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Standardization of Siddha Drug Eachuramooli Chooranam

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ABSTRACT:

Siddha text prescribed several lakhs of medications and formulation that are indicated for various diseases**.** Standardization of traditional medicine become highly essential as there is possibilities of frequent adulteration and contamination in most of the indigenous medicines. Siddha system of medicine demand more quality analysis for globalization. The sample was subjected to investigation of Loss on drying, Total ash value, Acid insoluble, Water-soluble ash value, Estimation of alcohol and water soluble extractive values. Analysis of Heavy metals Mercury, Arsenic, Lead, Cadmium were carried out.Eachuramooli chooranam (EC) in dry powdered form was subjected to microscopic analysis. Powder microscopy revealed the correct selection of plant species for the research purpose.

Keywords: Siddha, Eachuramooli chooranam, standardization.

INTRODUCTION:

Siddha text prescribed several lakhs of medications and formulation that are indicated for various diseases**.** Standardization of traditional medicine become highly essential as there is possibilities of frequent adulteration and contamination in most of the indigenous medicines. Siddha system of medicine demand more quality analysis for globalization. Recently implement guideline on AYUSH makes more viable analysis of the formulation for commercial values. Product with standard possess maximum therapeutic efficacy and also surely gains the confidence of the people which become a mode of gaining popularity for the Indian traditional medicines [1].

Quality control on chooranam, chenduram, parpam, legium, ennai, nei, dhravagam and related formulations reveals some evidence based data on the nature, stability, genunity, organoleptic, functional groups and phytochemical nature of the formulations. This data would be preferentially used for comparing the test drug with the standard one and ascertain

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the quality in each batch at the manufacturing site [2]. Siddha text prescribed several lakhs of medications and formulation that are indicated for various disease, continuous process of drug standardization provides an ideal monograph of limits on quality parameter's that could be high useful for the fellow researchers and also for manufacturer in the forthcoming generation's.

As most of the raw drugs used for formulating siddha preparation are having frequent chance of soil contamination like environmental pesticide residues imparted on the test drug. Other contamination includes presence of aflatoxins, soil oriented heavy metal absorption and other possible contaminates like basic and acid radicals including pathogenic organism. As per the recent research outcome it was evident that majority of people including western population are utilizing the alternated medicines has their drugs of choice in management of health issues [3], this could be an emerging scope for the researcher in the field of traditional therapy to validate more medicines in the process of disease prevention as prophylaxis.

MATERIALS AND METHODS:

Plant

Aristolochia Indica Linn commonly known its vernacular name called as Indian birthwort (family: Aristolochiaceae). In southern parts of Asia this herb is considered to be possessing potential medicinal properties

Distribution

This plant is a perennial climber with greenish white woody stems found in low hills and plains of India, Nepal and lower Bengal to Chittagong in Bangladesh.

Phytocomponents

This is plant is known to have p-coumaric acid, β-caryophyllene and α-humulene, Aristolactone[4,5]

Pharmacological Activity

As per the literature this herb is known for its versatile pharmacological acitiy the some of which included below

S.No	Pharmacological Activity	References
1.	Anti-Proliferative activity[6]	Kupchan and Doskotchet; 1962.
2.	Anti-oxidant[7]	Thirugnansampandan et al., 2008.
3.	Anti-Microbial activity[8]	Shafi et al., 2002
4.	Anti-inflammatory, anti-pyretic and analgesic activity[9]	Das et al., 2010
5.	Anti-Diabetic Activity[10]	J. Marlin Cynthi et al.,2012

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Collection and Authentication

Plant materials were collected from the southern zone of Tamil Nadu. Collected specimen was identified and authenticated the Director, Plant Anatomical Research Centre, Chennai, Tamil Nadu, India. The specimen voucher was deposited in the National institute of Siddha, Chennai 600047, Tamil Nadu, India.

Formulation of *Eachuramooli Chooranam*

Collected plant materials was washed thoroughly and were extended for shade dry procedure followed by formulation as per the standard formulation method.

Dose: 1-2 gram twice daily

Indications: Heart diseases *(Irudaya rogam)*

Powder microscopy

In order to confirm the genunity and authenticity of the test material EC in dry powdered form was subjected to microscopic analysis at Captain srinivasa murthy research Foundation, Chennai and the results of the same is listed for the reference as a pictogram.

Standardization of Eachuramooli Chooranam

In order to confirm the standard and stability of the prepared formulation EC. The sample were subjected to investigation of Loss on drying, Total ash value, Acid insoluble, Water soluble ash value, Estimation of alcohol and water soluble extractive values. The above parameters were analyzed using standard protocol mentioned in Pharmacopoeial Laboratory for Indian Medicine (PLIM) [11]

Preliminary Phytochemical screening tests (Qualitative)

Preliminary qualitative phytochemical estimation of test drug EC was carried out using optimized procedures established by Brain KR, Turner T et al[12] for identification of alkaloids, flavonoids, saponin, glycosides, terpenoids, steroids, tannins, phenols, coumarins, sugar, proteins, anthocyanins etc.

TLC/HPTLC Analysis

The test sample EC was subjected to TLC analysis in the twin trough chamber using the designated solvent system. Once the run plates have dried and been visually scrutinised Light long-wave UV light at 365 nm and short-wave UV light at 254 nm13.

High performance thin layer chromatography (HPTLC) techniques highly reliable method for quantifying the relative intensity of existence of phytocompounds the values were calculated based on the peak intensity and percentage area[14]. Plates were scanned under UV at 254 and 366nm under running condition on mobile phase toluene: Ethyl acetate (7:3). Through the

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CAMAG programme, the scanning-derived data were integrated. Each sample's phytoconstituents were identified using a chromatographic fingerprint, and their corresponding Rf values were tabulated.

Aflatoxins Assay (B1, B2, G1, G2)

Aflatoxin analysis of specific toxins such as B1, B2, G1, G2 were estimated using qualitative TLC technique [15], Standard aflatoxin was applied in volumes of 2.5 µL, 5 µL, 7.5 µL and 10 µL to the surface of a pre-coated TLC plate. The test sample was similarly put and Dry the spots, then develop the chromatogram until the solvent front has moved at least 15 cm from the origin in an unsaturated chamber using a solvent system composed of a chloroform, acetone, and isopropyl alcohol (85: 10: 5) mixture. Mark the solvent off, then take the plate out of the developing chamber and let it air dry. Examine the plate under 365 nm UV light to find the spots.

Pesticide residue Analysis

Presence of pesticide residues in particular to organophosphates, carbamates and organochlorides were quantified using GCMS technique by Acetone and Toulene extraction method[16].

Test for specific Pathogen and Microbial contamination (Sterility)

The test sample EC was dissolved in saline and inoculated using the pour plate method into the appropriate pathogen medium (EMB, DCC, Mannitol, Cetrimide). For observation, the plates were incubated at 37°C for 24–72 hours. The presence of various diseases is shown by their distinctive colour in relation to the colony formation pattern in each differentiating medium.

Heavy metals analysis

Analysis of Heavy metals Mercury, Arsenic, Lead, Cadmium were carried out as per the test method of PLIM guideline[17].

RESULT ANALYSIS:

Results of Powder microscopy analysis

Powder microscopic evaluation of the sample EC tested for the genunity on the plant with respect to its morphological structure on leaf, stem and roots. The results were pictorially represented in figure 1 – 47.

Results of Physiochemical Evaluation of EC

According to the results of the physicochemical analysis of EC, the loss on drying value at 105oC was determined to be 3.63%. Similarly, total ash value of was found to 6.22 % w/w in which acid insoluble ash value is 0.36% w/w and acid insoluble ash was found to be 0.225%

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w/w respectively. Extractive value is an index of total soluble material that impart the genunity of the raw drug. The results of water soluble extractive value of EC was 18.45% w/w and the alcohol soluble extractive was found to be 5.34%w/w. The findings were summarised in Table 1.

Results of Preliminary Phytochemical analysis

Phytochemicals are the real active bio components responsible for expected pharmacological action and also for exerting/mediating the enzymatic reaction. Following the results of the phytochemical evaluation of EC, there are phytocomponents such as alkaloids, proteins, sterols, carbohydrates, and tannins detected. Table 2 shows the results.

Result interpretation of TLC/HPTLC analysis

The results of HPTLC analysis of the sample EC reveals the presence of 09 prominent peaks corresponds to 14 different compounds' with Rf value ranging from 0.07 to 0.96 with percentage area of 2.17 to 38.02%. The results were tabulated in Table 3 and illustrated in Figure 49 and 50.

Results of Aflatoxins Assay (B1, B2,G1,G2)

The results shown that there was no spots were been identified in the test sample EC loaded on TLC plates when compare to the standard , which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2 as represented in the table 4.

Pesticide residue Analysis

The results of pesticide residue analysis of the sample EC showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus and pyrethroids in the sample. The results were tabulated in Table 5.

Test for specific Pathogen and Microbial contamination (Sterility)

Result analysis of specific pathogen test reveals no growth after incubation period. Indicates absence of specific pathogen. Further the sterility analysis report suggested that the total bacterial and fungal count of the sample was found to be $< 10^3$ CFU's. The results were tabulated in Table 6.

Quantification of Heavy metals analysis

Impacts of the current investigation have clearly shown that the sample EC has traces of heavy metals Mercury, Arsenic and Cadmium. Further the results show the presence of lead at 0.57 ppm whose level is below the prescribed limit. The results were tabulated in Table 7.

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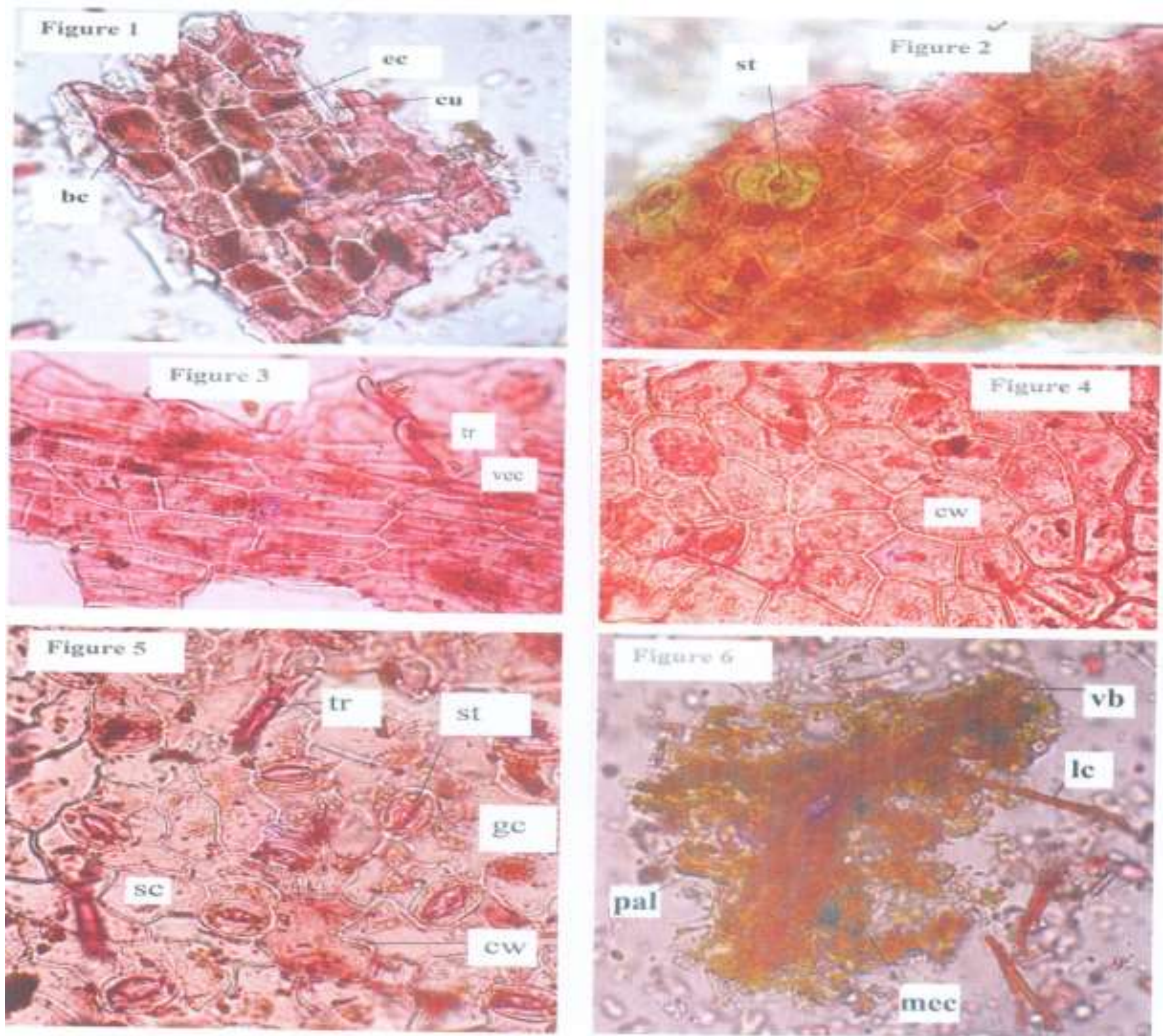
Pictograms of Powder microscopy of the Sample EC



Powder Microscopy of *Aristolachia indica* L.

Figure 1: Stem epidermal cells in surface view embedded with brownish content, covered with thick cuticle.

Figure 2: Stem epidermal cells in surface view embedded with stomata

Figure 3: Leaf midrib epidermal cells in surface view attached with trichome.

Figure 4: Leaf upper epidermal cells in surface view covered with cuticle.

Figure 5: Fragment of leaf lower epidermal cells in surface embedded with anamocytic stomata

Figure 6: Fragment of leaf vascular bundle associated with palisade parenchyma and mesophyll cells with chlorophyll

Figure 1 – 6 Abbreviation: **ee**, epidermal cells; **cu**, cuticle; **be**, brownish content; **st**, stomata; **cw**, cell wall; **tr**, trichome; **vee**, vein epidermal cells; **gc**, guard cell; **sc**, subsidiary cells; **pal**, palisade; **mec**, mesophyll cells; **vb**, vascular bundle; **lc**, laticiferous canals.

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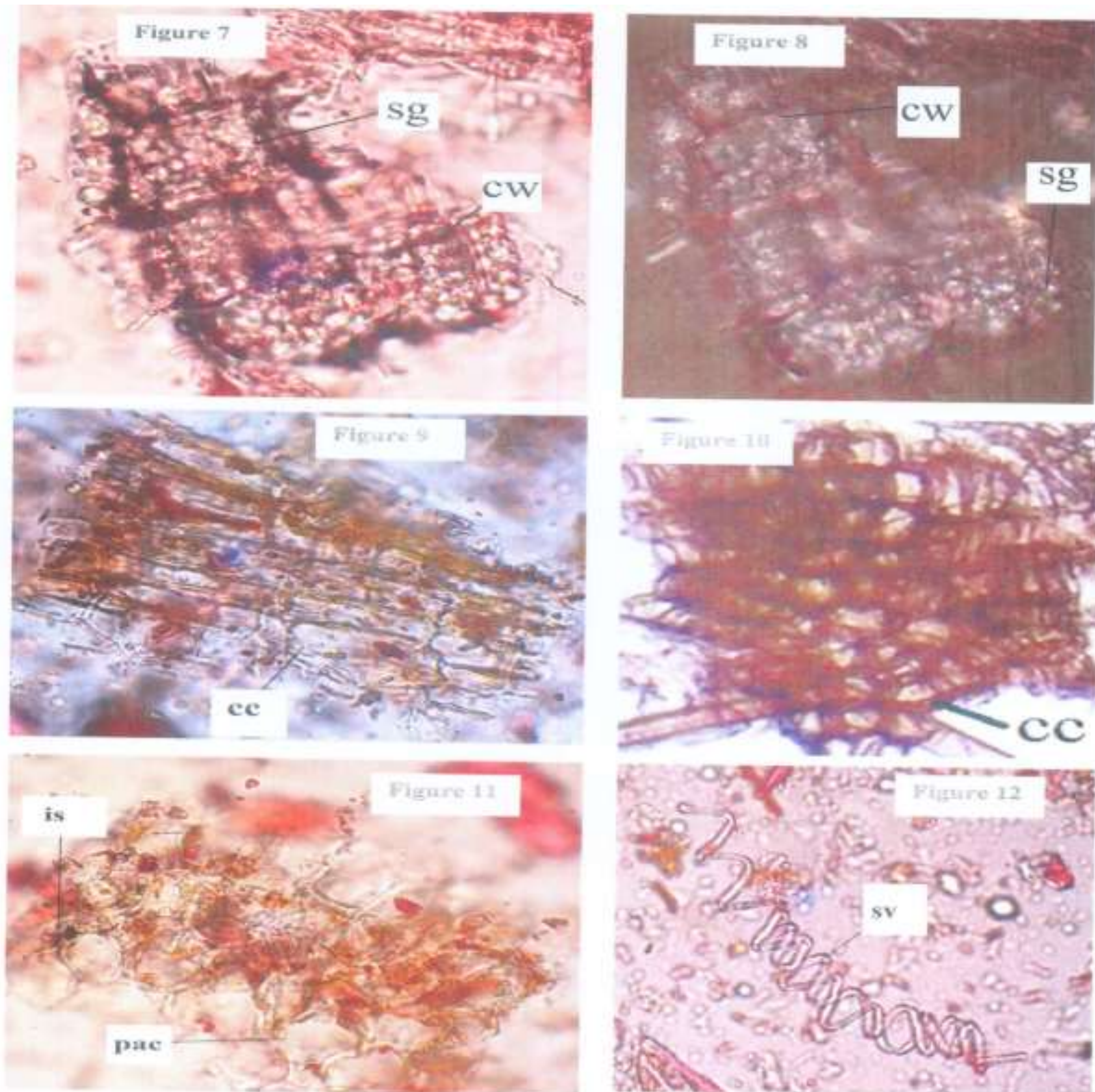

Powder Microscopy of *Aristolochia indica* L.

Figure 7: Fragment of parenchymatous cells embedded with starch grains.

Figure 8: Fragment of parenchymatous cells embedded with starch grains under polarizer light.

Figure 9: Fragment of root cork cells in sectional view

Figure 10: Fragment of root cork cells in surface view

Figure 11: Fragment of parenchymatous cells with inter cellular space

Figure 12: Spiral vessel.

Figure 7 – 12 Abbreviation: cc, cork cells; **sg**, starch grain; st. stomata; cw, cell wall; **is**, inter cellular space; **pac**, parenchymatous cell; sv, spiral vessel.

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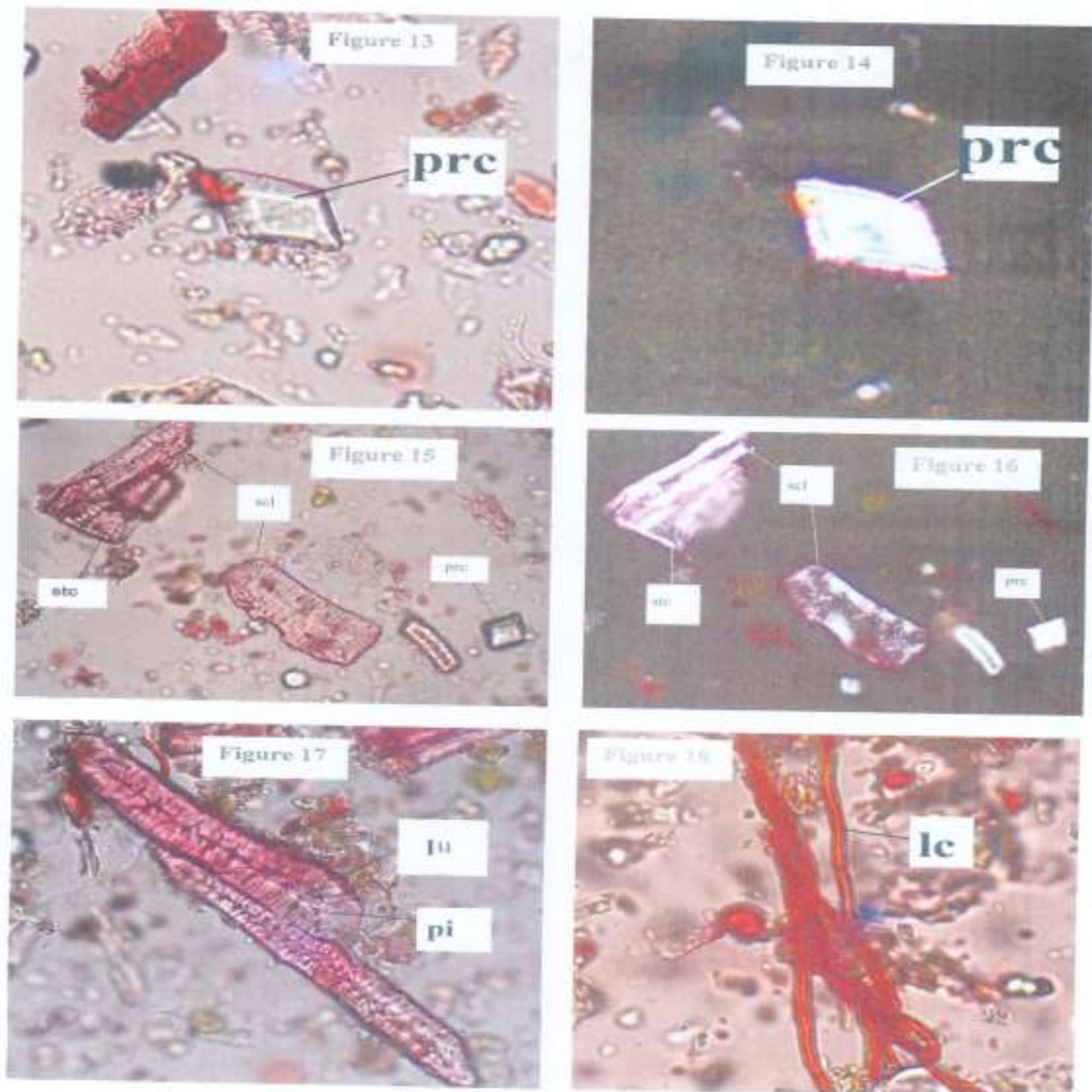

Powder Microscopy of *Aristolochia indica* L.

Figure 13: Prismatic crystals of calcium oxalate

Figure 14: Prismatic crystals of calcium oxalate seen under polarizer light

Figure 15: Stone cells, scleraids and prismatic crystals

Figure 16: Stone cells, sclereids and prismatic crystals seen under polarizer light

Figure 17: Scleraids

Figure 18: Laticiferous canals

Figure 13 – 18 Abbreviation: prc, prismatic crystals of calcium oxalate; **scl**, sclereidal cell; **stc**, stone cell; **lc**, laticiferous canals; pi, pits; lu, lumen.

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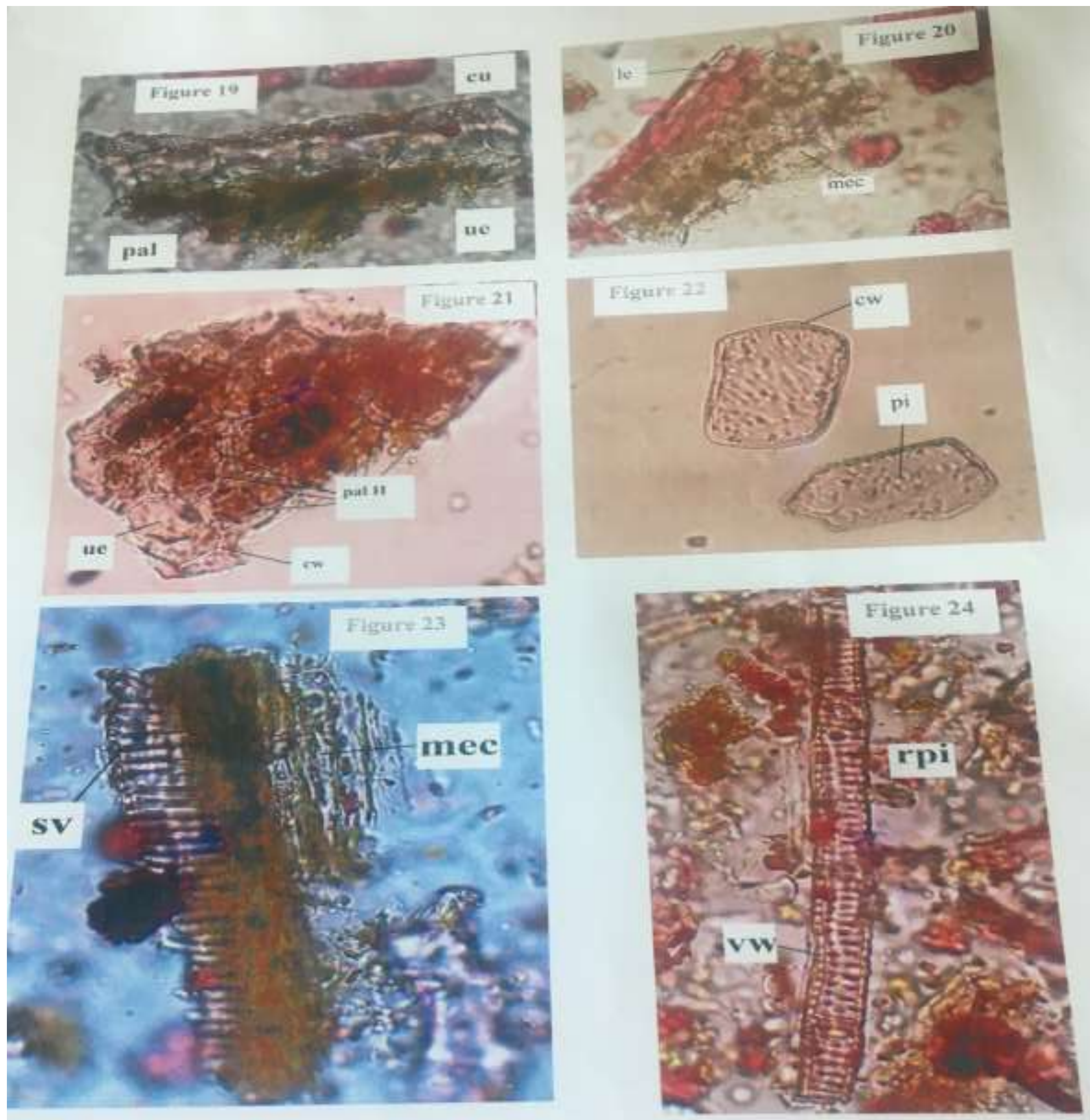

Figure 19: Transversely cut fragment of lamina with a layer of palisade underneath the upper epidermis, covered with cuticle

Figure 20: Transversely cut fragment of lamina with mesophyll cells

Figure 21: Fragment of upper epidermal cells in surface view underneath palisade cells

Figure 22: Pitted parenchyma cells

Figure 23: Fragment of fibre associated with spiral vessel and mesophyll cells

Figure 24: Reticulated vessels

Figure 19 – 24 Abbreviation: pal, palisade; **pal H**, palisade head; **ec**, epidermal cell; **cu**, cuticle; **mec**, mesophyll cell; **ue**, upper epidermis, **le**, lower epidermis; **sv**, spiral vessels; **cw**, cell wall; **vw**, vessel wall; **rpi**, reticulated pits; **pi**, pits.

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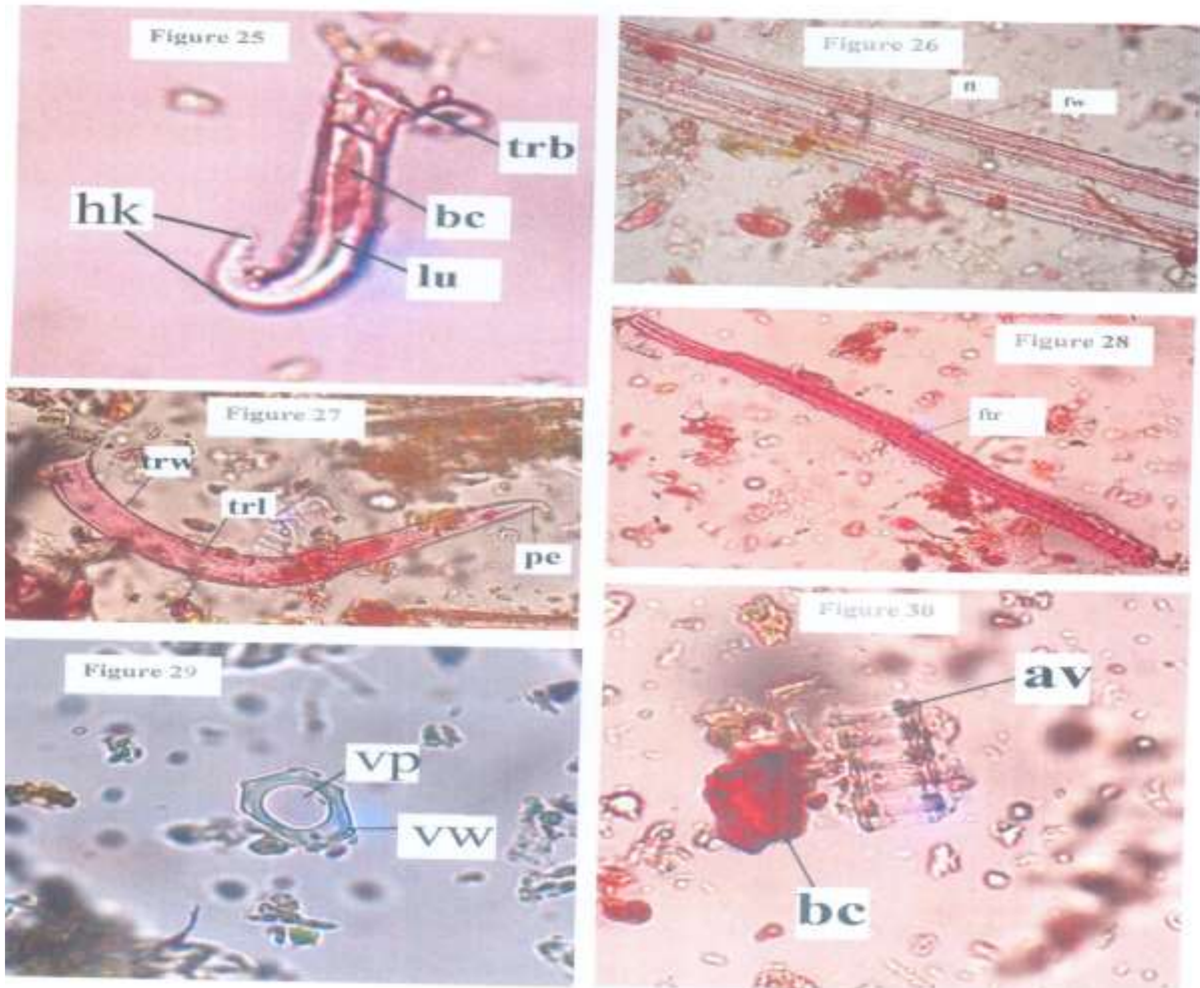

Figure 25: Fragment of hooked trichome with sharp end

Figure 26: Tibres with thin walled and wide lumen.

Figure 27: Trichome with pointed end

Figure 28: Fibre tracheids

Figure 29 &30: Annular vessels

Figure 25 – 30 Abbreviation: **trw**, trichome wall; **trl**, trichome lumen; **trb**, trichome base; **lu**, lumen; **hk**, hooked end; **fl**,fibre lumen; **fw**, fibre wall; **pe**, pointed end; **ftr**, fibre tracheid; **vp**, vessel perforation, **av**, annular vessel; **vw**, vessel wall; **bc**, brownish content.

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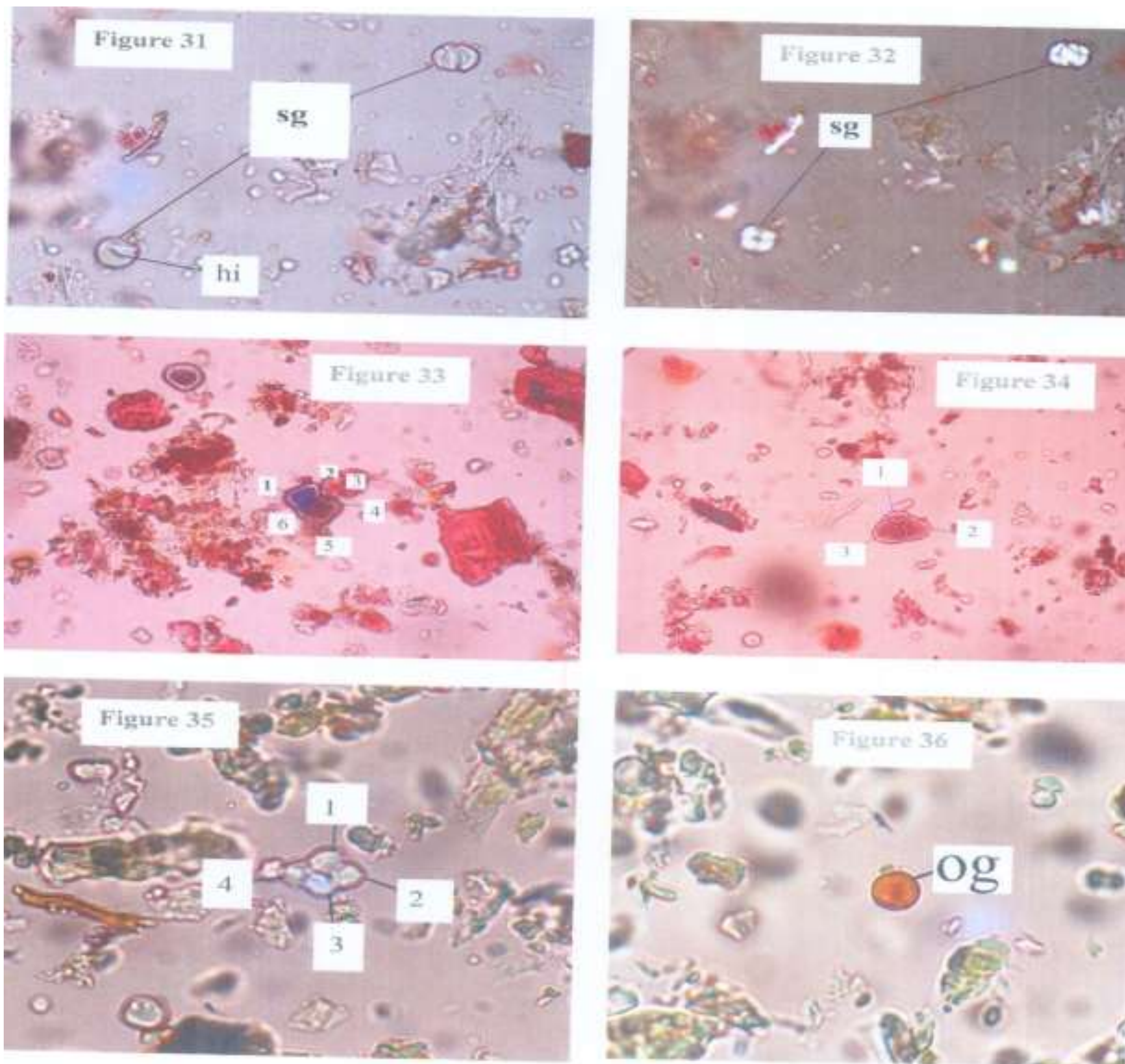

Figure 31: Component Starch grains having simple and bi-component

Figure 32: Component Starch grains having simple and bi-component under polarizer light.

Figure 33: Component Starch grains having six component

Figure 34: Component Starch grains having three component

Figure 35: Component Starch grains having four component

Figure 36: Oil globule

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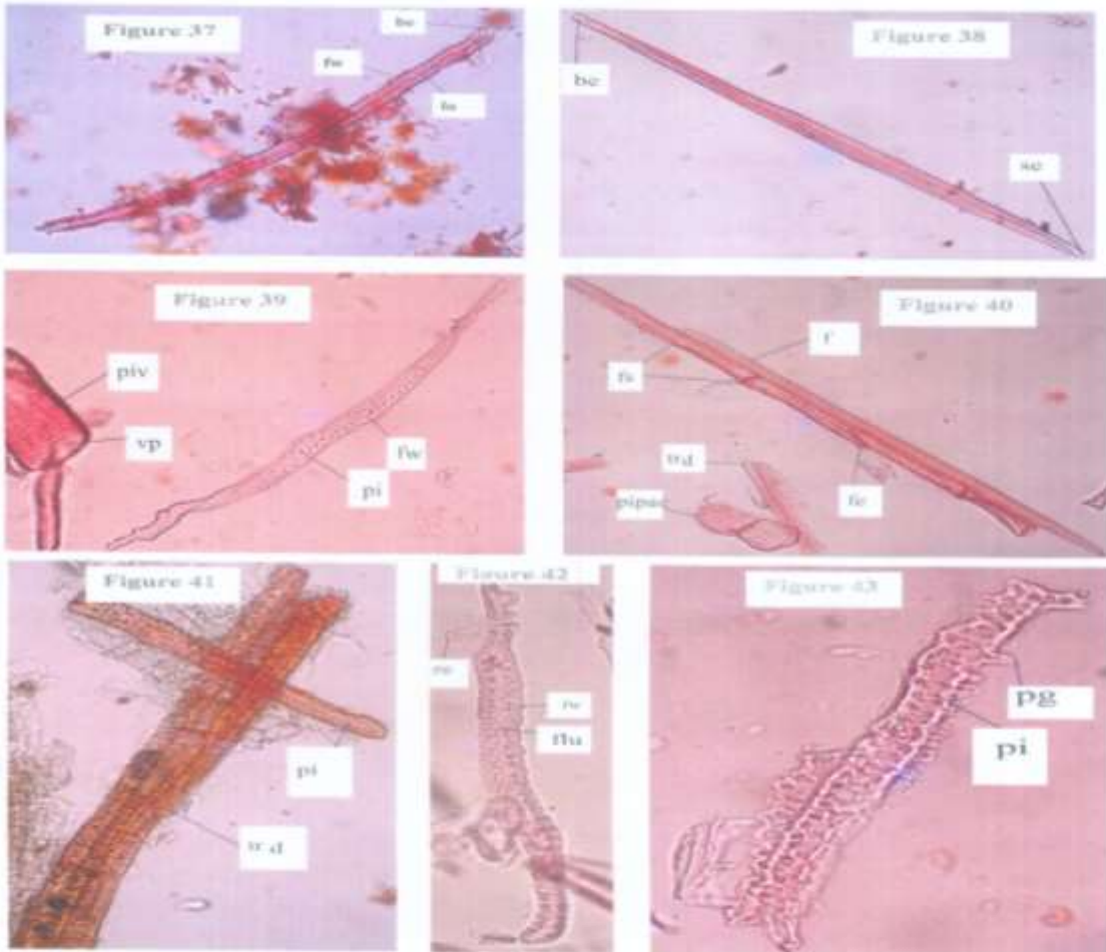

Figure 37 &38: Fibre differing morphology

Figure 39: Fibre tracheid with pits and pitted vessels

Figure 40: Septet fibre

Figure 41: Tracheids

Figure 42: Fibre sclereids

Figure 43: Tracheid

Figure 25 – 30 Abbreviation: piv, pitted vessel; **trd**, tracheid; be, blunt end; **lu,** lumen; **se,** sharp end; **fl**, fibre lumen; fw, fibre wall; pe, pointed end; ftr, fibre tracheid; vp, vessel perforation; **pi**, pits; **fe**, fibre end; **pipac**, pitted parenchyma; f, fibre, **fs**, fibre septum; pg, pegged out growth.

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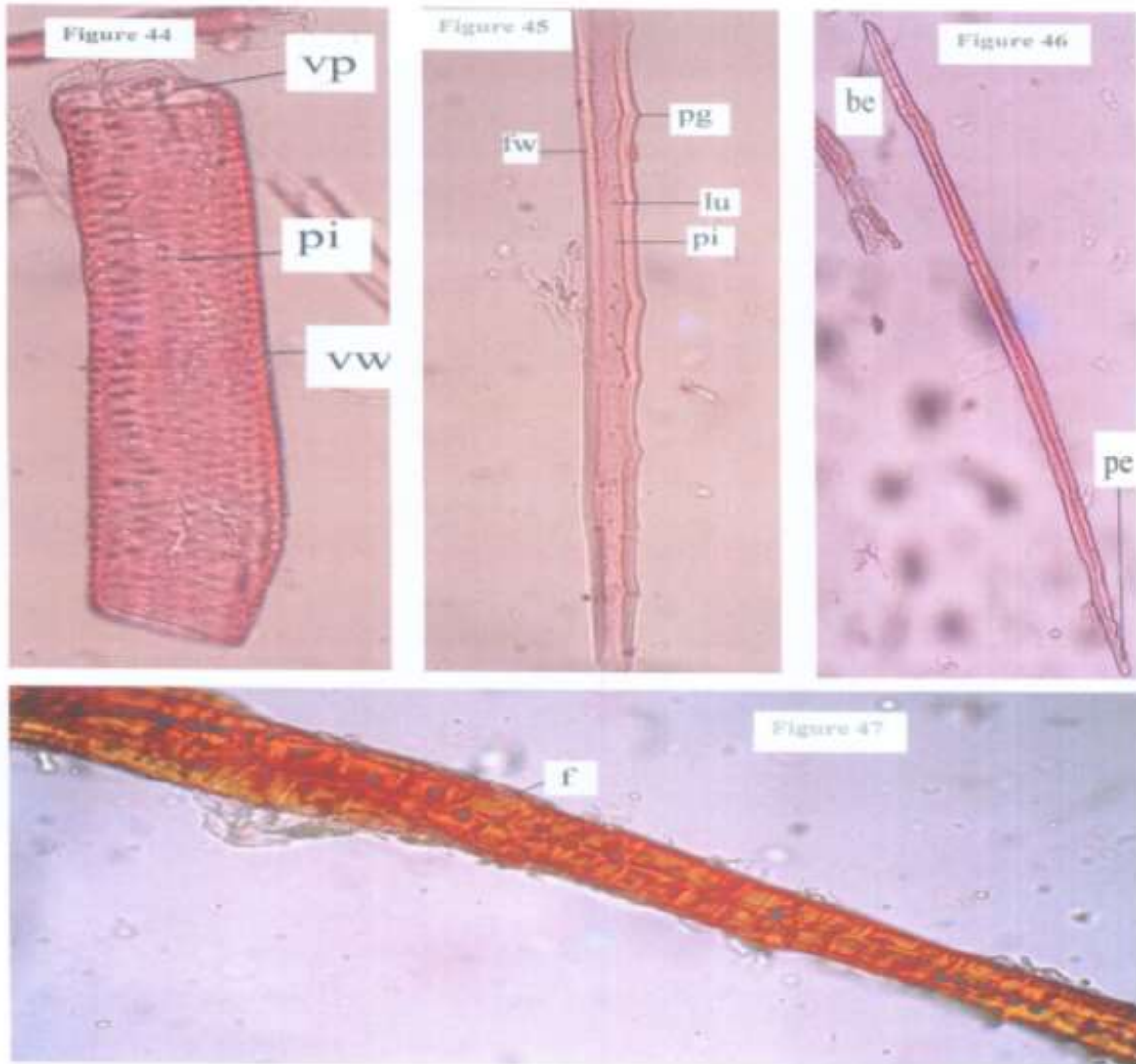

Figure 44: Pitted vessels with simple perforation

Figure 45: Fibre enlarged view shows pegged out growth

Figure 46: Thin walled fibres show blunt end and pointed end

Figure 47: Thick walled fibre

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Powder Microscopy of *Aristolochia indica* L. (Whole plant)

Aristolochia indica L. habit

Aristolochia indica whole plant powder

Figure 48: Pictorial representation of Whole plant and powdered *Eachuramooli*

Table 1: Physicochemical Evaluation of the sample EC

S.No	Parameter	Mean
1.	Loss on drying at 105^0C	3.63 (%)
2.	Total Ash	6.22 (%)
3.	Acid insoluble ash	0.36 (%)
4.	Alcohol soluble extractive	5.34
5.	Water soluble extractive	18.45

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Table No 2 : Preliminary qualitative phytochemical Evaluation of the sample EC

Phytochemicals	Observation
1. Alkaloids	+
2. Carbohydrates	+
3. Reducing sugars	-
4.Glycosides	-
5. Cardiac glycosides	-
6. Saponins	-
7. Tannins	+
8. Phenols	-
9. Phytosterols	+
`10. Diterpenes	-
11. Triterpenes	-
12. Flavanoids	-
13. Proteins and amino acid	+
14. Quinones	-

+ Presence and - Absence

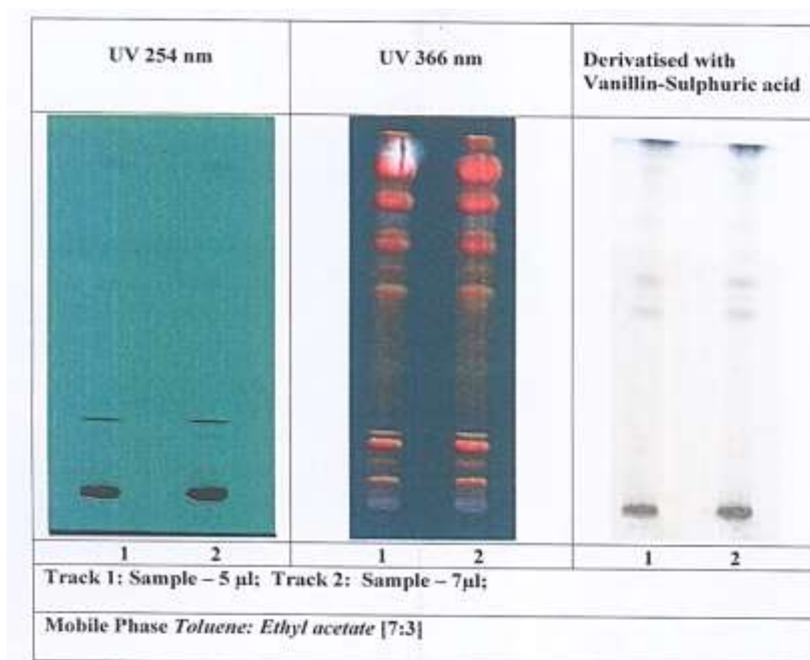

Figure 49: TLC finger printing of Sample EC

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Table No 3: Results of Peak Table of HPTLC analysis of EC

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.3 AU	0.09 Rf	13.8 AU	2.26 %	0.11 Rf	0.9 AU	176.5 AU	2.17 %
2	0.17 Rf	0.6 AU	0.19 Rf	33.6 AU	5.53 %	0.21 Rf	1.2 AU	457.6 AU	5.64 %
3	0.22 Rf	1.3 AU	0.24 Rf	382.9 AU	62.92 %	0.26 Rf	0.3 AU	3086.0 AU	38.02 %
4	0.44 Rf	5.7 AU	0.47 Rf	21.1 AU	3.46 %	0.51 Rf	1.8 AU	515.6 AU	6.35 %
5	0.66 Rf	8.6 AU	0.70 Rf	59.7 AU	9.81 %	0.74 Rf	7.0 AU	1438.1 AU	17.72 %
6	0.74 Rf	7.1 AU	0.77 Rf	19.3 AU	3.18 %	0.80 Rf	11.9 AU	534.1 AU	6.58 %
7	0.81 Rf	12.3 AU	0.84 Rf	21.6 AU	3.54 %	0.88 Rf	1.6 AU	621.3 AU	7.65 %
8	0.90 Rf	0.1 AU	0.93 Rf	21.7 AU	3.57 %	0.96 Rf	0.7 AU	464.8 AU	5.73 %
9	0.96 Rf	1.1 AU	0.99 Rf	34.9 AU	5.73 %	1.03 Rf	5.9 AU	823.4 AU	10.14 %

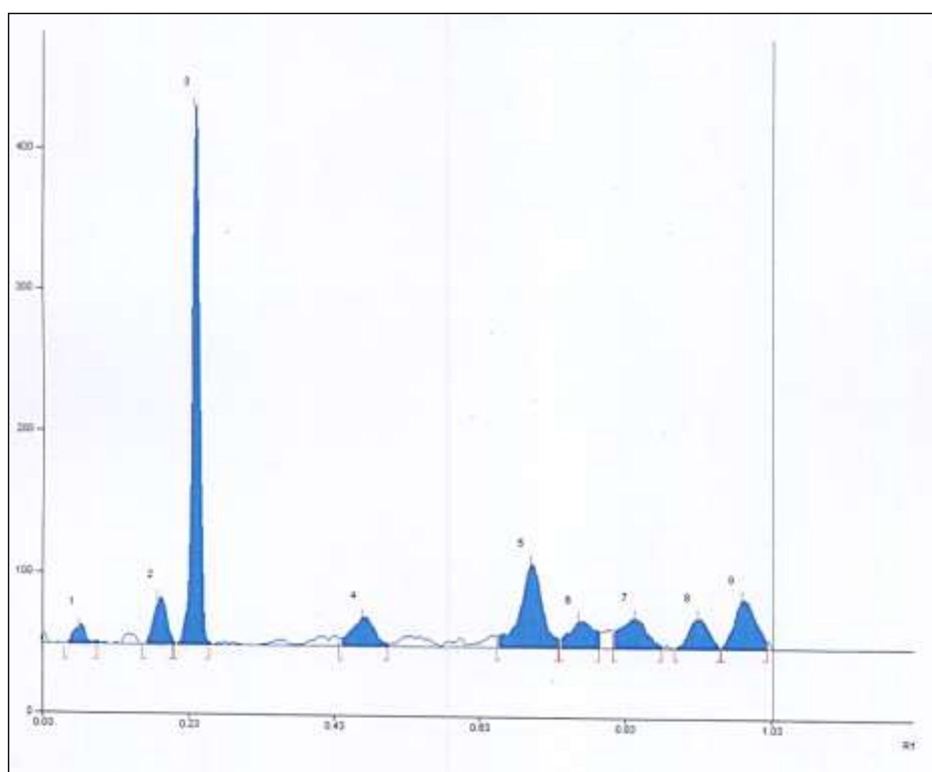

Figure 50: HPTLC finger printing of Sample EC

Table 4: Aflatoxin assay of the sample EC

Aflatoxin	Sample MRK	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm
B2	Not Detected - Absent	0.1 ppm
G1	Not Detected - Absent	0.5 ppm
G2	Not Detected - Absent	0.1 ppm

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Table 5 : Test Result Analysis of the Sample EC

Pesticide Residue		**AYUSH Limit (mg/kg)**
I.Organo Chlorine Pesticides	**Sample EC**	
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

Table 6: Test for specific Pathogen analysis of the sample EC

Organism	**Specification**	**Result**	**Method**
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	

Table 7: Heavy metal Analysis of the sample EC

Name of the Heavy Metal	**Result Analysis**	**Maximum Limit**
Mercury	BDL	1 ppm
Lead	0.57 ppm	10 ppm
Arsenic	BDL	3 ppm
Cadmium	BDL	0.3 ppm

BDL- Below Detection Limit

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DISCUSSION:

Indian system of traditional medicine like siddha pioneers the art of healing from ancient time as it is systematically documented and well established practice guideline framed by the traditional healers otherwise called as siddhars (ancient physicians). Medicine for therapeutic ailments claims high level of sterility as it is intended for the internal purpose. To ensure the sterility of the formulation the sample were periodically subjected for test for specific pathogens as prescribed by the WHO and AYUSH for traditional formulations.

Botanical values of the herbs offer greater deal of information on the arrangement of the morphological structure that confirms the justification made during the process of authentication. In the present study powder microscopy revealed the correct selection of plant species for the research purpose.

Physiochemical evaluation is a good indicator for projecting the physical and chemical properties of the test drug. Total ash value is the exhaustive limit on revealing the purity of the formulation, further extractive values showcase the quality of the raw drugs. The outcomes acquired from the physicochemical evaluation of EC revels that the loss on drying value at 105°C was fond to be 3.63%. Similarly, total ash value of was found to 6.22 % w/w in which acid insoluble ash value is 0.36% w/w and acid insoluble ash was found to be 0.225% w/w respectively. Extractive value is an index of total soluble material that impart the genunity of the raw drug. The results of water soluble extractive value of EC were found to be18.45% w/w and the alcohol soluble extractive was found to be 5.34%w/w.

Alkaloids, flavonoids, glycoside and phenols are the major metabolites[18] who are involved in the receptor interaction since these functional group wither has hydroxyl / quaternary ammonium side chain that can easily form bonds with the surface amino acids on the receptors. Interaction of these molecules in the biological sites have brings about expected pharmacological action, when multiple therapeutics came into play the chance of synergism maybe possible. The results obtained from the phytochemical analysis of EC reveals the presence of phytocomponents such as alkaloids, tannins, carbohydrates, sterols and proteins.

Instrumental methods like HPTLC is a age old analytical techniques still works reliable in the field of photochemistry. Basic fingerprinting analysis is a primary tool for enumerating the phyto components present in the formulation, further selection of mobile phase seems high important as the separation compounds on the TLC plate depends on the polarity of the components with respect to that of the separation medium[19]. The HPTLC analysis of the sample EC reveals the presence of 09 prominent peaks corresponding to 14 different compounds' with Rf values ranging from 0.07 to 0.96 with a percentage area of 2.17 to 38.02%. Aflatoxin are categorized as carcinogens. Some toxins have tendency to causes potential health hazards[20]. Majority of the cases toxins are derived from the microbes under suitable condition. Plant being rich in nutritive values are highly prone for aflatoxin contamination, hence it become mandate for herbal preparations to confirm the absence of

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aflatoxin. In the cycle of infection, the source of livestock feed contamination is the primary one.

Primary organ targeted by aflatoxins are liver, kidney and brain. The clinical symptoms include fevers, vomiting, body pain, immunosuppression etc. The results showed no spots in the test sample EC loaded on TLC plates compared to the standard, indicating that the samples were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2. Heavy metals can cause traumatic incidence even at traces. Prolong consumption of drugs contaminated with metals have been lodged in to the vital organs like liver, kidney and in rare case at brain. Outcomes of the present examination have shown that the sample EC has glimmers of heavy metals Mercury, Arsenic and Cadmium. Additionally, the results show the fact of lead at 0.57 ppm, whose level is below the specified limit. Pesticide from the agricultural land absorbed by soil root mode. Pesticide causes serious adverse such as ganglionic blockage, GIT irritation, hepatic disorders and also collapse the metabolic pathway. Pesticides have cause higher level of impact on the nervous system which will readily dissociate the lipoidal membrane and also alters the ion mediated impulse conduction and propagation[21]. The sample EC's pesticide residue study showed no traces of pesticide residues such as Organochlorine, Organo phosphorus and pyrethroids in the sample.

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