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Comparative Phyto-pharmacognostical profile of stem of *Ixora coccinea* Linn. and *Ixora arborea* Roxb

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ABSTRACT

Ayurveda dating back to 1500-800 BC has been an integral part of Indian culture. The term comes from the Sanskrit root *Ayu* (life) and *Veda* (knowledge). *Ixora* is said to be native to Asia and whose name derives from an Indian deity. Till date there is no scientific data is available regarding the phyto-pharmacognostical profile of stem of *Ixora arborea* Roxb. *AndIxora coccinea* Linn., hence present study two plants has been selected to evaluate comparative morphological, pharmacognostical and phytochemical profile. The microscopic features of each T.S and each powder were studied under 4X, 10X and 40 X resolutions under microscope and the pictures were taken by camera. Pharmacognostical evaluation of both the plant stem T.S showed that group of stone cell present in Pith region. Powder microscopy showed that Annular & spiral vessels present in *I. arborea* where is absent in *I. coccinea*. Water soluble extractive showed 9.44% w/w & 14.86% w/w in *I. coccinea* and *I. arborea* respectively. The spectral comparison of stem shows 6 similar Rf values.

Keywords: Ayurveda, *Ixora arborea*, *Ixora coccinea*, Pharmacognosy.

INTRODUCTION

Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda is most developed and widely practiced in India. Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and /or reduced toxicity [1]. There are about 400 species of *Ixora* spread from Africa to India to Southern Asia [2]. *Ixora coccinea* Linn. and *Ixora arborea* Roxb. both are large shrub or small tree. *I. coccinea* Linn called "Rati Nevari"in Gujarati and "Bandhuka" in Sanskrit. *I. arborea* Roxb. Called "Nevri" in Gujarati and "Nevali" in Sanskrit. The wood of *I. arborea* Roxb. are used for tool handles, pegs and bed stead legs [3]. The decoction of bark of *I. arborea* is used for anemia and general debility [4]. Leaf and stem extract of *I. coccinea* showed Broad-spectrum antimicrobial activity and *in vitro* antioxidant effect [5,6]. *I. coccinea* stems are used for sprains, eczema, boils and contusions [7]. Till date there is no scientific data available regarding comparative study of *I. coccinea* Linn and *I. arborea* Roxb. The present study is evaluated to assess comparative phyto-pharmacognostical and HPTLC profile of stem of *Ixora coccinea* Linn. and *Ixora arborea* Roxb.

MATERIALS AND METHODS

Collection and authentification of raw drug

Ixora coccinea Linn. and *Ixora arborea* Roxb. selected plants were collected from natural habit from botanical garden of Jamnagar in month of November-December. Pharmacognostical identification and authentication was done in Pharmacognosy lab, IPGT & RA. Fresh samples were used for various Pharmacognostical evaluations. Healthy uninfected samples were made into herbarium IPGT & RAPhm 6105/2015 and 6106/2015 *Ixora coccinea* Linn. and *Ixora arborea* Roxb. respectively and kept in the Lab for further reference. Stem was separated and dried in shed, powdered at 80 # for further phyto-pharmacognostical studies. All experiment is completely randomized design and repeated at least thrice and mean taken into consideration.

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Macroscopic Study

The collected samples were identified and authentified by studying their characters systematically as per the methods described in the textbooks of pharmacogosy [8,9].

Organoleptic characters of the powder

Organoleptic characters i.e. colour, odour, tasteand nature of powder were noted done by sensory observations [8].

Microscopic Study

Fresh samples were taken for detailed microscopic study. Free hand sections were taken; cleared with chloral hydrate and then, the sections were stained with phloroglucinol and hydrochloric acid and observed for lignified elements like fibres, stone cells, vessels etc. Microphotographs were taken by using Carl Zeiss Trinocular microscope attached with camera. Same procedure was followed for detailed powder microscopy [8,9].

Histochemical evaluation

Thick sections of stem sample and powder samples of both the plants were subjected to histochemical tests to catch out starch, tannin etc. by treating various reagents [8,9].

Physicochemical Evaluation

Physico-chemical Parameters like loss on drying, total ash, alcohol soluble extractive (90% methanol), watersoluble extractive and pH values were determined as per the API guidelines for the powdered samples [10].

Preliminary PhytochemicalEvaluation

Preliminary phytochemical examination of methanolic extract of sample drugs was carried out for Carbohydrate, proteins, Steroids, glycosides, tannins,flavonoids, alkaloids etc. according to standard procedure [11,12].

RESULT

MICROSCOPIC STUDY

Transverse section of *Ixora coccinea*Linn.stem

Diagrammatic T.S. of stem was oval to wavy in outline. Outer epidermis was covered with thick cuticle and some of the epidermal cells lead into simple unicellular trichomes, followed by hypodermis, cortex, endodermis, pericycle, vascular bundle and central parenchymatous large pith with several groups of stone cells.

Detailed T.S. showed the outer most layers compactly arranged some of the epidermal cells lead into simple unicellular trichomes. Epidermis covered with thick cuticle. Hypodermis 2-3 layered compactly arranged collenchymas cells. Some of the hypodermal cells filled with red colouringmatters followed by cortical cells made up of 6-9 layers of simple parenchyma cells without any intercellular spaces, mostly filled with chlorophyll pigments. Some of the cells loaded with rosette crystals and cluster crystals of calcium oxalate, oil globules and simple starch grains. Cortex is followed by single layer of endodermis. 2-3 layers of compactly, circulary arranged, pericyclic fibers which are lignified.vascular bundles are open and collateral, radially arranged. Metaxylem is facing towards cortical region and protoxylem to words central pith region with xylem parenchyma and fibres. Xylem is separated by uni-serrate medullary rays. Phloem is situated above the xylem with sieve elements and fibres. Larger region of the section is occupied by pith made up of parenchymatous cells. In the pith region 3-5 isolated groups of pitted stone cells. Pith cells are filled with oil globules, rarely simple starch grains and rosette and cluster crystals of calcium oxalate. (Figure 1)

Transverse section of *Ixora arborea*Roxb.Stem

Diagrammatic T.S. of stem was oval to wavy in outline. Outer epidermis was followed by hypodermis, cortex, pericycle, endodermis, vascular bundle and central parenchymatous large pith with several groups of stone cells.

Detailed T.S. showed the outer most layers compactly arranged epidermal cells. Epidermis covered with thick cuticle. Hypodermis 2-3 layered compactly arranged collenchymas cells. Some of the hypodermal cells filled with red colouring meters and tannin content followed by cortical cells made up of 6-9 layers of simple parenchyma cells without any intercellular spaces, mostly filled with chlorophyll pigments. Most of the cells loaded with rosette crystals and cluster of calcium oxalate, oil globules and simple starch grains. Cortex is followed by single layer of endodermis. 2-3 layers of compactly circularly arranged discontinued lignified pericyclic fibers which are lignified.Vascular bundles are open and collateral, radially arranged. Metaxylem is facing towards cortical region and protoxylem to words central pith region with xylem parenchyma and fibres. Xylem is separated by uni-serrate to biserriate medullary rays, mostly filled with simple and compound starch grains. Phloem is situated above the xylem with sieve elements and fibres.Larger region of the section is occupied by pith made up of parenchymatous cells. In the pith region 5-7 isolated groups of pitted stone cells. Some of the parenchyma adjacent to vessels were pitted and lignified. Pith cells are filled with oil globules, simple starch grains and rosette and cluster crystals of calcium oxalate. (Figure 2)

Comparative study on Powder microscopy

Regarding comparative similar and dissimilar Organoleptic and microscopic characters of powder were scientifically observed. (Table 1 & 2) (Figure 3&4).

Histochemical evaluation:

Both Samples are subjected to histochemical tests to find out starch, tannin, calcium etc. by treating various reagents. (Table 3)

Comparative physico-chemical parameters:

Stem powder of *Ixora coccinea* and *Ixora arborea* were subjected to various physico-chemical analyses such as loss on drying, ash value, acid insoluble ash, extractive values etc. estimated. (Table 4)

PRELIMINARY PHYTOCHEMICAL EVALUATION

Stem powder was evaluated for the presence of different phyto-constituents like alkaloids, saponins, tannins and Steroid etc. (Table 5)

CHROMATOGRAPHIC ANALYSIS (HPTLC) [13]:

Chromatographic techniques were carried out as per the standard materials & methods.Solvent system which were designed for TLC i.e. Toluene: Ethyl acetate: Acetic acid (7: 2: 0.5) was used for HPTLC studies. (Table 6) (Figure 5&6)

- Track- 1: Stem of ***Ixora arborea*** (1 mg/ml)
- Track- 2: Stem of ***Ixora coccinea*** (1 mg/ml)

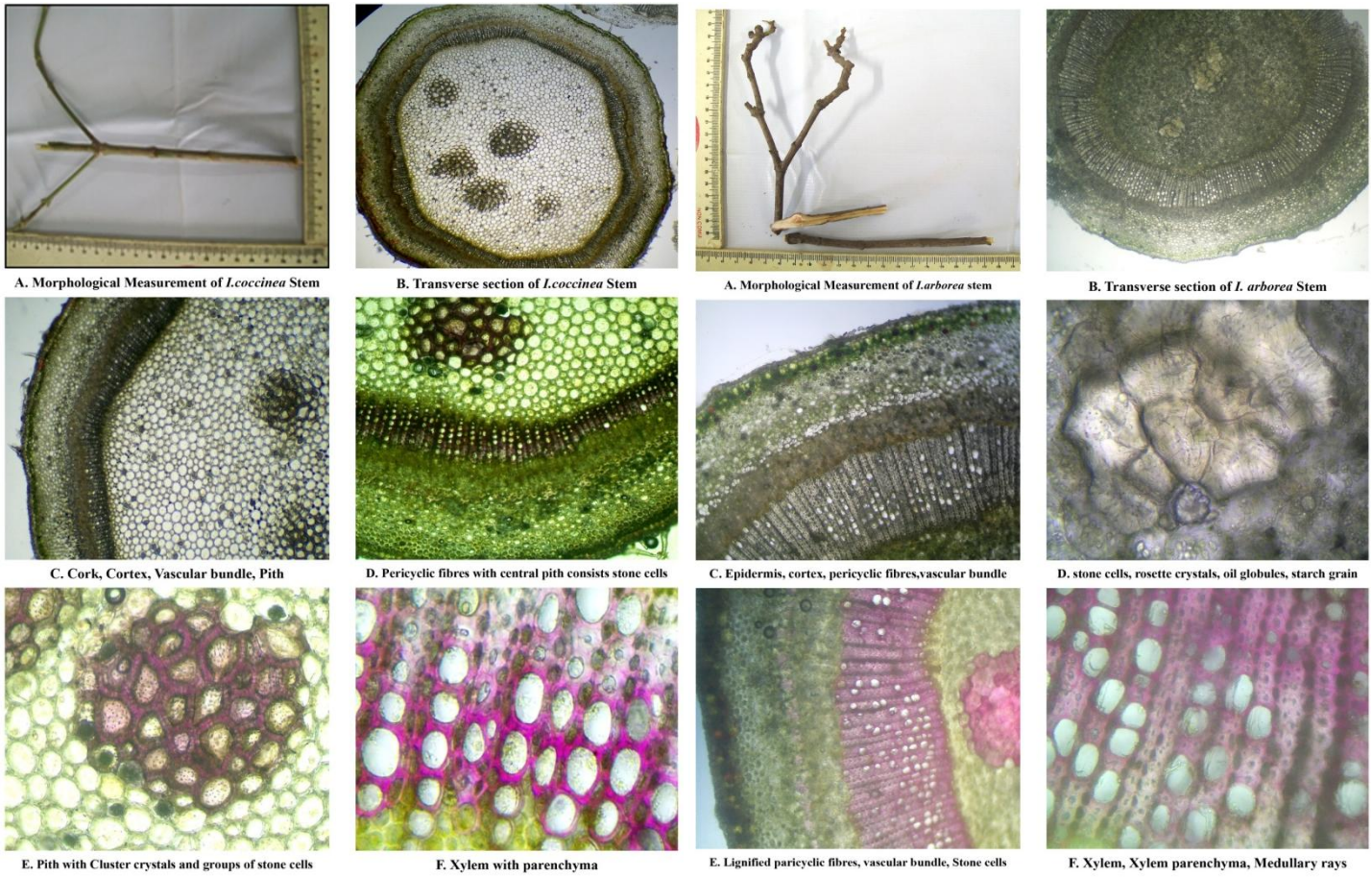

Figure 1: *Ixora coccinea* Linn. stem

Figure 2: *Ixora arborea* Roxb. stem

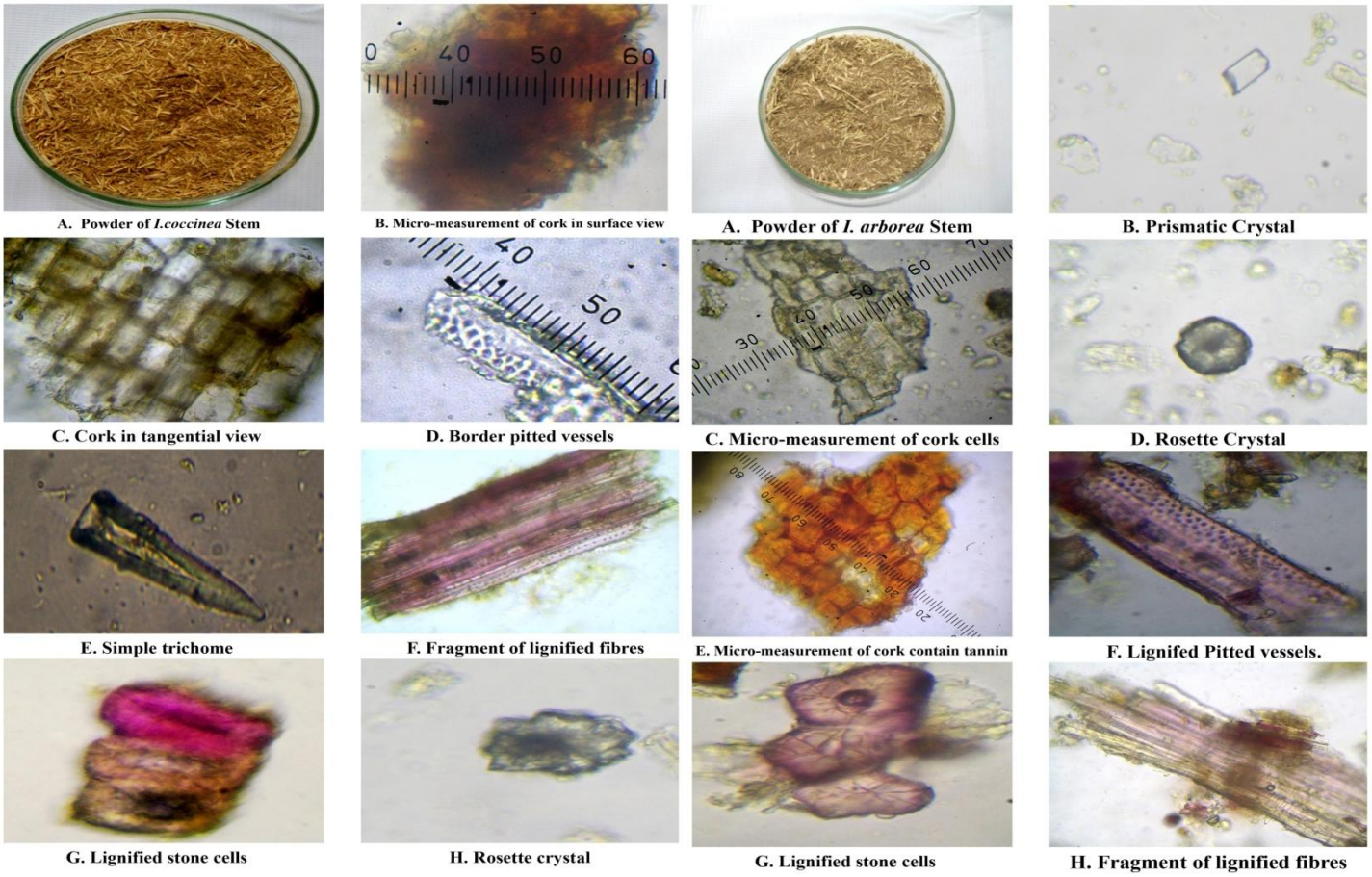

Figure 3: *Ixora coccinea* Linn. stem powder

Figure 4: *Ixora arborea* Roxb. stem powder

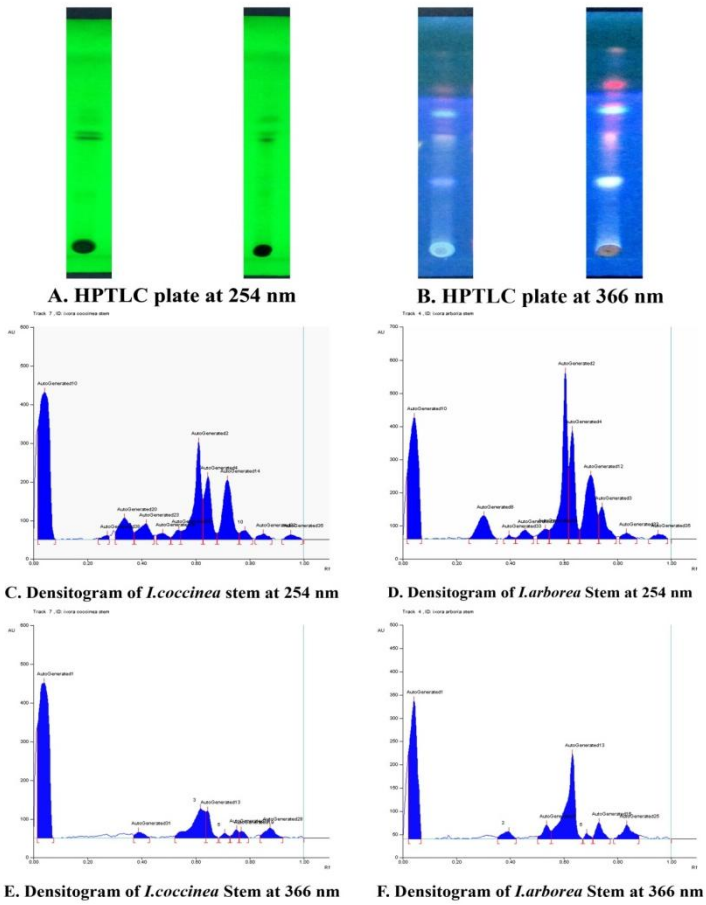

Figure 5: HPTLC profile of *I. coccinea* and *I. arborea*

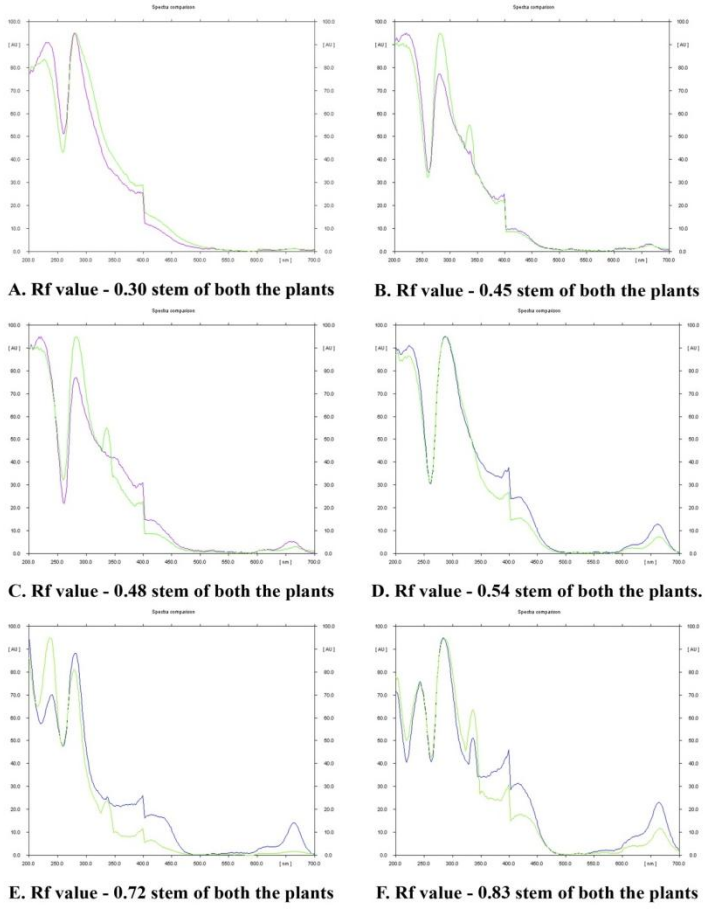

Figure 6: Spectral comparition of both the stem plant

Table 1: Organolepticcharacters of stem powder of *I. coccinea* and *I. arborea*

Characters	***I.coccinea***	***I. arborea***
Colour	Light brown	Brownish yellow
Odour	Aromatic	Astringent
Taste	Characteristic	Characteristic
Nature of powder	Corse	Smooth

Table 2: Comparative powder microscopy of *I. coccinea* and *I. arborea* stem powder

S No	**Characters**	***Ixora coccinea***	***Ixora arborea***
01	Group of Stone cells	+	+
02	Simple Starch grain	+	+
03	Tannin content	+	+
04	Cluster, Rosette crystal of Ca. oxalate	+	+
05	Fragments of Lignified fibres	+	+
06	Fragment of Epidermal cells	+	+
07	Oil globules	+	+
08	Annular, Spiral vessels	-	+
09	Pitted, Border pitted vessels	+	+
10	Simple Trichomes	+	+

Table 3: Histochemical Results of stem of *I. coccinea* Linn. and *I. arborea* Roxb.

S 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	**++**	**++**

Table 4: Physicochemical parameters of stem of *I. coccinea* Linn. and *I. arborea* Roxb. Stem powder

S No	Parameters	***Ixora coccinea***	***Ixora arborea***
1	Loss on drying (%w/w)	8.37	7.39
2	Ash value(%w/w)	2.45	8.61
3	Water soluble extractive (%w/w)	9.44	14.86
4	Alcohol soluble extractive (%w/w)	11.35	11.51
5	pH	6.2	6.0

Table 5: Preliminary phytochemical evaluation of stem of *I. coccinea* Linn. and *I. arborea* Roxb. Stem powder

S No	Name of the test	***Ixora coccinea***	***Ixora arborea***
		Stem	**Stem**
1	Carbohydrates	++	++
2	Starch	++	++
3	Mucilage	--	--
4	Protein	--	--
5	Amino acid	--	--
6	Steroid	++	++
7	Saponin glycoside	++	++
8	Flavonoid	++	--
9	Tannin	++	++
10	Alkaloid	++	++

Table 6: HPTLC Studies of methanolic extracts at 254nm and 366nm

Sample	254 nm		366 nm	
	No.of Spots	**Rf Value at 254 nm**	**No.of Spots**	**Rf Value at 366 nm**
Track –1	**10**	0.07, 0.35, 0.42, 0.49, 0.55, 0.62, 0.66, 0.73, 0.87, 0.99	**7**	0.07, 0.42, 0.55, 0.67, 0.71, 0.77, 0.88
Track – 2	**12**	0.08, 0.28, 0.37, 0.45, 0.51, 0.54, 0.63, 0.68, 0.76, 0.81, 0.88, 1	**8**	0.07, 0.43, 0.64, 0.68, 0.73, 0.76, 0.80, 0.92

DISCUSSION

T.S of *I.coccinea*showed single layered epidermal cell with unicellular trichomes where as *I.arborea* showed without trichomes. T.S of the stem in both of the species showed presence of similar kind of rosette and cluster crystals and oil globules. Hypodermis, pericyclic fibers, medullary rays similar in structure. Pith region in both of the species contained oil globules and starch grain, specific character i.e. 4-5 groups of stone cells observed.

Results obtained from organoleptic study emphasized the more or less similar in characters like color, odour, taste and touch in both of the species. Both the species Stem powder showed similar characters i.e. epidermal cells, oil globules, brown contents, lignified fibres etc. Both the species showed similar results when subjected to various histochemical tests i.e. Presence of tannin, starch grains, oil globules etc.Preliminary phytochemical evaluation by methanol and water soluble extract showed that Carbohydrate, alkaloids, starch, saponin and tannin etc. are present in both plants, whereas mucilage, amino acids, protein are absent in both plants extract;Flavonoids only present in *I. coccinea*extract.

Result obtained from physicochemical study represented that both the stem showed similar pH value nearby 6.0 showed weak acidic in

nature. L.O.D study revealed both the species contained more or less same amount of moisture contain. Ash value isdifferent in both of the species; the variation may be due to the effects of inorganic matters. Water soluble extractive value varies May due to the different water soluble chemical moieties. Alcohol soluble extractive value is nearby same.The spectral comparison of stem shows six similar $R_f$values i.e. 0.30, 0.45, 0.48, 0.54, 0.72, 0.83, This may show most common chemical moiety present in both the stem sample of plants.

CONCLUSION

Taxonomically both the plants belong to the same family Rubiaceae, slightly variability in anatomy and bioactive principles. This study is helpful for further research work. Further explorations are required to DNA Finger printing and isolate the bioactive principles.

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Conflict of interest – None declared.

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