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Pharmacognosic Studies of Ipomoea Sepiaria Roxb. with Two Other Specious

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Abstract: ***Three different species of ipomea studied to confirm the genuine material common name Thiruthali used in number of classical medicine. The study area is Moovatupuzha. Morphologically and anatomically these three species shows number of similarities and dissimilarities. The powder microscopy revealed the presence of fibers, different kind of thickening. Preliminary photochemical analysis of the water and methanol extract reveals the presence of flavanoids, sterols and terpinoids, phenolic compound, glycosides, tannin, carbohydrate. Water extract give negative result in protein, starch, aminoacids and alkaloids. TLC studies reveal that Ipomea separia with more phytocompounds than the other two species. I. sepiaria rich with I-Dodecen. It has halide activity. It is a good mental stress retardant. It also one of the major raw herb in classical mental stress relaxation medicine Manasamithra vatakam. The plants are identified and deposited in Kerala Forest Research Institute (KFRI). Ipomea triloba L. Assetion number 17697, Ipomea marginata (Desr.) verc. Assetion number 17698, Ipomea sepiaria L. assetion number 17699.***

Keywords: *Ipomoea sepiaria*, Adulterant, Anatomy, Powder microscopy, preliminary Phytochemical screening, TLC

1. Introduction

The use of classical herbal medicine is gaining momentum in this era due to the side effect of synthetic pharmaceutical products and the safety, efficiency and promising potential of plant derived medicine (7). To get the good quality and efficiency of the product, it is very important to ensure the quality and genuinely of the raw herbs which used in products (4).

Ipomoea sepiaria Koening ex. Roxb. Is a source of ayurvedic medicine called as Lakshmana. In folklore practice this herb is known as a good antidote to arsenic poisoning, uterine tonic, aphrodisiac and anti-ulcer drug (8). The plant also shows anti microbial activity and deposition of number of phytochemical constituents (9). The Ayurvedic texts Basavarajeeyam (18th Century) it is mentioned leucorrhoea property of I. Sepiaria (11). It is also one of the major raw herbs in classical mental stress relaxation medicine Manasamithra vatakam. It is a brain and nerve tonic drug out of ayurvedic classics. For people all age groups hermanizez the body through the thoughts, feelings and instincts of the mind. It is very useful in the treatment of psychosomatic disease, neuroses, anxiety and stress. It also effects in children and impove theire academic performance. It is a stress buster. It reduces mental fatigue and improves concentration. Now day's women's are affected by pre menstral tension it helps to cure these problems. . The plant ipomea sepiaria widely used in herbal industry in different products like Thali, Hair protector creems etc. In I. sepiaria various phytochemicals have been identified. It showed the presence of I-Dodecene, I-Hexadecanol, I-Hexadecene, and 2-hexadecanol etc. From that I-Dodecene has halide activity (14). It helps to retard mental stress. Due to the presence of active compounds Ipomea sepiaria is used in Manasamithra vatakam.

Ipomoea is the largest genus in the flowering plant, family convolvulacea. It commonly known as morning glory. Most Morning-Glory is a perennial growing vine that thrives in full sun and little water (6). The genius includes 500-600 specious (2). This family shows twining and climbing woody or herbaceous plants that often have heart-shaped leaves and funnel shaped flowers (3). Ipomea genius shows the property of phytotoxicity, which means suppressing the growth of other plants including invasive weeds. Te genius is widely used in local traditional medicine in many countries for the treatment of number of diseases (10). Number of species Ipomoea in the world varies from 600 to 700 species (2). All species propagate by seeds and some of them are multiplied by vegetative parts. The World Health Organization (WHO), 1978 has estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. In India about 65 % of the population relies on ethno medicine, which is the only source of their primary health care needs (13)

Adulteration occurs most of the time because of incorrect evaluation. Ipomea sepiaria is shows similarity with Ipomea triloba & Ipomea marginata. So it needs a keen observation to conform their genuinity. This study aimed to compare this tree specious in pharmacognostic view using Stem flower and leaf. The present paper attempt to evaluate and find the genuine Thiruthali at moovattupuzha area through Microscopic, Organoleptic, Preliminary phytochemical screening and TLC analysis of I. sepiaria, I. trilobaandI. margin

2. Materials and Method

Three specious of Ipomea collected from Muvattupuzha area washed with water shade dried and powdered was subjected to pharmacognostic analysis; the parameters included Morphological study, microscopic evaluation, preliminary phytochemical analysis, TLC analysis.

Collection of plant material

Morphological Study using simple Hand lance:-colour, shape and structure of the three specious where studied.

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Anatomical Study using Trinocular microscope: C. S of Stem, leaf, petiole where taken and stained using sfranin observed under trinocular microscope find the cell arrangements and cell structure.
Powder microscopy: Dried plant powdered and observed under microscope and finds the cell structure and different kind of thickening

2.1 Preliminary phytochemical screening (12, 1, 5, 15)
2.1.1. Tests for alkaloids
2.1.1.1 Dragendorff's Test: 2 ml of the extract was treated with a few drops of Dragendorff's reagent (solution of potassium bismuth iodide). Formation of orange brown precipitate indicates the presence of alkaloids.
2.1.1.2 Mayer's Test 2 ml of the extract was treated with a few drops of the Mayer's reagent (Potassium mercuric iodide solution). Formation of a cream coloured precipitate indicates the presence of alkaloids.

2.1.2 Test for Glycosides
2.1.2.1 Keller-Kiliani Test The extract was mixed with 2 ml of glacial acetic acid containing 1 or 2 drops of freshly prepared ferric chloride solution. The mixture was shaken well and was carefully poured into a test tube containing concentrated sulphuric acid along the sides. Formation of a brown ring at the junction indicates the presence of cardiac glycosides.

2.1.3 Test for flavonoids
2.1.3.1 Shinoda Test Crude extract was mixed with a few small pieces of Magnesium ribbon for a minute and a few drops of concentrated HCl was added drop wise into this mixture. Development of pink scarlet colour or light red colour after a few minutes indicates the presence of flavonoids.
2.1.3.2 Lead acetate Test: Small quantity of the extract was treated with a few drops of lead acetate solution. Formation of yellow colour or yellow creamy precipitate indicates the presence of flavonoids.
2.1.3.3 Alkaline reagent Test: The extract was mixed with 2% NaOH solution. Intense yellow colouration which loses the intensity on the addition of dilute acid indicates the presence of flavonoids.

2.1.4 Test for tannins
2.1.4.1 Ferric chloride Test: 2 ml of freshly prepared ferric chloride solution was added to 2 ml of the concentrated extract. Formation of dark blue or green or black colour indicates the presence of tannins

2.1.5 Test for phenols
2.1.5.1 Ferric chloride Test: To 2 ml of the extract, 2 ml of freshly prepared ferric chloride solution was added. The development of blue-green or black colour indicates the presence of phenols.

2.1.6 Test for saponins
2.1.6.1 Froth test: 2 ml of the extract was mixed with 20 ml of distilled water in a graduated test tube and shaken well for 10 minutes. Formation of 1 cm thick froth indicates that the sample contains saponins.

2.1.7 Test for sterols
2.1.7.1 Liebermann-Burchard Test: 2 ml of the extract was mixed with a few drops of acetic anhydride. It was boiled and cooled and concentrated sulphuric acid was added along the sides of the test tube carefully. A brown ring at the junction of two layers and the upper layer turning green indicates the presence of sterols.

2.1.8 Test for carbohydrates
2.1.8.1 Molisch's test: 2 ml of the extract was taken in a test tube and few ml of Molisch's reagent was added along the sides. Formation of violet ring at the junction indicates the presence of carbohydrates.
2.1.8.2 Fehling's test: 1 ml each of Fehling's solution A and B were mixed and boiled for one minute. Equal volume of the extract was added and then boiled in a water bath for 5 minutes. Formation of reddish brown colour indicates the presence of reducing sugar.
2.1.8.3 Iodine test: 1 or 2 drops iodine solution was added to 1ml of the extract. Formation of dark blue colour indicates the presence of carbohydrates.

2.1.9 Test for proteins and amino acids
2.1.9.1 Ninhydrin test: 3 ml of the extract was boiled with 3 drops of 5% Ninhydrin solution. Formation of blue or violet colouration indicates the presence of amino acids.
2.4.9.2 Xanthoproteic test: The extract was treated with a few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins.

TLC comparative study
Methanol extracts of Ipomoea specious were subjected to thin layer chromatography studies, to find out the probable number of compounds present in them. Used solvent system-Methanol: Chloroform 4

3. Result

3.1 Morphological analysis:-morphological characters of leaf, stem, petiole and flower are checked using hand lance observations were noted (Table I)

Table I

S. No.	Part	Specious Name	Character
1	Stem	Ipomea marginata	vine, Green with Brown margin, Pubescent, Girth 0.7cm
		Ipomea sepiaria	vine, Green with Brown margin, Pubescent, Girth 0.7cm
		Ipomea triloba	Vine, Violet in colourwith green marking, pubescent, Girth 0.6cm
2	Leaf	Ipomea marginata	Simple, pubescent, young leaf heart shaped large ones shows 2-3 teethes on the lower end, petiolate, Stipulate, 4-4.5cm length, 3.5-4cm breadth, petiole 4.5cm length
		Ipomea sepiaria	Simple, heart shaped, Smooth, Petiolate Stipulate, 3.5-4cm length, 4.5-5cmdreadth, petiole 3.5 cm length

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		Ipomea triloba	Simple, Pubescent, Lamina with 3 teeth or lobes, Petiolate, stipulate, Length 4-4.5cm, Breadth 2.5-3 cm, petiole with 3cm length
3	Flower	Ipomea marginata	simple cyme, Gamosepalous –fushion seen at lower potion, gamopetalous, pink colour
		Ipomea sepiaria	Single, Pedicalate, ivory in color with yellow separation, pentamerous gamopetalous, gamosepalousCorolla tube with violet marking
		Ipomea triloba	simple cyme, Gamosepalous –fushion seen at lower potion, gamopetalous, pink colour

3.2 Anatomy: Thin hand section of Leaf, stem, petiole are taken stained with safranin Then observed under Trinocular microscope and characters are noted (Table II & Pict I)

Table II

Anatomy

S. No.	Part	Specious Name	Character
1	Stem	Ipomea marginata	Single layer epidermis, cortex cells closely arranged, single layer endodermis, closely packed vascular bundles, pith cells are closely packed parenchyma
		Ipomea sepiaria	Single layer epidermis, cortex cells Single layer epidermis, cortex cells closely arranged with 2-3 cavities , single layer endodermis, closely packed vascular bundles, pith cells are closely packed parenchyma with schizogenous cavity
		Ipomea triloba	closely arranged with 2-3 cavities , single layer endodermis, closely packed vascular bundles, pith cells are closely packed parenchyma
2	Leaf	Ipomea marginata	Single layer of epidermis with cuticle, closely packed palisade tissue and loosely packed spongy tissue, Midrib region with vascular bundle, Parasitic stomata, Epidermal cells are Hexagonal in shape
		Ipomea sepiaria	Epidermis with cuticle, Closely packed palisade and spongy tissue, Midrib region with vascular bundle, Parasitic stomata, Epidermal cells are wavy out line
		Ipomea triloba	Epidermis with cuticle, Closely packed palisade and spongy tissue, Midrib region with vascular bundle, Parasitic stomata, Epidermal cells are wavy out line
3	Petiole	Ipomea marginata	Single layered epidermis with cuticle, Cortical cells are with some kind of deposition, Vascular bundles are 5Nos.
		Ipomea sepiaria	Single layer of epidermis with cuticle, Cortical cells are with some kind of deposition, Vascular bundles are 4 Nos.
		Ipomea triloba	Single layered epidermis with cuticle, Cortical cells are with some kind of deposition, Vascular bundles are 5Nos.

3.3 Preliminary photochemical analysis

Aqueous and methanol extracts of three species were subjected to preliminary photochemical analysis and the result of various photochemical constituent depicted in Table III.

Aqueous and methanolic extract of these three species showed the presence of Flavanoid, Sterol& Tarpinoid, Phenolic compound, Glyscoide, Tannin and Carbohydrate. Alkaloid, Protien and Starch are not present.

Preliminary phytochemichal screening

Sl. No.	Class of compound	Ipomea sepiaria		Ipomea marginata		Ipomea triloba	
		Water extract	Methanol extract	Water extract	Methanol extract	Water extract	Methanol extract
1	Flavanoid	+ ve	+ve	+ ve	+ve	+ ve	+ve
2	Alkaloid	-ve	-ve	-ve	-ve	-ve	-ve
3	Sterol& Tarpinoid	+ve	+ve	+ve	+ve	+ve	+ve
4	Saponin	+ve	-ve	+ve	-ve	+ve	-ve
5	Phenolic compound	+ve	+ve	+ve	+ve	+ve	+ve
6	Glycoside	+ve	+ve	+ve	+ve	+ve	+ve
7	Tannin	+ve	+ve	+ve	+ve	+ve	+ve
8	Carbohydrate	+ve	+ve	+ve	+ve	+ve	+ve
9	Aminoacid	-ve	+ve	-ve	+ve	-ve	+ve
10	protien	-ve	-ve	-ve	-ve	-ve	-ve
11	Starch	-ve	-ve	-ve	-ve	-ve	-ve

3.4 Powder microscopy

The whole plant dried and powdered and observed under micro scope. I. marginata shows light green colour with high fiber content, simple trichomes are seen spiral thickenings are also seen. I. sepiaria shows fine powder compares to marginata& triloba and light brown colour. Less fiber content compare to marginata and simple trichomes with internal separation and also shows spiral thickening. I. triloba shows annular thickening (Pic-II)

3.5 TLC Studies using silica gell plate and Rf value calculated (Table IV & Pict III)

Table IV

Sl. No.	Specious Name	Visualization	RF Value

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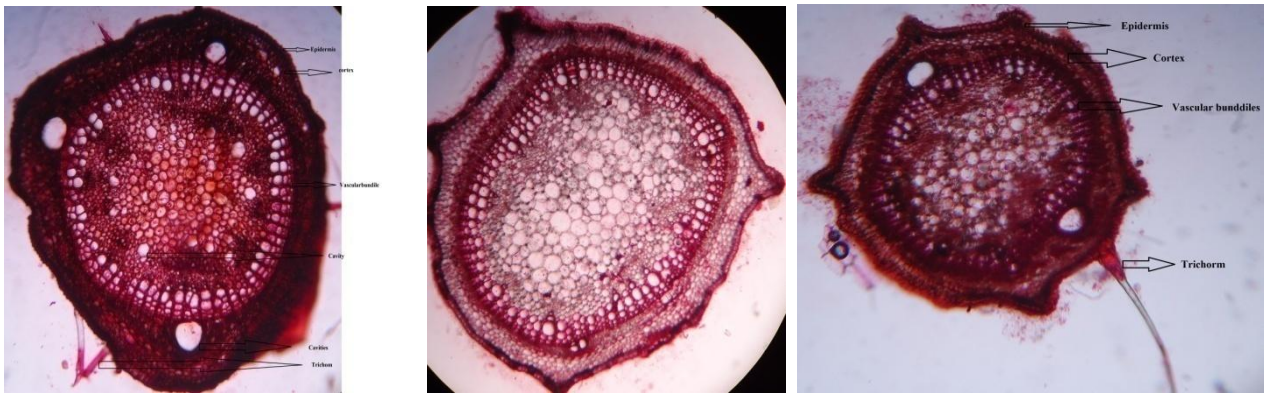
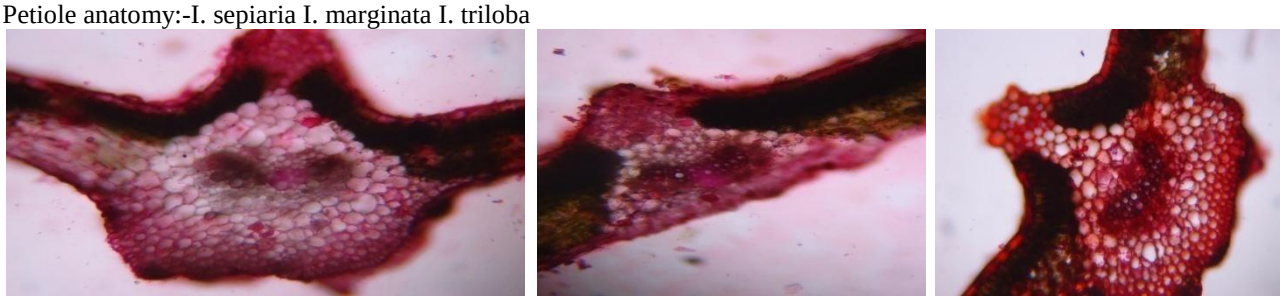
1	Ipomea sepiaria	L-Wave	0.96, 0.75, 0.59, 0.53, 0.37, 0.318, 0.181, 0.075
		S-Wave	0.96, 0.75, 0.59, 0.530, 0.439, 0.378, 0.318, 0.181
		Florescent light	0.969, 0757, 0.590, 0.530, 0.378, 00303, 0.151, 0.075
2	Ipomea marginata	L-Wave	0.96, 0.530, 0.75, 0.181
		S-Wave	0.96, 0.75, 0.59, 0.530, 0.181
		Florescent light	0.969, 0.757, 0.530, 0.151
3	Ipomea triloba	L-Wave	0.96, 0.59, 0.45, 0.181, 0.075
		S-Wave	0.96, 0.65, 0.59, 0.585, 0.53, 0.457, 0.43, 0.214
		Florescent light	0.96, 0.75, 0.6, 0.457, 0.214

Pict-I



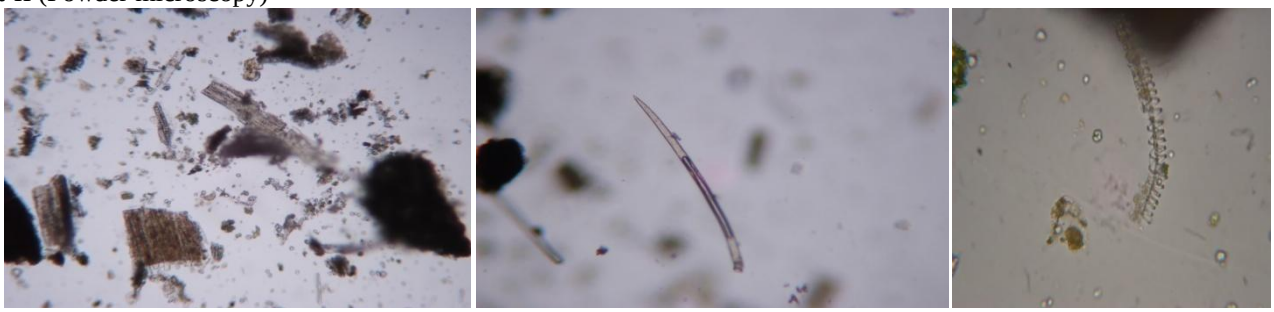
Stem anatom:-I. sepiaria I. marginata I. triloba

Petiole anatomy:-I. sepiaria I. marginata I. triloba



Leaf anatomy:-I. sepiaria I. marginata I. triloba

Pict-II (Powder microscopy)



I. sepiaria

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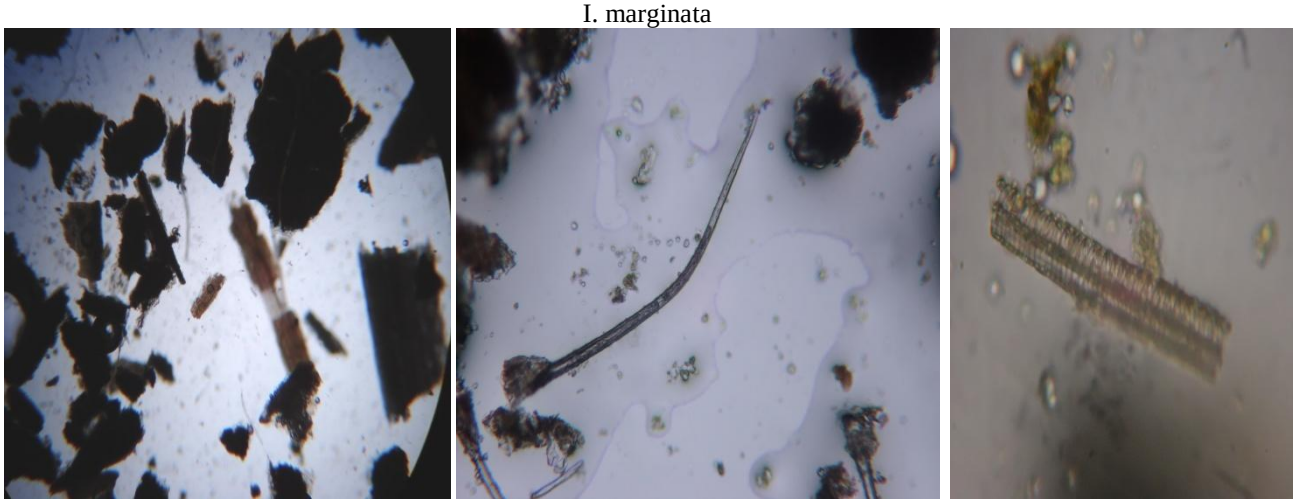
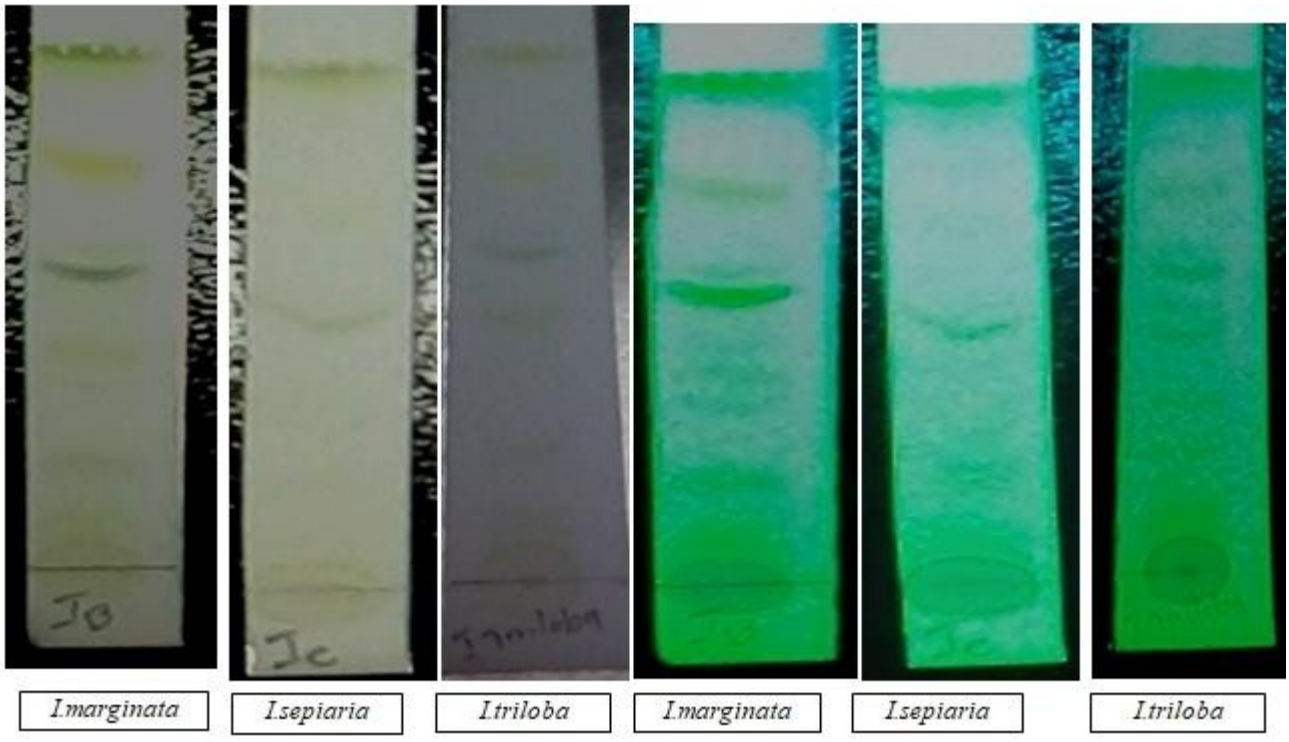
I. marginata



I. triloba

Pict-III



S-Wave
Flurasent

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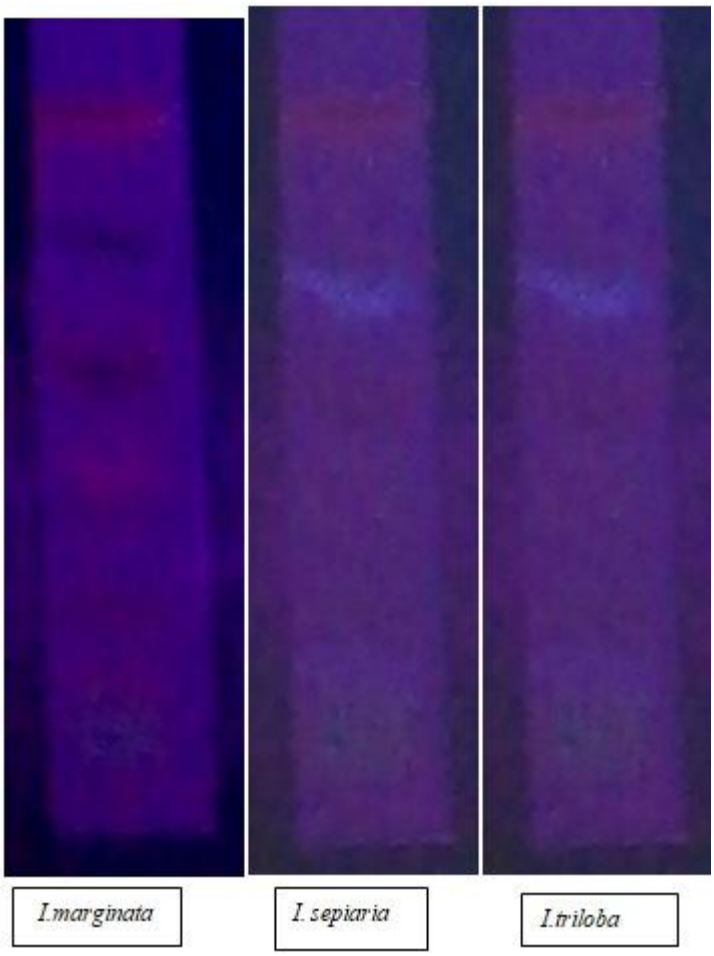

L-Wave

4. Discussion and Conclusion

These three specious are available in Muvatupuzha region very commonly. Both specious are locally known as thiruthali. Due to unawareness of the correct specious need for the product and herbs are collected by local people so the chance to come adulterant is very common. The specious Marginata and sepiaria shows similarity in stem morphology and leaf morphology. Specious triloba flower shows similarity with Marginata. Stem anatomy of marginata shows similarity with sepiaria. These three specious show similarity in there petiole morphology and anatomy. All the three specious shows similar result in Preliminary phytochemichal screening. But shows difference in TLC study. The actual specious need for the product is sepiaria and the formulation didn't tell any substitute. To conform collected specious is correct one need to done all the analysis. Adulteration is common problem in herbal industry so check all the characters of the herbs which we collected and ensure its genuinety. It helps to give good quality and efficacy to the product. To prevent the herbal adulteration it is very important to done the cultivation at least which the industry want.

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