

PHARMACOGNOSTIC EVALUATION OF *CURCUMA NEILGHERRENSIS* WT.

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ABSTRACT

Objectives: *Curcuma* species are the important Indian spice ingredients of food items and in traditional uses very much important component on all auspicious occasions. Due to its high utilization since ages *Curcuma longa* has been domesticated very much in India and also earning high income through exports. For the past few decades all herbal medicines have been characterized pharmacognostically for their validation. Hence *Curcuma* species also having many medicinal values needs scientific evaluation to prove its purity to minimize adulteration of drugs.

C. neilgherrensis is a wild *Curcuma* species of Eastern Ghats distributed along Seshachalam Hill ranges at higher altitudes known as "adavi Pasupu" by the local herbalists and the tribes. Rhizomes and leaves are used to cure skin disorders, cold and cough, ulcers, bone fractures and to reduce cholesterol levels; also possesses many phytoconstituents with effective microbial activities. Hence the powders of crude drug are subjected for their physico- chemical evaluations in terms of ash values, moisture content, extractive values and histochemical studies.

Methods: All physico chemical studies were carried out by the standard methods of Anonymous, Khandelwal, Trease, Kokate and Johanson, and the results were given in tables and figures.

Results: Rhizomes branched and brown with fusiform elongated tuberous, white roots with slightly aromatic odour and bitter in taste; stem with 6-9 leaves covered with trichomes; inflorescence cylindrical stout panicle with 15-20 big flowers surrounded by coma of bracts and bracteoles pink, petals yellow, labellum violet. Leaf with cuticle and paracytic stomata on both surfaces. Rhizome tissues with starch grains and oil glands. The major phytoconstituents are lignins, calcium oxalate crystals, alkaloids, tannins and saponins. Powder drug with trichomes Paracytic stomata, scalariform tracheids, spiral and pitted vessels. Fluorescence studies with significant colors in natural light greenish yellow, short wave light reddish yellow to brownish green in UV light reddish black.

Conclusion: *C. neilgherrensis* is close with *C. amada* in total ash content and in cold water extracts, but differs with *C. caesia*, *C. haritha* and *Z. zerumbet* with low water soluble ash and high acid insoluble ash. Moisture content is very high than *C. caesia*. Hence every herbal drug has to be characterized for their pharmacognostic studies in quality, quantity and adulteration check.

Keywords: Adulteration, Physico-chemical, Histochemical, Ash Values, Moisture Content, Extractive Values.

INTRODUCTION

Zingiberaceae is the largest family of Zingiberales comprises nearly 50 genera and 1000 species with a large group of rhizomatous and aromatic plants characterized by the presence of volatile oils and oleoresins. Members of this family comprises spices, dyes, perfumes, medicines and a number of ornamental species. Medicinal species have acquired great importance in present-day with its anti aging, anticancer, anti- alzheimers, anti oxidant and a variety of other therapeutic medicinal properties. *Curcuma neilgherrensis* (Adavi Pasupu) is one such wild endemic medicinal plant from Araku valley and also from Tirumala and Talakona of Seshachalam Hill Ranges along the Eastern Ghats.

According to the traditional data from the local herbalists, and from Yanadi tribes rhizomes were used to treat cuts, boils, wounds, skin diseases, pimples, bone fractures, common cold and ulcers. It is also used in their common diet to control cholesterol levels. Rhizome and leaf yielded a good number of secondary metabolites like alkaloids, flavonoids, phenols, steroids, glycosides, tannins, indols, carbohydrates, proteins and amino acids mainly with aqueous, alcohol and methanol extracts [1].

MATERIALS AND METHODS

Plant Material Collection

C. neilgherrensis (Zingiberaceae) plant material was collected from Tirumala and Talakona during the months of April – September, 2011, and authenticated by Prof. N.Yasodamma the voucher specimens DC 921, DC 922 were prepared and preserved in the herbarium (SVUTY) Department of Botany, S.V.University, Tirupati as per the standard method [2].

Macroscopic - Morphological studies

The characters like colour, odour, taste, size and shape of leaf, scape, rhizome, roots and flowers were noted.

Microscopic - Anatomical studies

Cross sections and photography was taken and observed the distinguished characters of the tissue systems of the leaf, scape, rhizome and root using digital microscope attached with computer system (Olympus Mic -D). Epidermal membranous layers (in fragments) were cleaned with chloral hydrate, mounted and observed under microscope.

Histochemistry

Hand sections of fresh leaf, stem, fruit and root were stained with a series of histochemical reagents. a) Safranin (1% safranin in 50% alcohol); lignins b) Iodine solution; cellulose c) Ruthenium red; mucilage d) Iodine; starch e) Wagner's reagent; alkaloids e) Dilute FeCl₃ solution; tannins f) Millon's reagent; proteins g) conc. H₂SO₄; saponins h) Sudan-III; fat/oil globules i) 20% aq.NaOH; sugars j) conc. HCl; calcium oxalate crystals k) heating ; strong KOH; sulphuric acid [3].

Powder drug microscopy

All powders were evaluated for microscopic structures, each of them were separately stained with the reagents such as phloroglucinol 1% and conc. HCl (lignified structures), H₂SO₄ (350g/L) (calcium oxalate crystals), iodine solution (starch granules), sudan red G (cuticular cell walls) and sudan red G in acetic acid and ethanol (essential oils, resins, fats and fatty oils) were used on bleached powders [4-5]. All samples were observed under Microscope.

Physico-chemical analysis

For the determination of ash values, leaf, scape, rhizome and flower powders were sifted through sieve no. 20 and the following tests were performed as per the methods of Anonymous, Khandelwal, Evans and Trease. [6-8]

Total ash

About 3 g of each powder is accurately weighed and taken separately in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

Acid insoluble ash

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Water soluble ash

The ash obtained as described for the total ash, was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min. and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried parts respectively.

Sulfated ash

A silica crucible was heated to red for 10 min. and was allowed to cool in desiccators and weighed. A gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml of concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at $800^{\circ}\text{C} \pm 25^{\circ}\text{C}$ until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool. A few drops of concentrated sulfuric acid were added and heated ignited as before and was allowed to cool and weighed.

Swelling index

It is defined as the volume in milliliters occupied by 1g of a drug. The drug is treated with 1.0 ml ethanol (96%) and 25 ml water in a graduated cylinder, shaken every 10 minutes for 1 h and allowed to stand. The drugs have mucilage (swell after absorbing plenty of water) as a Phyto-constituent may have different swelling index and therefore, provide the useful information.

Moisture content: Air dried material of 10g was dried in an oven at 105°C . The loss of weight was calculated and values were tabulated.

Foreign matter

100g of the powdered drug is taken and spread out in a thin layer on a slide and observed free from foreign matters like soil, insect parts

or animal excreta. They are separated and weighed and the percentage was calculated.

Extractive values

Coarsely powdered air-dried material 20 g was placed in a glass stopper conical flask with 200 ml of solvents shaking frequently, and then allowing it to stand for 18 hours. Filter it rapidly through Whatmann No. 1 filter paper, taking care not to lose any solvent. Transfer 25 ml filtrate to flat-bottom dish and evaporate it on a water bath. Dry at 105°C for 6 hours, cool in a desiccators for 30 minutes and weigh it immediately. Calculate the content of extractable matter in% of air-dried material by the standard method from the book Kokate. [9]

Fluorescence Analysis

A small quantity of dried and finely powdered leaf, scape, rhizomes, roots and flowers were placed on a grease free clean microscopic slide and added 1-2 drops of Conc. Sulphuric acid, 50% Sulphuric acid, Conc. Hydrochloric acid, 50% Hydrochloric acid, Conc. Nitric acid, 50% Nitric acid, 10% Sodium hydroxide, 5% Ferric chloride, 5% Potassium hydroxide, Water and Acetic acid, gently tilting the slide and waited for 1-2 min. Then the slide was placed inside the Ultra Violet viewer chamber and viewed in Day light, Short (245nm) and long (360nm) Ultra Violet radiation. The colors observed by application of different reagents in different radiations were recorded as per the method from the book Kokate. [9]

RESULTS

Macroscopic - Morphological studies: [Plate I]

Rhizome small, 3×1 cm, conical brownish; roots many, some ending in root tubers, 3.5×0.7 cm, fusiform, white inside. Leafy shoot 20-30 cm tall, pseudo stem 8-19 cm tall. Leaves 6-9, distichous; petiole 10-15 cm, green; lamina $12-18 \times 6-9$ cm, ovate-elliptic to obovate, base sub equal, tip acute, lower surface sparsely pubescent, ligule small, 5 mm long, hyaline. Inflorescence both lateral and axial central, stout panicle, surrounded by 15-20 flowers, $8-20 \times 5-6$ cm, with a distinct-coma, coma bracts 5-7, oblong-lanceolate, fused at the base, light to dark pink turn to violet. Fertile bracts 6-15, $2.5-3 \times 1.1-1.5$ cm, fused upto $1/3$, slightly recurved, margin wavy, green later turn to pink with violet spots at the tip, densely pubescent. Bracteoles $2-3 \times 2$ mm, triangular. Flowers longer than the bracts, $4.5-5.5$ cm; bract $3-4$ cm; light yellow.

Calyx $1-1.2$ cm, 3 lobed at apex, violet dotted, densely pubescent. Corolla tube light yellow, lobes unequal, pubescent; dorsal lobe 1.8 cm long, hooded at tip, hood 2 mm long; laterals, 1.7×0.7 cm, linear-elliptic, outer surface sparsely hairy. Labellum 2×2 cm, with a deep yellow median band. Lateral staminodes 2.2×1.3 cm, yellow, glabrous, anther-lobes 5 mm long, parallel, hooded, glandular hairy on back surfaces, spurred; spurs 2 mm long, downwardly pointing. Epigynous glands two, 3 mm long, linear. Ovary 4 mm long, trilobular with many ovules, on apparently axial placenta, style long, filiform; stigma bilipped, appressed within the case. Fruit capsule 1.3×1 cm, obovate, yellowish-green, fruit wall semitransparent, pubescent, with persistent calyx. Seeds $3.5-5$ mm long, obovate; aril white, lacerate.

Plate I: Macroscopic - morphological studies

INFLORESCENCE



LEAF WITH SCAPE



RHIZOMES WITH ROOTS



Table I: Macroscopic characters

| S. No. | Character | Leaf | Scape | Rhizome | Root | Flower |
|--------|-----------|-----------------|-------------------|--------------------------|-------------------|--------------------|
| 1 | Color | Thick Green | Greenish Pink | Thick Brown | Light Brown | Yellow |
| 2 | Shape | Lanceolate | Cylindrical | oblong-palmate Irregular | Long, cylindrical | Funnel |
| 3 | Size | 20-30 cms | 15- 20 cms | 3-4 cms | 8-9 cms | 5-7 cms |
| 4 | Texture | Smooth | Smooth | Rough | Smooth | Smooth |
| 5 | Taste | Slightly bitter | Bitter | Bitter | Bitter | Pungent and Bitter |
| 6 | Odour | Aromatic | Slightly aromatic | Slightly aromatic | Slightly aromatic | Slightly aromatic |

Microscopic Anatomical studies: [Plate II, III, IV& V]

Leaf: Leaf isobilateral and consists of upper and lower identical, single layered, oval – rectangular **epidermal** cells covered with cuticle perforated by *Paracytic* stomata. Trichomes and stomata are present on both sides. **Mesophyll:** **palisade** and **spongy** parenchyma are not demarcated. They are compactly arranged thin walled, isodiametrical, chlorophyllous with **scattered oil cavities** made up of epithelial cells. **Vascular Bundles:** alternating with **oil cavities**; collateral and closed. Xylem is towards upper side and phloem towards lower side surrounded by bundle sheath; **Xylem** consists of vessels and **phloem** with sieve tubes and companion cells. Sclerenchyma cells in patches on both sides of midrib region to give mechanical support of the vascular bundles. **Scape:Epidermis:** single layer, covered by thin cuticle. **Cortex:** parenchymatous cells, vascular bundles covered by an arch of sclerenchyma over the xylem, consists of oil cavities alternating with foliar **Vascular bundles**, both covered by sheathing leaf base.

Rhizome: Single layer **epidermis** covered with thick cuticle, Cortex: 3-4 layers, thick walled collenchymatous, and filled with

starch grains. **Endodermis:** single layer with compact collenchymatous filled with starch grains. **Pericycle:** single layer. **Vascular Tissue:** conjoint and collateral, **xylem** exarch, Protoxylem towards endodermis, metaxylem towards pith. **Phloem** composed of sieve tubes and phloem parenchyma. **Pith:** occupied major portion with parenchymatous filled with starch grains.

Root: single layered **epidermis** covered by cuticle. **Cortex:** large parenchymatous cells **Endodermis:** stele surrounded by endodermis with very thick cells. **Pericycle:** single layered. **Vascular tissue:** conjoint, collateral and radially arranged. **Xylem** consists of vessels and xylem parenchyma. **Phloem** composed of sieve tubes and phloem parenchyma. **Pith:** parenchymatous.

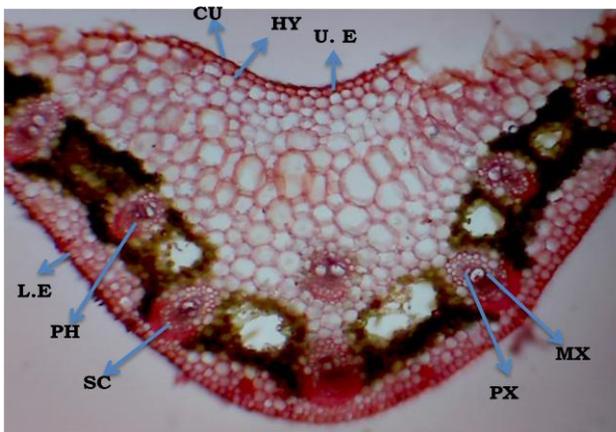
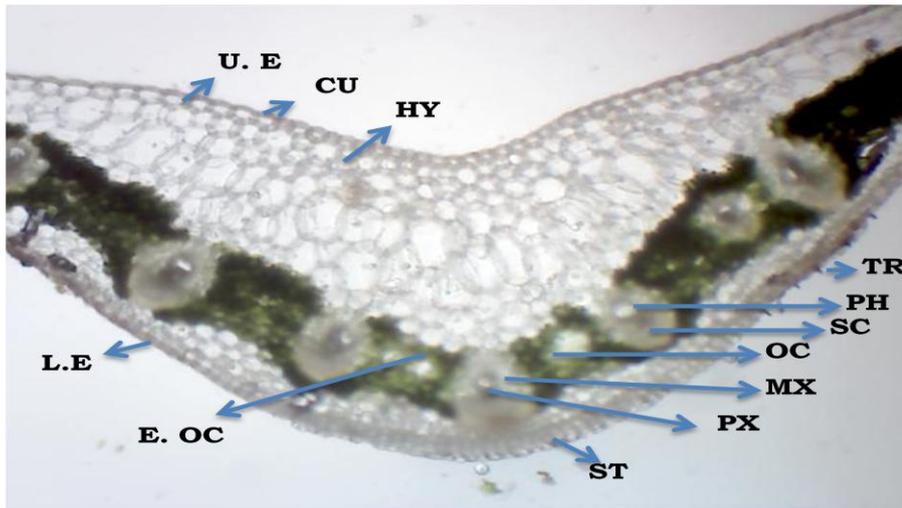
Histochemistry: [TABLE- II]:

Histochemically all parts were tested with various reagents for their accumulation of phytoconstituents in tissue systems. **Lignins** present in leaf and root, **calcium oxalate crystals** only in rhizomes; **alkaloids** absent in leaf, **saponins** absent in root, **starch** absent in leaf, scape and root. **Tannins** and mucilage are present in all parts.

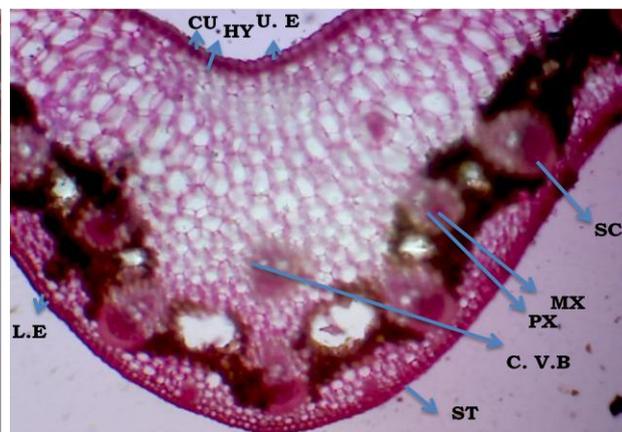
Table II: Histochemistry

| Test for | Reagent used | Nature of change | Leaf | Scape | Rhizome | Root |
|---------------------------------|--|--------------------|--|---|--|---|
| Lignins | Safranin (1%) safranin in 50% alcohol) | Red | Upper Epidermis, Lower Epidermis, Hypodermis, Mesophyll tissue, Vascular Bundle. | - | - | Cortex, Endodermis, Pericycle and Vascular Tissue. |
| Mucilage | Ruthenium Red | Pink | Upper Epidermis, Lower Epidermis, Hypodermis, Mesophyll tissue, Vascular Bundle. | Foliar Vascular Bundles, Sclerenchyma. | Hypodermis, Cortex, Cortex, Endodermis, Pericycle, Pith. | Cortex, Endodermis, Pericycle, Vascular Bundles, Pith |
| Starch | Iodine | Blue | - | - | Cortex, Pith | Cortex, Pith |
| Alkaloids | Wagner's Reagent | Orange | - | Foliar Vascular Bundles, Central Vascular Bundles, Sclerenchyma | Cortex, Pith | Cortex |
| Tannins | Dil. FeCl ₃ solution | Bluish Black | Upper Epidermis, Lower Epidermis, Sclerenchymatous Cells | Cortex, Sheathing Leaf base. | Cortex, Endodermis, Pericycle, Vascular tissue. | Vascular Tissue. |
| Saponins | Conc. H ₂ SO ₄ | Light Yellow | Upper Epidermis, Lower Epidermis. | Foliar Vascular Bundles, Vascular Bundles. | Endodermis, Pericycle, Vascular Tissue. | - |
| Calcium oxalate crystals | Conc. Hcl | Bright effervesces | - | - | Cortex, Pith | - |

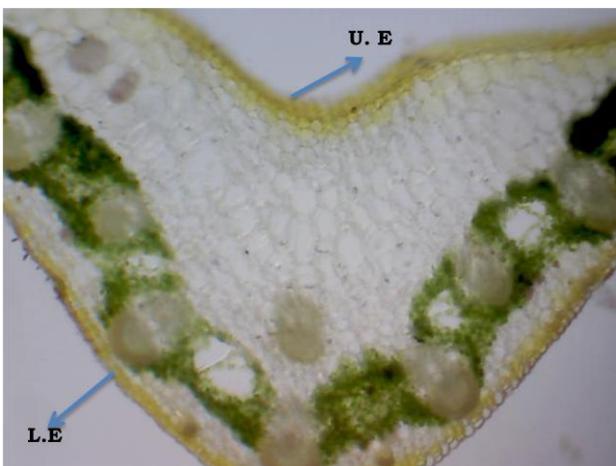
Plate II: Histochemical studies: Leaf



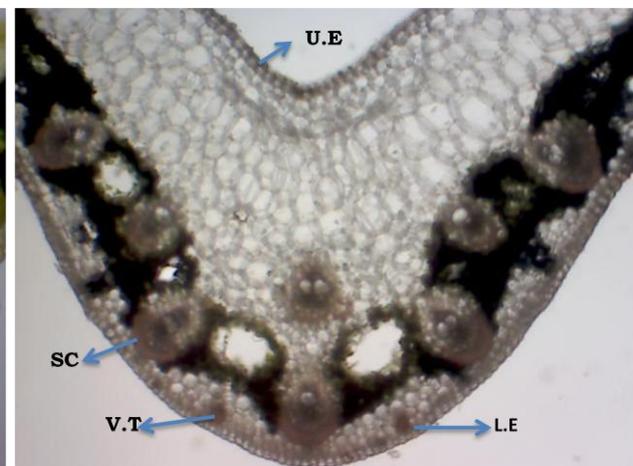
Lignins



Mucilage



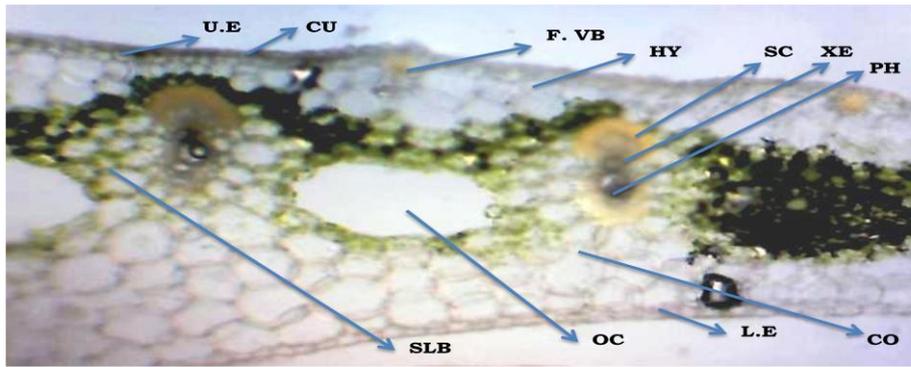
Saponins



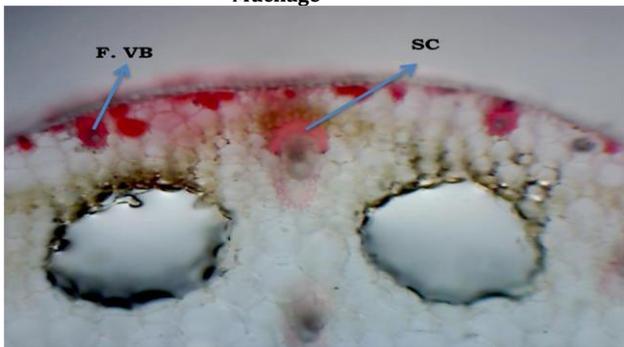
Tannins

U.E: Upper Epidermis; L.E: Lower Epidermis; CU: Cuticle; TR: Trichomes; ST: Stomata; HY: Hypodermis; OC: Oil Cavity; PX: Proto Xylem; MX: Meta Xylem; SC: Sclerenchyma; PH: Phloem; V.T: Vascular Traces; C.V.B: Central Vascular Bundles.

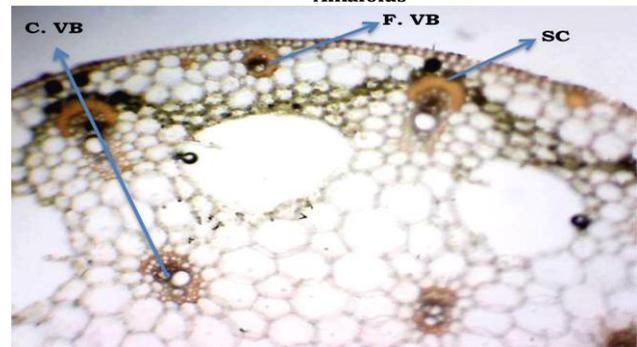
Plate III: Histochemical studies: Scape



Mucilage



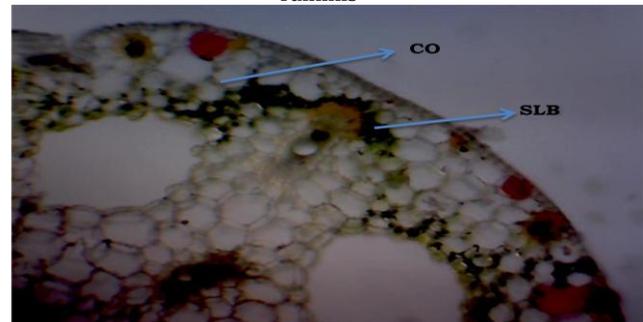
Alkaloids



Saponins

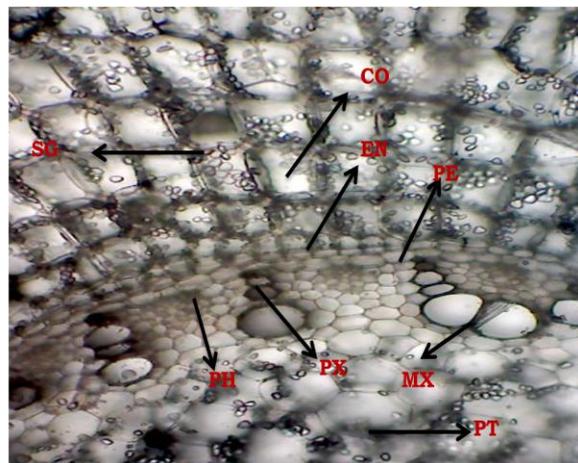
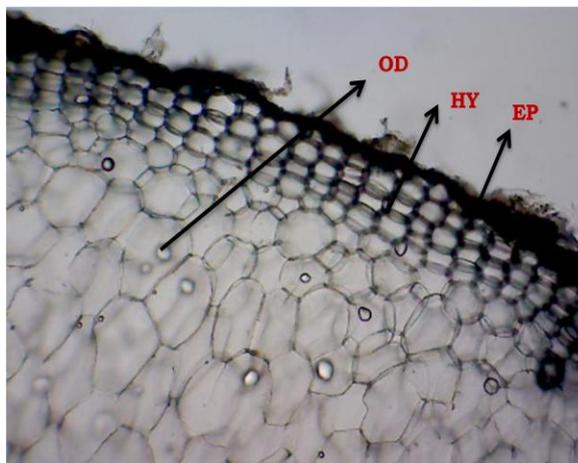


Tannins

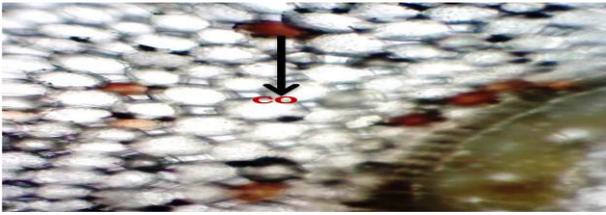


U.E: Upper Epidermis; L.E: Lower Epidermis; CU: Cuticle; HY: Hypodermis; CO: Cortex; SLB: Sheathing Leaf Base; OC: Oil Cavity; XE: Xylem; PH: Phloem; SC: Sclerenchyma; F. VB: Foliar Vascular Bundles; C.VB: Central Vascular Bundles.

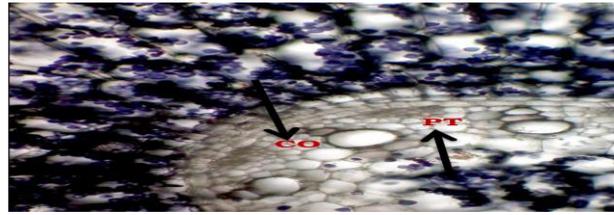
Plate IV: Histochemical studies: Rhizome



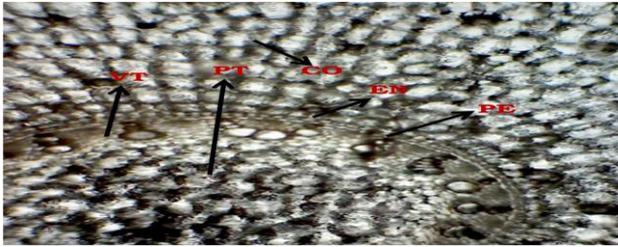
Alkaloids



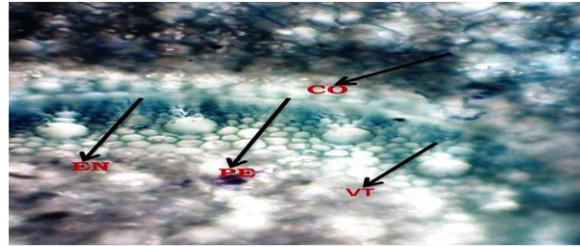
Strach



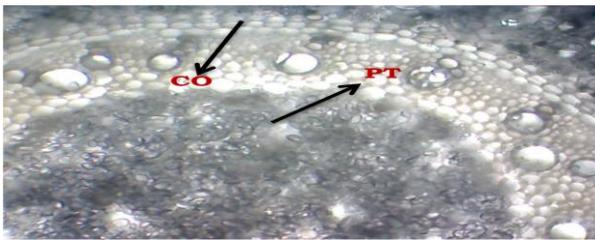
Tannins



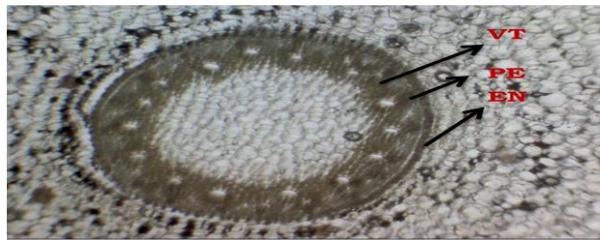
Mucilage



Calcium oxalate crystals

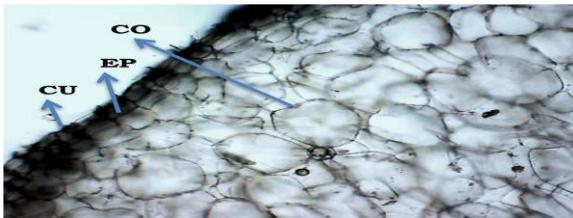


Saponins

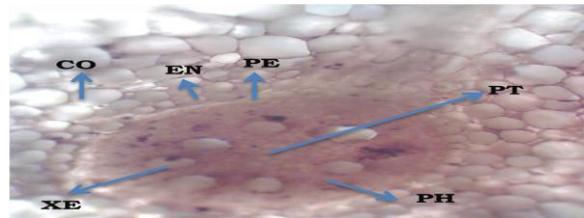


EP: Epidermis; HY: Hypodermis; OD: Oil Droplets; CO: Cortex; SG: Starch Grains; EN: Endodermis; PE: Pericycle; PX: Proto Xylem; ME: Meta Xylem; PH: Phloem; PT: Pith

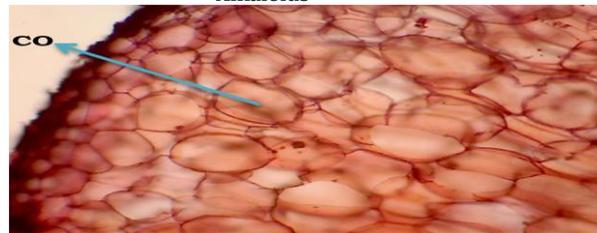
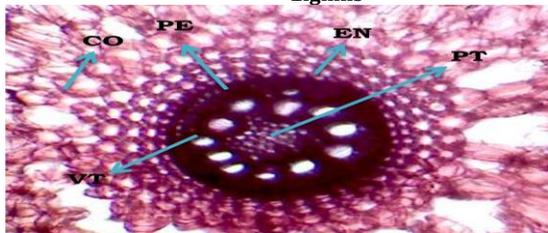
Plate V: Histochemical studies: Root



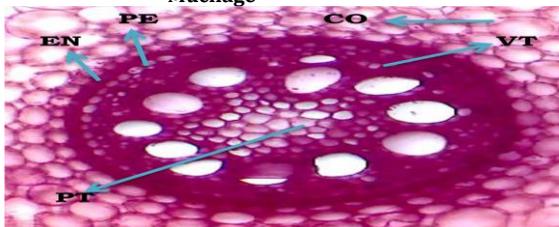
Lignins



Alkaloids



Mucilage



Suberins



EP: Epidermis; CU: Cuticle; CO: Cortex; EN: Endodermis; PE: Pericycle; VT: Vascular Tissue; XE: Xylem; PH: Phloem; PT: Pith.

Powder drug microscopy: [PLATE: VI, VII, VIII] Leaf, rhizome and root consists epidermal cells, cortical tissues, scalariform, spiral and

pitted vessels. Rhizome consists epidermal hairs, starch grains and oil glands, leaf with parasitic stomata.

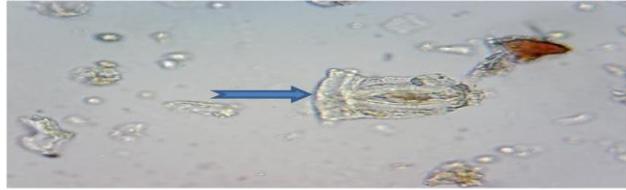
Plate VI: Powder drug microscopy

Leaf

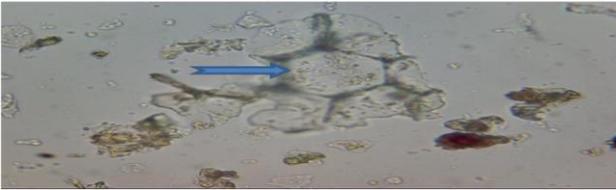
EPIDERMAL CELLS



STOMATA



MESOPHYLL CELLS



SCALARIFORM TRACHIDES



SCAPE

CORTICAL CELLS



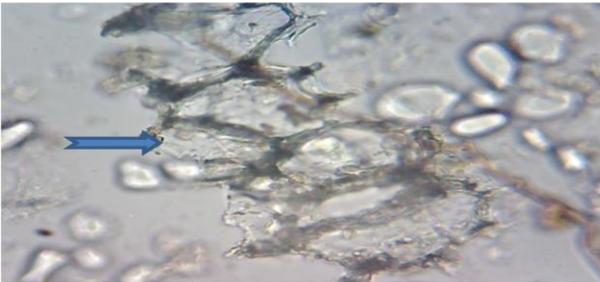
SPIRAL VESSELS



Plate VII

Rhizome

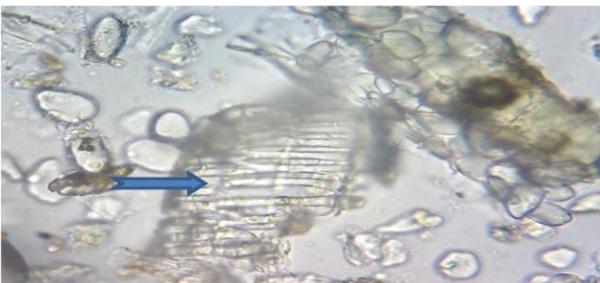
PARENCHYMATOUS CELLS



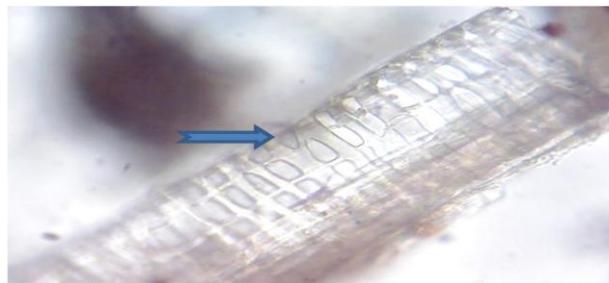
EPIDERMAL HAIRS



SCALARIFORM VESSELS



PITTED VESSELS



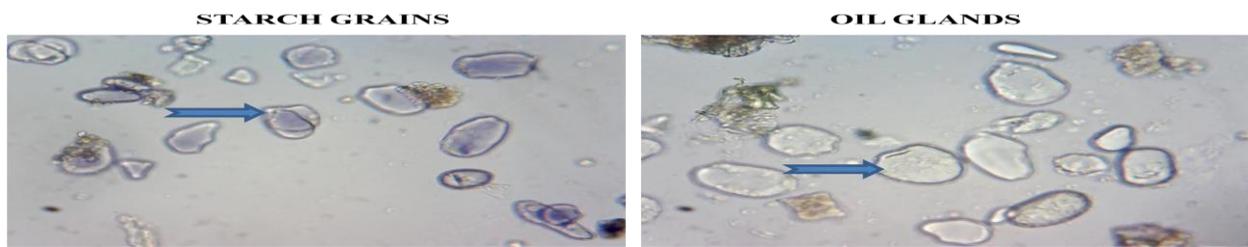


Plate VIII

Root

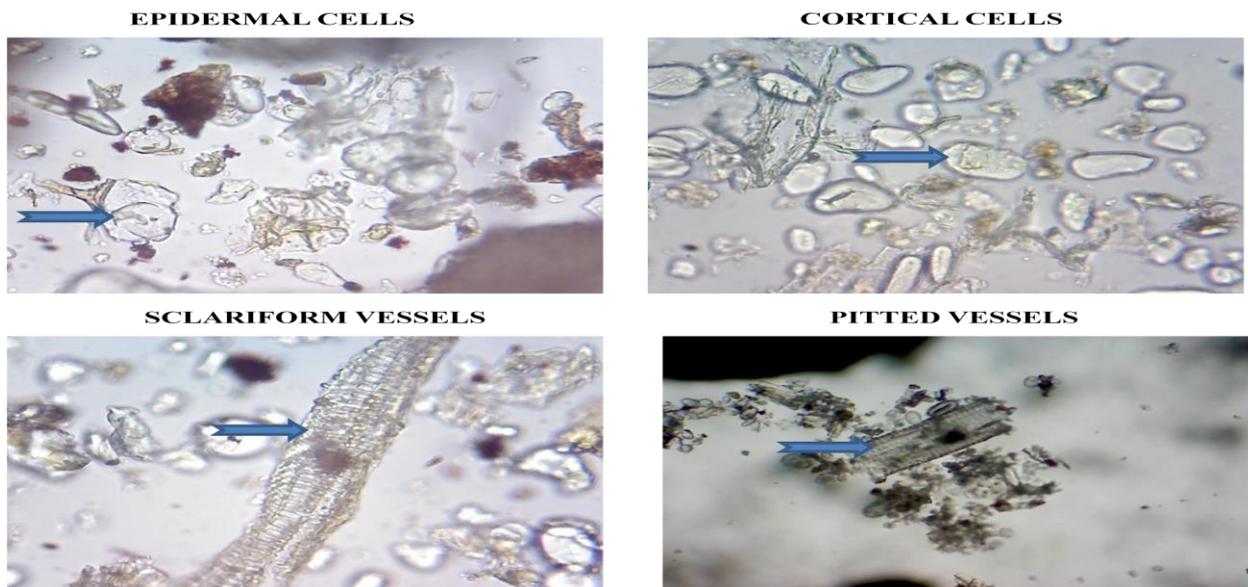


Table III: Physico chemical Analysis

| Parameters | Leaf | Scape | Rhizome | Root | Flower |
|--------------------|-----------|-----------|-----------|-----------|-----------|
| Total ash | 16.6% w/w | 13.3% w/w | 20.0% w/w | 13.3% w/w | 10.0% w/w |
| Acid insoluble ash | 8.0% w/w | 11.5% w/w | 16.6% w/w | 7.6% w/w | 7.4% w/w |
| Water soluble ash | 4.0% w/w | 7.69% w/w | 8.3% w/w | 3.8% w/w | 3.7% w/w |
| Sulphated ash | 2.0% w/w | 10.0% w/w | 25.0% w/w | 10.0% w/w | 8.0% w/w |
| Moisture Content | 30.0% w/w | 40.0% w/w | 50.0% w/w | 50.0% w/w | 10.0% w/w |

Physico Chemical Analysis: [Table-III]

Total ash 20%, acid insoluble ash 16.6%, water soluble ash 8.3%, sulphated ash is high in rhizome followed by scape, leaf, root and flower. Moisture content is very high nearly 50% in rhizome and roots, followed by scape 40%, leaf 30% and in flower 10%.

Extractive Values: [Table: IV]

Extractive values are high in aqueous, alcohol and methanol extracts ranging from 4.5 to 7.21mg and very low in ethyl acetate, chloroform, benzene and hexane extracts as 0.15 to 1.25mg. Over all rhizome yielded highest extracts followed by root, leaf, scape and flower.

Table IV: Extractive Values: (mg)

| Parameters | Leaf | Scape | Rhizome | Root | Flower |
|---------------|------|-------|---------|------|--------|
| Cold Water | 6.52 | 5.90 | 7.21 | 6.97 | 2.65 |
| Hot Water | 5.70 | 5.15 | 6.94 | 6.84 | 1.30 |
| Alcohol | 3.25 | 4.60 | 4.67 | 4.0 | 2.00 |
| Methanol | 4.43 | 3.41 | 2.38 | 2.04 | 2.17 |
| Ethyl acetate | 0.60 | 0.97 | 0.94 | 0.99 | 0.54 |
| Chloroform | 0.92 | 1.35 | 1.25 | 1.09 | 0.19 |
| Benzene | 0.51 | 0.84 | 0.79 | 0.64 | 0.20 |
| Hexane | 0.43 | 0.63 | 0.55 | 0.59 | 0.15 |

Fluorescence Analysis [Table V]

Powder drugs of all parts were treated with different acids of various concentration observed for the colors under day light, short and long waves of ultra violet rays. The main colors of the powders

in natural light of leaf and scape blackish green, rhizome and root yellow, flower light green; in short wave black, reddish yellow, brown and green; where as in long wave black, reddish black, blackish yellow respectively.

Table V: Fluorescence Analysis

| Chemical analysis | Day light | | | | | 250-270nm | | | | | 360-390nm | | | | |
|--------------------------------------|-----------|-----|------|------|------|-----------|-----|-----|-----|-----|-----------|-------|------|------|-------|
| | L | S | Rh | Rt | F | L | S | Rh | Rt | F | L | S | Rh | Rt | F |
| Powder as such | B.G | G | Y | Y | L.G | G | Y | P.G | P.G | L.G | T.A | P.G | L.G | L.G | P.G |
| 50% H ₂ SO ₄ | Br | G | B | B | T.Br | B | R.Y | Br | Br | B | B | B | B | B | B |
| Conc. Hcl | L.Br | G | B | B | T.Br | B | R.Y | B | B | Br | B | B.Br | B | B | B |
| 50% Hcl | Br | G | B | B | Br | G | Br | G | G | B | B | Br | T.Br | T.Br | R.Br |
| 50% HNO ₃ | Y | L.Y | A | A | T.Br | P.Y | P.Y | Y | Y | G | Br | Y | G.Y | G.Y | G |
| 10% NaOH | T.Br | G | R.Y | R.Y | T.Br | B | R.B | B.R | B.R | G | R.B | L.Br | Br | Br | Br |
| Conc. H ₂ SO ₄ | B | B.G | R.B | R.B | B | B | R.B | B | B | G | R.B | B | B | B | B |
| Conc. HNO ₃ | Y | L.G | A | A | Y | P.Y | P.Y | T.Y | T.Y | L.G | Br | L.Y | G.Y | G.Y | Y |
| 5% FeCl ₃ | G | G | B | B | G | B | B | G | G | B | G | T.Br | B | B | B |
| 5% NaOH | R.G | G | B | B | R.G | G | Br | B.G | B.G | G | T.Br | B | B | B | B |
| With Water | G.Y | G | L.Br | L.Br | G.Y | P.G | G.Y | G | G | L.G | G.Y | L.B.Y | Y.G | Y.G | L.Br |
| Acetic Acid | Br | G | L.Br | L.Br | Br | L.G | Br | Y.G | Y.G | G | B | L.Y | Y | Y | L.B.Y |

L: Leaf; **S:** Scape; **Rh:** Rhizome; **Rt:** Root; **F:** Flower

B.G: Blackish Green, G: Green, Y: Yellow, L.G: Light Green, P.G: Pale Green, L.G: Light Green, T.A: Thick Ash, Br: Brown, B: Black, T.Br: Thick Brown, R.Y: Reddish Yellow, B.Br: Blackish Brown, L.Br: Light Brown, R.Br: Reddish Brown, L.Y: Light Yellow, A: Ash, P.Y: Pale Yellow, G.Y: Greenish Yellow, R.B: Reddish Brown, L.Br: Light Brown, P.Y: Pale Yellow, T.Y: Thick Yellow, R.G: Reddish Green, L.Br.Y: Light Brownish Yellow, Y.G: Yellowish Green, L.B.Y: Light Blackish Yellow, B.R: Blackish Red.

DISCUSSION

C.amada rhizome pale brown with nodes and internodes, branched obliquely, with scars of undeveloped buds covered with leafy scales. Pharmacognostic studies reveals that the total ash 20% and water soluble ash 19.5%, extractive values with water 7.2 mg, petroleum ether 4.9mg, and all extracts are sticky; water and chloroform extracts are powdery. Histochemically phytoconstituents are mainly localized in xylary elements and in the cortical tissues. Blackish brown and pale yellow colored in fluorescence analysis [10].

C.caesia rhizome tuberous with camphoraceous sweet odor, irregularly shape, laterally flattened covered with adventitious roots and scars with longitudinal circular zones, bluish black. water soluble ash 13.6%, total ash 6%, moisture content 9%, alcohol insoluble ash 6%, acid insoluble ash 4%; extractive values highest in methanol 4.6%, followed by n-hexane 2.2%; Alkaloids, carbohydrates, proteins, fixed oils, flavonoids, phenols and tannins. Cineole, camphor and α -turmeone are the main volatile oils in methanol extract [11].

C.domestica rhizome irregular in shape, orange yellow; surface rough, pungent and slightly bitter aromatic. Total ash 8.5%, alcohol insoluble ash 7.4% and moisture content 12.5%; fluorescence analysis develops yellowish to reddish brown. Rhizome yielded mainly glycosides, steroids and alkaloids in all solvents. Tannins and saponins are absent [12].

C.charita rhizome laterally flattened, longitudinally wrinkled, branched, non-aromatic, pale yellowish green inside, sweet taste, tubers sessile, fleshy, finger shaped, branch roots branching sympodial, horizontal, thread like yellowish brown; water soluble extracts 19.5%, alcohol soluble 8.5%, starch 42.8%, sugars 5%, total ash 7.4%; extractive values high in aqueous 35.6 mg followed by hexane, methanol, chloroform and acetone extracts. Main essential oils are bluish violet to reddish, with camphoraceous odour; monoterpenoids 48.5%, sesquiterpenoids 51.49%, curcumins are very less [13].

C.neilgherrensis leaf powder grey, bitter in taste and slightly aromatic. Leaf covered with single layered epidermis, with cuticle and trichomes, paracytic stomata present on both surfaces. Leaf moisture content 4.5%, total ash 8%, water soluble ash 6.4%, alcohol soluble ash 8%, methanol extracts constitutes flavonoids, alkaloids, carbohydrates, tannins, organic acids and glycosides [14]. *Zingiber zerumbet* rhizomes are irregularly branched with nodes and internodes, scales present at the nodal region, surface smooth, covered

with trichomes and fibers, light greyish yellow, hard and brittle, fragrant, aromatic, spicy and slightly bitter, with abundant starch grains and oxalate crystals; water soluble ash highest 24% than alcohol insoluble ash 9.5% with an 11% of moisture content. Main phytoconstituents are glycosides, triterpenoids, saponins, tannins, carbohydrates, proteins, amino acids, sterols and volatiles are present only in petroleum ether extracts [15].

CONCLUSION

Macro and microscopic studies of *C.neilgherrensis* reveals that the rhizome was conical in shape, brownish with mild aromatic flavor to that of *C.caesia*. But it is different in having fusiform long tuberous roots, with secondary branching from other *Curcuma* species. Leaves with Paracytic stomata on both sides with trichomes and with oil cavities; rhizome with starch grains consisting spiral, pitted and scalariform xylary elements; mainly with phytoconstituents like mucilages, starch, alkaloids, tannins, calcium oxalate crystals and saponins. In other *Curcuma* species saponins are absent. Pharmacognostic studies reveals that the total ash content is equal (20%) to that of *C. amada*, where as in other *Curcuma* species it is very less (7 to 9%). Sulphated ash values are (25mg/g); water soluble ash is (8.3mg/g) less than other species (14-25mg/g); acid insoluble ash is high (16.6mg/g) than other species (0.7 to 4.3mg/g); moisture content is nearly 50mg/g. Extractive values are equal to *C. amada* (7.2mg/g) in cold water. Hence each *Curcuma* species is having specific pharmacognostic characters to be identified its quality and quantity in the drug formulations, and also to check the adulterations.

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