both Leaves Samples (T.S as well as powder) subjected to histochemical tests to find erigastic substances (starch, Crystal) and metabolites (tannin, lignin) by treating various reagents and the results are tabulated in table 5.

Physico-chemical parameters

Leaf powder of Ixora coccinea and *Ixora arborea* were subjected to various physico-chemical parameters and the observed results are illustrated in the table 6.

Preliminary phytochemical tests

Leaves powder of both the sample was tested for the presence or absence of different phyto constituents. The observed results are shown in the table 7.

High Performance Thin Layer Chromatography (HPTLC)

Chromatographic techniques were carried out as per the standard protocol. Solvent system which were designed for TLC i.e. Toluene: Ethyl acetate: Acetic acid (7: 2: 1 V/V) was used for HPTLC studies. The results are shown in the table.no. 8. (Figure no. 3)

Sample Loading

Track 1- Methanolic extract of *I. coccinea* Leaf (5µl)
Track 2- Methanolic extract of *I. arborea* Leaf (5µl)

DISCUSSION

Taxonomically both the plants belong to the same family Rubiaceae, available all over India. Both the plants were shrubs with simple opposite, leaves with interpetiolar stipule. As compare to the *I.coccinea*, *I.arborea* was big in size. *I.coccinea* leaves smaller than that of *arborea.* Both the leaves were Dorsi-ventral, *I.coccinea* showed 3-5 layered palisade cells whereas 2-3 layered in *I.arborea*. Other characters i.e. to say spongy parenchyma, cellular constitute and vascular bundles were similar in nature. Quantitative microscopic data are found to be constant for a species. These values are exclusively useful for identifying the different species of genus and also helpful in the resolve of the authenticity of the plant. Both the species showed stomata in upper and lower surfaces. *I.coccinea* stomatal length and width of upper epidermis were 0.9 X 0.7mm, whereas lower epidermis showed 0.8 X 0.6mm. *I.arborea* showed upper and lower epidermis was 0.8 X 0.3mm. Both the species showed 33.33 Stomatal Index (SI) in lower and upper epidermis. Leaves powder showed similar characters i.e. spongy parenchyma cells, oil globules, prismatic, and cluster crystals of calcium oxalate, simple fibres, fragment of stomata, epidermal cells, covering trichomes, annular and spiral vessels in both the species. Rosette crystal was found only in *I. arborea* whereas pitted and border pitted vessels found in *I. coccinea* only. Both the species showed similar results when subjected to various histochemical tests i.e. Presence of tannin, starch grains, oil globules etc.

Physico-chemical parameter showed i.e. L.O.D study revealed both the species contained same amount of moisture contain, Ash value study revealed both of the species contained more or less same amount of inorganic contain and the variation may be due to the effects of inorganic matters. Water soluble extractive value more in *I.coccinea* leaf in compare to *I. arborea* leaf, which signify that more amount of hydrophilic chemical moiety present in *I. coccinea* leaf. Alcohol soluble extractive value more in *I. arborea* leaf in compare to *I. coccinea* leaf. The various phytochemical can be extracted depending on the polarity and solubility of the solvent. Preliminary phytochemical analysis showed that Carbohydrate, starch, and tannin are present in both plants leaf, whereas mucilage, amino acids, protein, Saponin, flavonoids are absent in both plants leaf. Alkaloids and steroids are only present in *I. coccinea* leaf.

CONCLUSION

The comprehensive literature survey revealed that *Ixora coccinea* and *I. arborea* both are the important medicinal plant which serve extensive pharmacological spectrum. The plant shows the presence of many chemical constituents which are responsible for varied pharmacological and medicinal property. The evaluation needs to be carried out on both the plant in order to their therapeutic uses and formulation of the plant in their clinical applications, which can be used for the well-being of the mankind.

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