Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 397-410
Received: 15-05-2018
Accepted: 20-06-2018

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Pharmacognostic studies in *Solanum capsicoides* all

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Abstract
Detailed analysis of morphological and anatomical features of *Solanum capsicoides* All. Was done that would be helpful for pharmacognostic identification. Phytochemical screening and histochemical test were performed for the confirmation and localization of the phytoconstituents present in the species. Among the morphological features anthocyanin pigmentation on leaf petiole, five different types of trichomes, The inflorescence with bisexual flowers, orange red fruit and winged seeds were found to be distinctive. Presence of sandy crystals and bicollateral vascular bundles, the unifying features of the genus, were observed in the plant. Absence of collenchymatous hypodermis in fruit exocarp was a distinguishing feature from other Solanum members. The phytochemicals identified in the plant were flavonoids, coumarins, alkaloids, tannins, steroids, saponins, phenol, resin, glycoside, protein and carbohydrate. The present study thus emphasis the pharmaceutical potential of the plant and the necessity for its conservation.

Keywords: anatomy, histochemical test, morphology, phytochemical screening

Introduction
Solanum is one of the species rich genera in angiosperm and is also the largest genera in Solanaceae [1]. Species coming under the genus *Solanum* includes vegetables [2], weeds and medicinal herbs [3]. *Solanum capsicoides* All. (Cockroach Berry) (Syn. *S. aculeatissimum*Jacq.) is a medicinal plant and is native to eastern Brazil. It is used as the source of Kantakari in ayurveda, an important therapeutic agent for dislodging tenacious phlegm.It is extensively used for the treatment of diverse ailments like cough, bronchitis, asthma, influenza and enteric fever.'Kanakasavam, kantakarighrtham, pulikaranjasavam and suranadileham' are the important ayurvedic formulations that use kantakari as a constituent [4]. Over exploitation and urbanization has drastically decreased the availability of this plant as a raw drug in the Indian Ayurvedic industry, especially in Kerala. Pharmacognostic standardisation of this valuable medicinal plant was not been reported so far. Hence, adulteration at raw drug level has become a problem in the industry.
Morphological and anatomical studies of medicinal plants are relevant for their identification. Now a days refined chemical and molecular methods are available for the identification of plant material. But morpho-anatomical documentation is the simplest qualitative method to avoid falsification and adulteration of the drug [5]. The structural analysis pinpoints idiosyncratic aspects that can be effective in determining the accuracy of medicinal plant species [6-8].
The medicinal potential of taxonomically related species can be studied using histochemical techniques [9-10]. This technique is quick and inexpensive and can be used in search of new pharmaceuticals [11-12]. The histochemical studies are rare in *Solanum* [13-14] though the members are used for medicinal purpose from ancient times [15].
Secondary metabolites present in a plant can be considered as its chemical individuality as their composition differ from species to species [16]. Phytochemical screening is crucial in the discovery of new sources of therapeutic agents that are economically important (Akrout *et al*., 2010) [17]. It is also essential for more pharmacological approaches.
The present paper reports morphological and anatomical characterization, histochemical localization and preliminary screening of phytochemical constituents of *S. capsicoides* as pharmacognostic tool for the raw drug industry.

Materials and Methods
Collection of Plant Materials
Mature plants with fruits were collected from Ernakulam district of Kerala State, India, identified and herbarium voucher specimens were deposited at the Herbarium of Kerala Forest Research Institute (KFRI), Peechi (KFRI-13056).

Structural Characterization
Morpho-anatomical analysis was executed using 30 cm ruler, graph paper, electronic balance, micrometer, Olympus trinocular microscope with Magnus microscope camera attached, TESCAN VEGA 3 SBH electron microscope and Olympus Stereo zoom microscope attached with Nikon camera.
Macroscopic features considered include plant habit, plant height, leaf colour, texture, phyllotaxy, leaf type, leaf blade shape, leaf area, root type, colour, texture, taste, inflorescence, flower colour, flower diameter, sepal length, petal length, stamen length, pistil length, fruit diameter, fruit weight, seed number, seed size and seed weight, where as the microscopic aspects numbered aretype of stomata, stomatal index, guard cell area, type of trichomes, trichome length, seed surface architecture and pollen viability.
Macroscopic measurements were executed using 30 cm ruler and electronic balance. Microscopic scaling was performed using a calibrated eye piece graticule.
The seed surface architecture was analysed using scanning electron microscope.

Leaf Structure Analysis
Mature healthy leaves were collected and used for studying various characters. Leaf area was measured using millimeter graph method [18]. For studying the venation pattern, leaves were cleared following the method of Gardner (1975) with minor modifications [19]. The epidermal features were studied using epidermal peels obtained from fresh leaves using pointed needle and forceps. The peels were stained using safranin and viewed under microscope.

Anatomical Studies
For microscopic analysis cross sections were prepared following free hand sectioning method and stained with toluidine blue.

Histochemical Localization
Histochemical localization was done on fresh plant sections using various reagents such as Lugol's solution for starch (Jensen, 1962), Aqueous NaOH for flavonoids (Johansen, 1940), Bromophenol Blue for protein (Mazia, 1953), Aqueous Ferric chloride for phenol (Johansen, 1940), Wagners Reagent for alkaloid (Furr and Mahlberg,1981) and Schiff's reagent for lignin (McLean and Cook, 1941) [20-24]. Plant sections not treated with any chemicals were used as negative control.

Powder Analysis
Leaf, fruit, stem and root were air dried and powdered. Fine powder was used for microscopic characterization and also for macroscopic analysis. Drug powder was treated with twelve different reagents and the colour change was noticed under day light.

Physico-Chemical Parameters
Parameters studied include pH value (1% solution and 10% solution), moisture content, extractive values (water soluble, ethanol soluble, methanol soluble, chloroform soluble and ethyl acetate soluble) and ash values (total ash, acid insoluble ash) following WHO guidelines.

Preliminary Phytochemical Screening
Powdered leaf, fruit, stem and root were soaked directly in extractive solvents such as ethyl acetate, chloroform and methanol in the ratio 1:5 and were kept for 48 hrs. Using Whatman Filter paper No.1, the extract was filtered and was concentrated in water bath. Evaporated extracts were used to investigate the presence of various phytochemical constituents following standard procedures (WHO, 2002) [25].

Results
Structural characterization
Habit: *Solanum capsicoides* is a short lived perennial plant and is suffrutescent. It is branching and acanceous with determinate growth reaching a height of 50cm- 1m.The stem is cylindrical, pubescentand green in colour (Fig 1). Plant possesses simple leaves arranged alternately at the base but as pairs in inflorescence portion.

Fig 1: Whole Plant

Inflorescence: The inflorescence of the plant is scorpioid cyme with 4 flowers (Fig. 2A) in extra-axiallary position (Fig. 2B).Flowers are stellate, pentamerous, actinomorphic, pedicellate with valvate aestivation and entomophilous. Both peduncle and pedicel are armed with prickles.Calyx: 5, gamosepalous, green in colour and is armed with prickles (Fig.2C) Corolla: 5, gamopetalous, white coloured and light greenish towards base (Fig.2D). Androecium: 5, equal, epipetalous, basifixed, and connivent anthers alternately arranged to petals. The filament tube is minute and pale green in colour. The antheris ovate, dark yellow coloured towards base which gradually fades to pale yellow at the tip (Fig.2E-F). Gynoecium: Ovary superior, bicarpellary, with terminal long style and a well developed green coloured bilobed stigma (Fig.2G-H). The ovary is bilocular with axile placentation (Fig.2I).

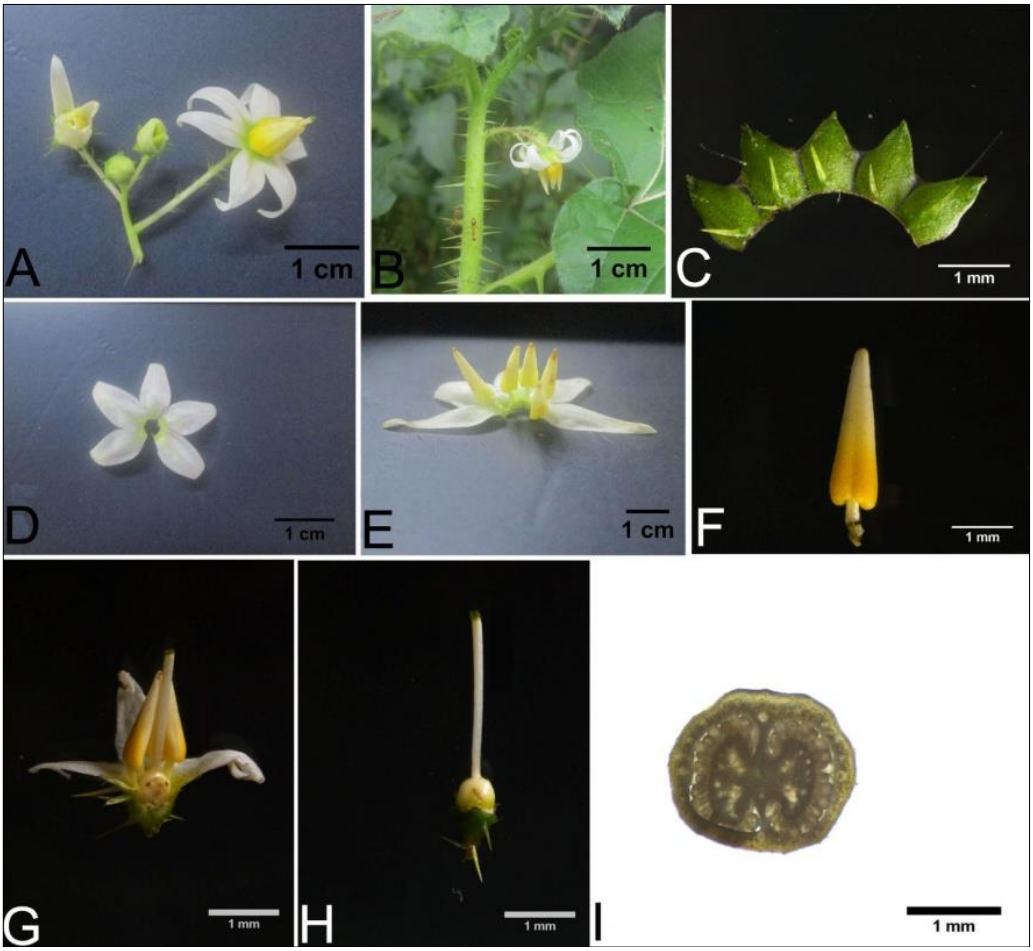

Fig 2: Inflorescence:(A) Inflorescence; (B) Extra-axillary position of inflorescence;(C) Calyx;(D) Corolla; (E) Epipetalous stamen; (F) Single Stamen; (G) Section of flower showing superior ovary; (H) Gynoecium; (I)Ovary T.S.

Fruits: Globose berry, but different from botanically defined berry because the fruit is only slightly juicy and the fruit release the seeds at maturity by dehiscence of pericarp. Fruit possess persistent calyx and the pedicel is curved. Both the pedicle and calyx are equipped with prickles. Usually 1 fruit is developed from each inflorescence. Young fruits are pale green at the base which gradually turns white towards the apex with green coloured longitudinal stripes (Fig. 3A). Mature fruits are bright orange- red in colour (Fig.3B). Inside portion of the fruit wall is white coloured and spongy. The flesh is slightly juicy but brittle and can be easily broken. Fruit is filled with numerous seeds. The septum present in the ovary has degenerated and two placental bodies are clearly visible (Fig.3C).

Seeds: The seeds are flattened, winged, considerably round and straw coloured. A little mucilage coating is present on the seed which make them sticky in texture (Fig. 3D-E). The seed surface exhibit reticulate pattern. The lumen is shallow and the convoluted cells displays sinuous pattern (Fig.3F-G).

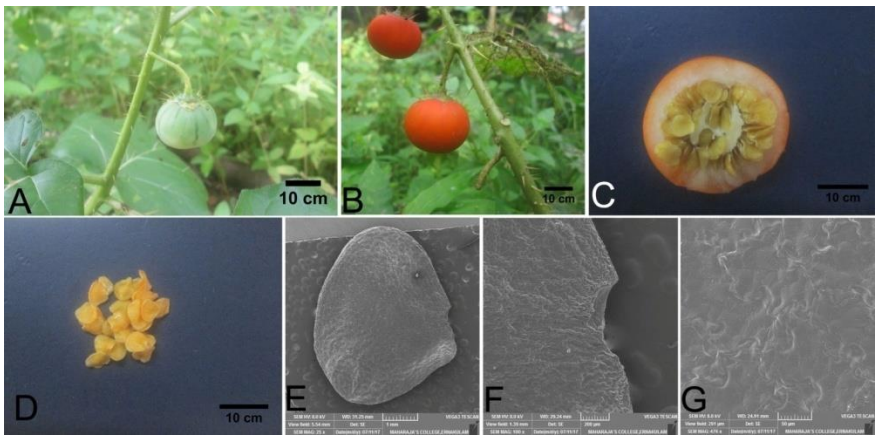

Fig 3: Fruit:(A)Young Fruit; (B) Mature Fruit; (C) Matured fruit halved showing inside of fruit wall, seeds and placental bodies; (D) Mature Seeds; (E)SEM image of seed; (F)Seed surface towards hilum showing reticulate architecture; (G) Lumen surrounded by convoluted cells with sinuous pattern

Root: Root is long, cylindrical and branched having long thin rootlets. External surface is rough in texture and brown in colour (Fig.4). Fracture not easy and the plane of fracture arefibrous. It tastes bitter and is odourless.

Fig 4: Root

Leaf structure analysis

Plants possess simple, dorsiventral, cordate membranous leaves, dark green coloured in the adaxial surface and pale green coloured in abaxial surface, arranged alternately at the base but as pairs in inflorescence portion. The pubescent lamina is symmetrical, with coarsely lobed margin, leptophyll in size and has marginal petiolar attachment. Petiole portion facing the adaxial surface of leaf is purple green in colour. Prickles are present along the major veins on both adaxial and abaxial surface (Fig. 5A-B).

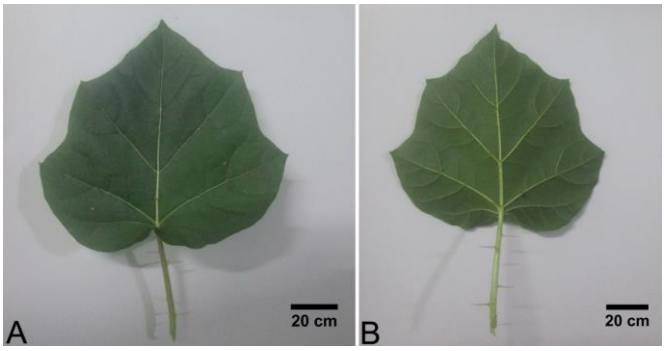

Fig 5: Leaf: (A) Adaxial surface; (B) Abaxial surface

Epidermal features: The epidermal cells are irregular in shape and anticlinal walls are undulating. The species is hypoamphistomatic in which bothanomocytic and anisocytic stomata are present (Fig.6A-B). Both glandular and non-glandular trichomes are present on entire plant. Two types of non-glandular trichomes were observed such as multicellular hair with pointed tip having 4-6 cells (Fig. 6C) and bicellular trichome with blunt end (Fig. 6D). Glandular trichomes are of three types, unicellular claviform trichome with bicellular base (Fig. 6E); multicellular capitate trichome with unicellular base (Fig. 6F); multicellular glandular trichome (Fig. 6G).

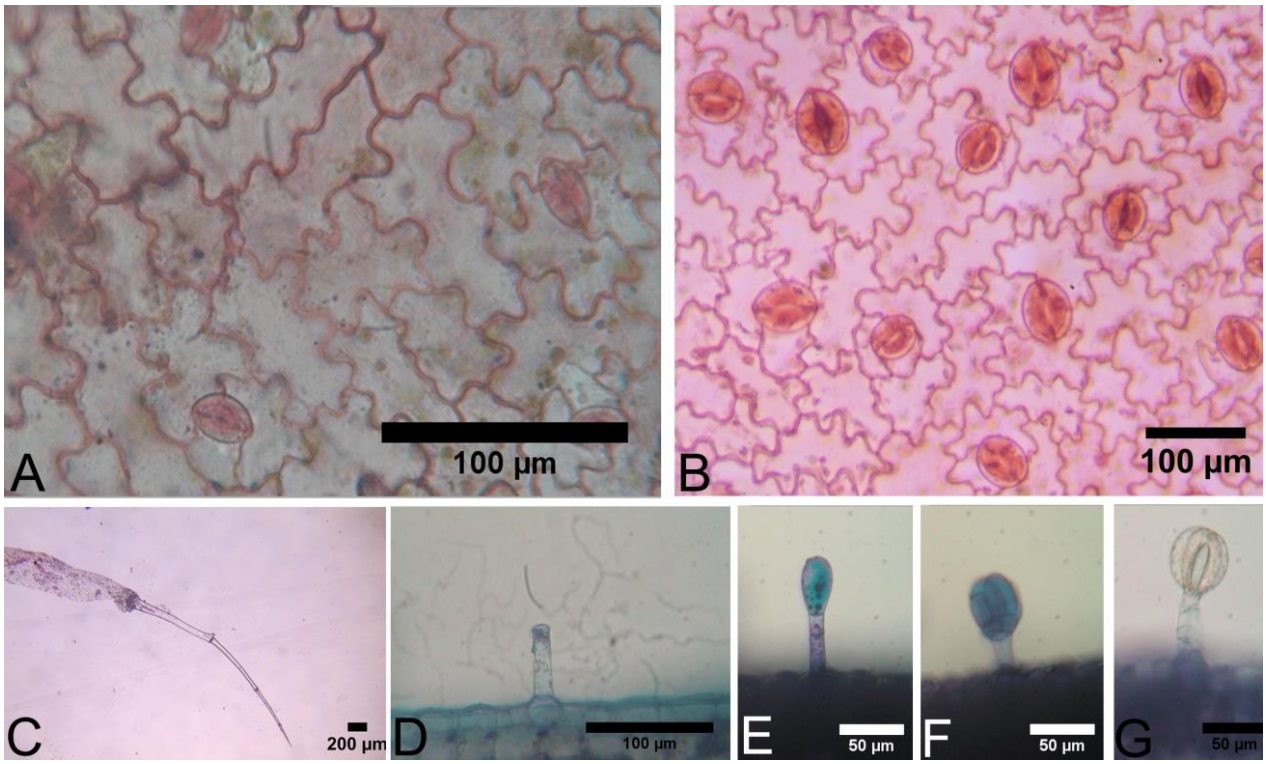

Fig 6: Epidermal Features: (A)Epidermal peel from adaxial surface showing anisocytic stomata; (B) anisocytic and anomocytic stomata on abaxial surface; (C) Multicellular eglandular trichome; (D)Bicellular eglandular trichome; (E)Claviform glandular trichome; (F)Capitate glandular trichome; (G) multicellular glandular trichome

Venation pattern: The venation is pinnate, ornamented and the primary vein is straight. The primary vein gives off secondary veins which number 5-8 on either side. The spacing between the secondary veins is not regular. Intersecondary veins are present. The highest order of the vein was observed as 6 degree. Marginal ultimate venation is looped and complete. The areoles are imperfect and random. They are square, quadrangular or pentangular in shape. Vein endings may or may not be present in the areole. Vein endings entering the areoles were simple or branched. Simple veinlets were either curved or linear. Branched veinlets observed were crescent shaped and Y shaped and was both symmetric and asymmetric. Veinlets are mostly uniseriate but at some junctions of veinlets they are biseriate. The tracheids are uniseriate, sometimes heavily thickened or less thickened. Both conventional and dilated tracheids were observed. Dilated tracheids were of two types; spindle shaped and gnarled (Fig. 7A-O).

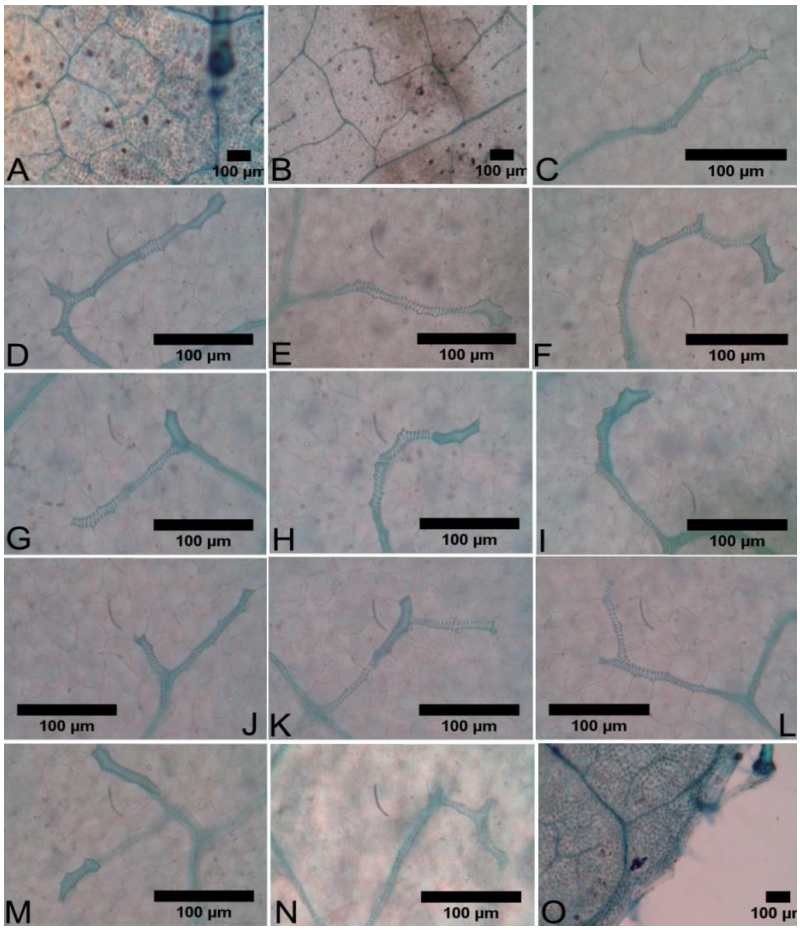

Fig 7: Leaf Venation Pattern:(A) areole lacking vein ending; (B) areole with vein ending; (C) curved conventional tracheid; (D) Principal asymmetric vein ending with heavily thickenedtracheids in between and at the tip of veinlet; (E) Veinlet biseriate at one portion bearing less thickened dilated tracheid; (F) Veinlet terminates in to two and one of them again divide dichotomously with one branch terminates in dilated tracheid; (G) Biseriate veinlet divides into two; spindle shaped tracheid and uniseriate veinlet with dilated end ; (H) Curved veinlet with conventional tracheid; (I) Gnarled tracheid; (J) Asymmetric Y shaped veinlets; (K) Conventional tracheid between veinlet; (L)Asymmetric Y shaped veinlet biseriate at junction; (M)Symmetric Y shaped veinlets with spindle shaped tracheids; (N)Crescent shaped veinlets; (O) Ultimate branching – complete and looped

Quantitative macroscopic and microscopic features are shown in Table I.

Table I: Quantitative microscopic and macroscopic features of *S. capsicoides* All.

Plant Parts	Feature Quantified		Measurement
LEAF	**Leaf Area (cm^2)**		**79.67±3.95**
	Trichome Length	Multicellular Eglandular (mm)	1.66-2.47
		Bicellular Eglandular (µm)	45-97.5
		Claviform Glandular (µm)	56.25-150
		Capitate Glandular (µm)	26.25-56.25
	Guard cell area (μm^2)	Upper epidermis	395.41±18.27*
		Lower epidermis	500.61±31*
	Stomatal Index (%)	Upper epidermis	6.25±0.61*
		Lower epidermis	15.46±0.53*
	Prickle Length (mm)	Upper epidermis	6-10
		Lower epidermis	4-9
STEM	Trichome Length	Multicellular Eglandular (mm)	4.05-4.75
		Bicellular Eglandular (µm)	48.75 -75
		Claviform Glandular (µm)	67.5 -97.5
		Capitate Glandular (µm)	56.25 -86.25
	Prickle Length (mm)		2-6
FRUIT	Fruit Diameter (cm)		7.2-7.9
	Fruit Weight (g)		3.78-5.53
	Seed Number		165-209
	Seed Size(mm)		5
	Seed Weight(g)		0.0017-0.0019
POLLEN	Pollen Viability (%)		92.05±1.34*
FLOWER	Flower Diameter (cm)		2-2.2
	Sepal Length (mm)		2-5

	Petal length (mm)	12-14
	Stamen Length (mm)	8-10
	Pistil Length (Long) (mm)	10-11
	Pistil Length (Short) (mm)	2-3

* Each value represents mean ± standard error
Abbreviations: mm- millimeter; cm- centimeter; µm- micrometer; g-gram

Anatomical Studies

Stem

Microscopic analysis revealed single layered barrel shaped epidermal cells covered with cuticle. The epidermal layer possesses appendages such as non-glandular and glandular trichomes but lack stomata. Epidermis is followed by a single layer of chlorenchyma cells. Cortex consists of 6 layers of angular collenchyma cells followed by 4-6 layers of parenchyma which are large sized and isodiammetric. The vascular bundle is bicollateral, amphiphloic siphonostele. The xylem is endarch, with large vessels. Both internal and external phloem is 4-5 layered and is covered with sclerenchymatous bundle sheath. Pith is parenchymatous. Cortex and pith encompass black powdery mass, the sandy crystals (Fig. 8A). As the secondary growth proceeds, the epidermis followed by the chlorenchymatous layer turned brown in colour. The parenchyma layer reduced in thickness and became flattened. The vasculature became continuous with the development of secondary xylem and secondary phloem and the bundle sheath expanded in thickness. The parenchyma in cortex and pith consists of sandy crystals and starch grains (Fig. 8B).

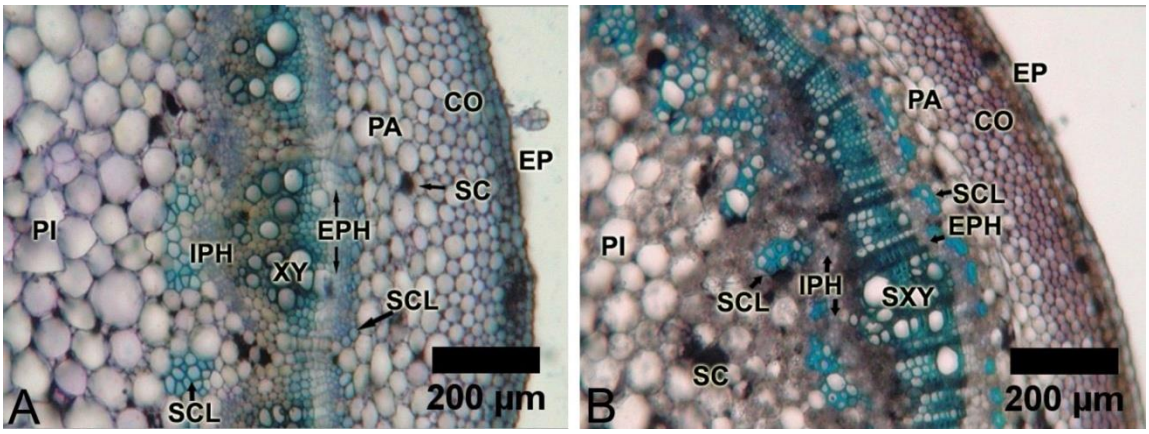

Fig 8: Stem anatomy: (A) Transverse section of stem in primary growth; (B) Stem in secondary growth. Abbreviations:EP-epidermis, CO-collenchyma, PA-parenchyma, SC-sandy crystals, EPH-external phloem, XY-xylem, IPH-internal phloem, SCL-sclerenchyma, PI-pith, SXY-secondary xylem, SPH-secondary phloem

Leaf

In the cross section, the epidermis is single layered with rounded cells and thin cuticle. Trichomes are present along the epidermal layer. In midrib the epidermis is followed by 2 layers of angular collenchyma and by parenchyma, the ground tissue. Single vascular bundle is present in the centre which is bicollateral and arc shaped. Sandy crystals are present in parenchyma and in the phloem (Fig. 9A). The microscopic view of lamina affirms the dorsiventral organization of leaf. The epidermis is followed by single layer of compactly arranged palisade parenchyma followed by 3 layers of loosely arranged spongy parenchyma. Stomata are visible on both upper and lower epidermis (Fig.9B).

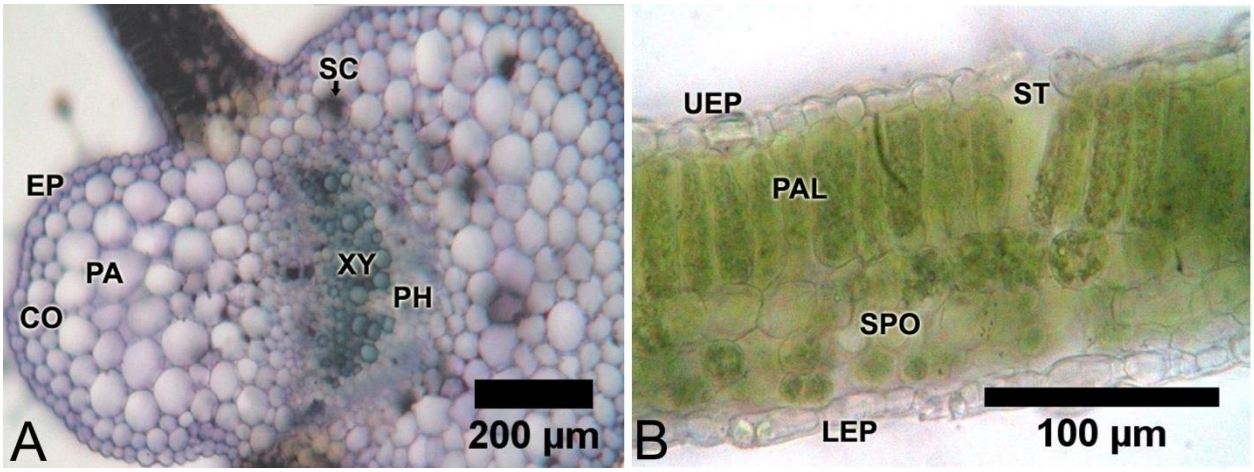

Fig 9: Leaf Anatomy: (A) Midrib cross section; (B) Transverse section of lamina. Abbreviations: EP-epidermis, CO-collenchyma, PA-parenchyma, SC-sandy crystals, PAL-palisade parenchyma, SPO-spongy parenchyma, ST-stomata, UEP-upper epidermal cell, LEP-lower epidermal cell

Fruit

In anatomical aspect the pericarp of fruits consists of three zones: epicarp, mesocarp and endocarp.

Epicarp: Cuticle is thick, sinuous and orange coloured. Epidermal cells are small, isodiammetric, and cuticular wedges are present along the tangential wall of epidermal cells. Epidermis is followed by hypodermis comprising 3 layers of radially compressed parenchymatous cells (Fig. 10A).

Mesocarp: Outer layer of mesocarp consists of 6-10 layers of medium sized loosely arranged parenchymatous cells with starch grains. The inner layer of mesocarp is juicy and consists of large cells which disorganize when the fruit ripe. The hypodermal cells and the cells in mesocarp consist of chloroplast in immature fruit. In ripe fruits, the chloroplasts disappears and the cells are radially compressed (Fig. 10B). Endocarp was not visible due to its very delicate nature.

Seed

Cross section of the seed exhibited testa, endosperm and embryo sac. The outer integument has a thin triangular cell followed by a layer of macrosclereids. The inner integument is made of crushed parenchyma cells. Hypodermis is absent and endosperm is made up of variously shaped thin walled cells filled with starch grains.Two embryo sacsare present (Fig. 10C-E).

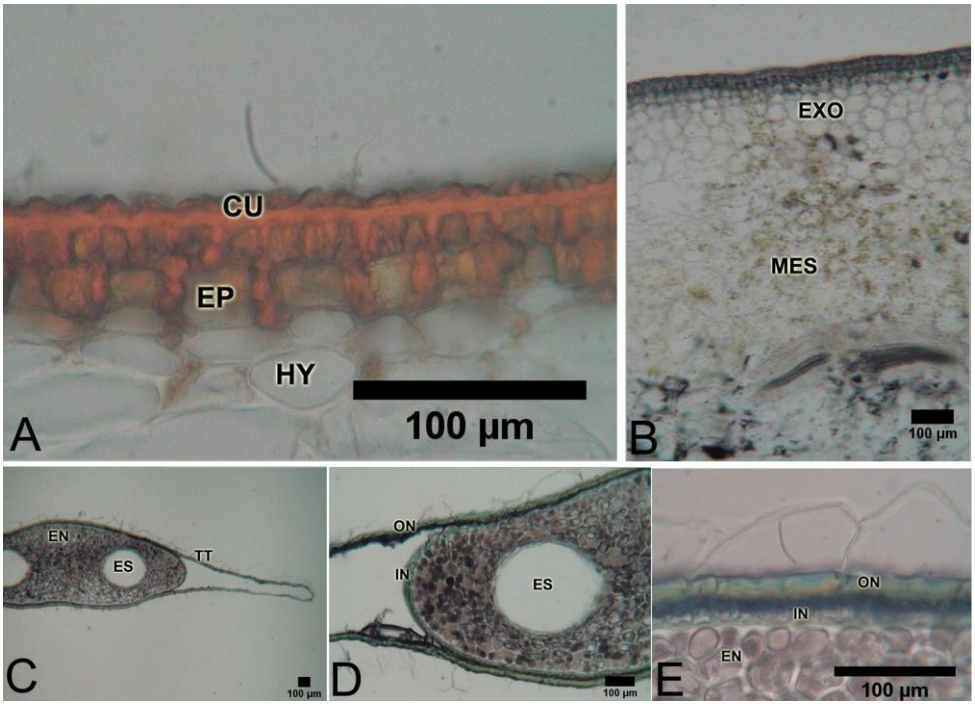

Fig 10: Fruit and Seed Anatomy: (A) Enlarged view of exocarp; (B) cross section of fruit; (C) Cross sectionof seed showing winged testa; (D) A portion of seed; (E) Enlarged view of testa

Abbreviation: EP-epidermis, CU-cuticle, HY-hypodermis, EXO-exocarp, MES-mesocarp,
EN- endosperm, ES- embryo sac, ON- outer integument, IN- inner integument, TT- testa

Root

A T.S. of the primary root is circular in outline with crushed parenchymatous epidermis which possesses abundant root hairs. The ground tissue consists of isodiammetric parenchymatous cells which encloses amphicribral vascular bundles. The xylem is monarch and exarch (Fig. 11A-B). As the root progress to secondary growth, the periderm forms the outer layer followed by tangentially elongated parenchymatous cortex. Cortical cells contain starch and sandy crystals. Secondary xylem is abundant with wide lumen arranged in solitary or in groups of two. Phloem surrounds the xylem (Fig. 11C)

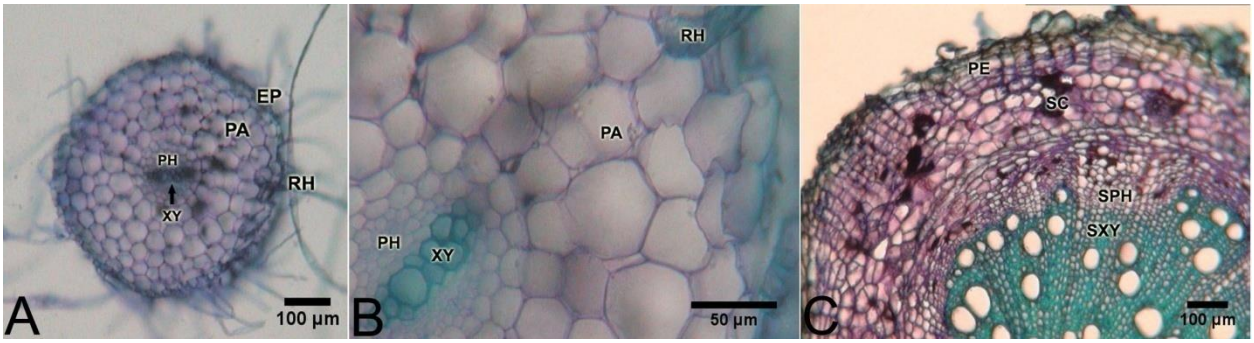

Fig 11: Root Anatomy: (A)Cross section of root in primary growth; (B) Enlarged view of root cross section; (C) Cross section of root in secondary growth. Abbreviations: EP-epidermis, PA-parenchyma, SC-sandy crystals, PH-Phloem, XY-Xylem, RH-root hair, SXY- secondary xylem, SPH-secondary phloem

Histochemical Localization

Histochemical test with Lugol's iodine solution revealed abundant deposition of starch grains in the cortex and pith of secondary stem, in the cortex of secondary root and also in the mesocarp of fruit (Fig. 12A-C). The presence of flavonoids in the xylem elements and cortex of primary stem and in the epidermal cells of secondary stem was indicated by aqueous NaOH (Fig.12D-E). Treatment of fresh sections with aqueous ferric chloride reported presence of phenol in the xylem element of primary stem, cortical cells and xylem of secondary stem and in the mesocarp cells of fruit (Fig.12F-I).

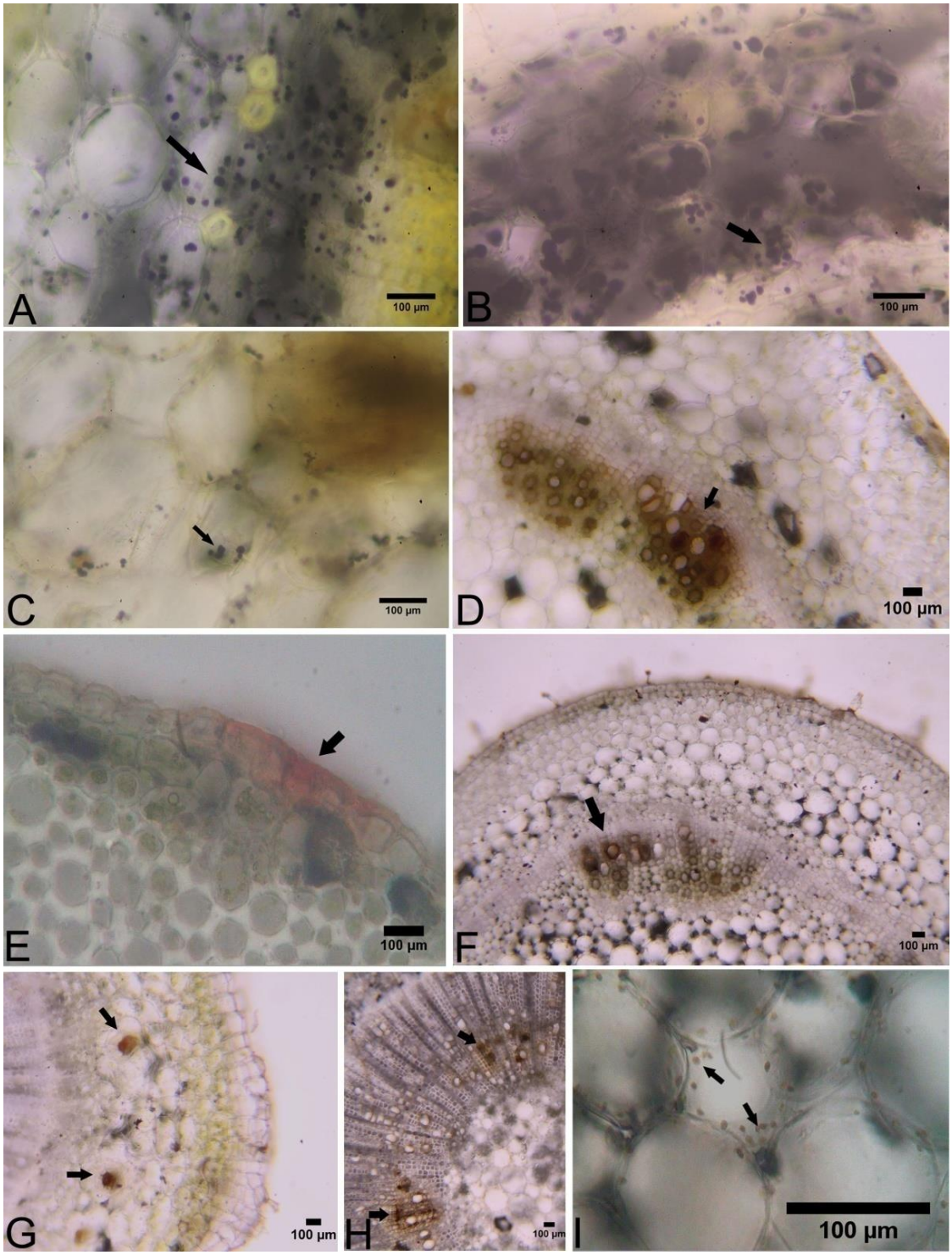

Fig 12: Histochemical Localization: (A-C)Starch grains stained blue-black with Lugol's Iodine: (A)Starch grains in secondary stem cortex; (B) Starch grains in secondary root cortex; (C) Starch grains in fruit mesocarp; (D-E) Flavonoidsstained wine red with aqueous NaOH: (D)Flavonoids in primary stem; (E) Flavonoids in secondary stem; (F-I) Phenol stained brown with Aqueous Ferric chloride: (F) Phenol in primary stem; (G-H)Phenol in secondary stem; (I)Phenol in fruit

Bromophenol blue tests confirmed the presence of protein in the stelar cells and also in the cortical cells near to the vascular region in the secondary root (Fig. 13A). Wagner's test revealed the presence of alkaloids in the exocarp of fruits and in the glandular trichomes (Fig. 13 B-C). Lignin depositions in the vascular region of secondary stem, primary stem, claviform glandular trichome, secondary root, and in the fruit wall wereconfirmed with Schiff's reagent test (Fig. 13D-H).

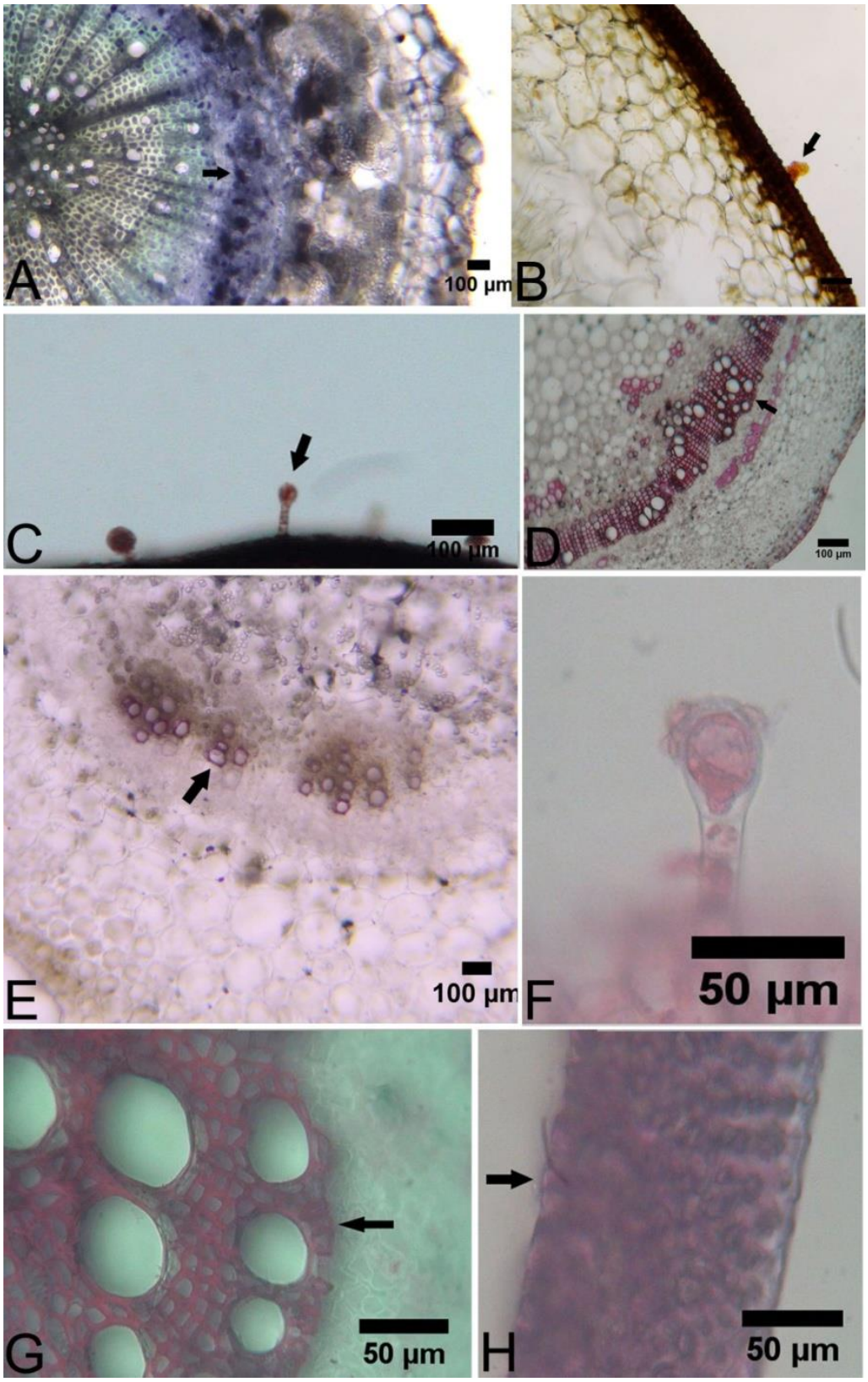

Fig 13: Histochemical Localization:(A)Protein stained blue with Bromophenol blue in secondary root; (B-C) Alkaloid stained reddish brown with Wagner's Reagent: (B)Alkaloid in fruit; (C) Alkaloid in trichome; (D-H) Lignin stained majenta with Schiff's reagent: (D)Lignin in secondary stem; (E) Lignin in primary stem; (F)Lignin in trichome; (G)Lignin in secondary root; (H)Lignin in fruit

Powder analysis

The powder form of stem and root is cream coloured where as leaf is in green colour and fruit in brown-orange colour (Fig. 14A-D).The stem powder under microscope revealed the presence of cells with crystals (Fig.14 E), starch grains (Fig. 14 F), spiral xylem vessel (Fig. 14 G) and fiber (Fig. 14 H). The leaf powder showed the presence of starch grains (Fig. 14 I), multicellular non-glandular trichome (Fig.14 J) and spiral thickening of xylem vessel (Fig. 14 K). The fruit powder confirmed cell with starch grains (Fig. 14 L), free starch grains (Fig. 14 M), fibers (Fig.14 N), endosperm cells (Fig. 14 O) and fruit wall (Fig. 14 P). The root powder under microscope presented fiber (Fig. 14 Q), starch grains (Fig. 14 R) and pitted vessels (Fig.14 S). The colour observed under day light after the reaction of drug powders to twelve different reagents is presented in Table II.

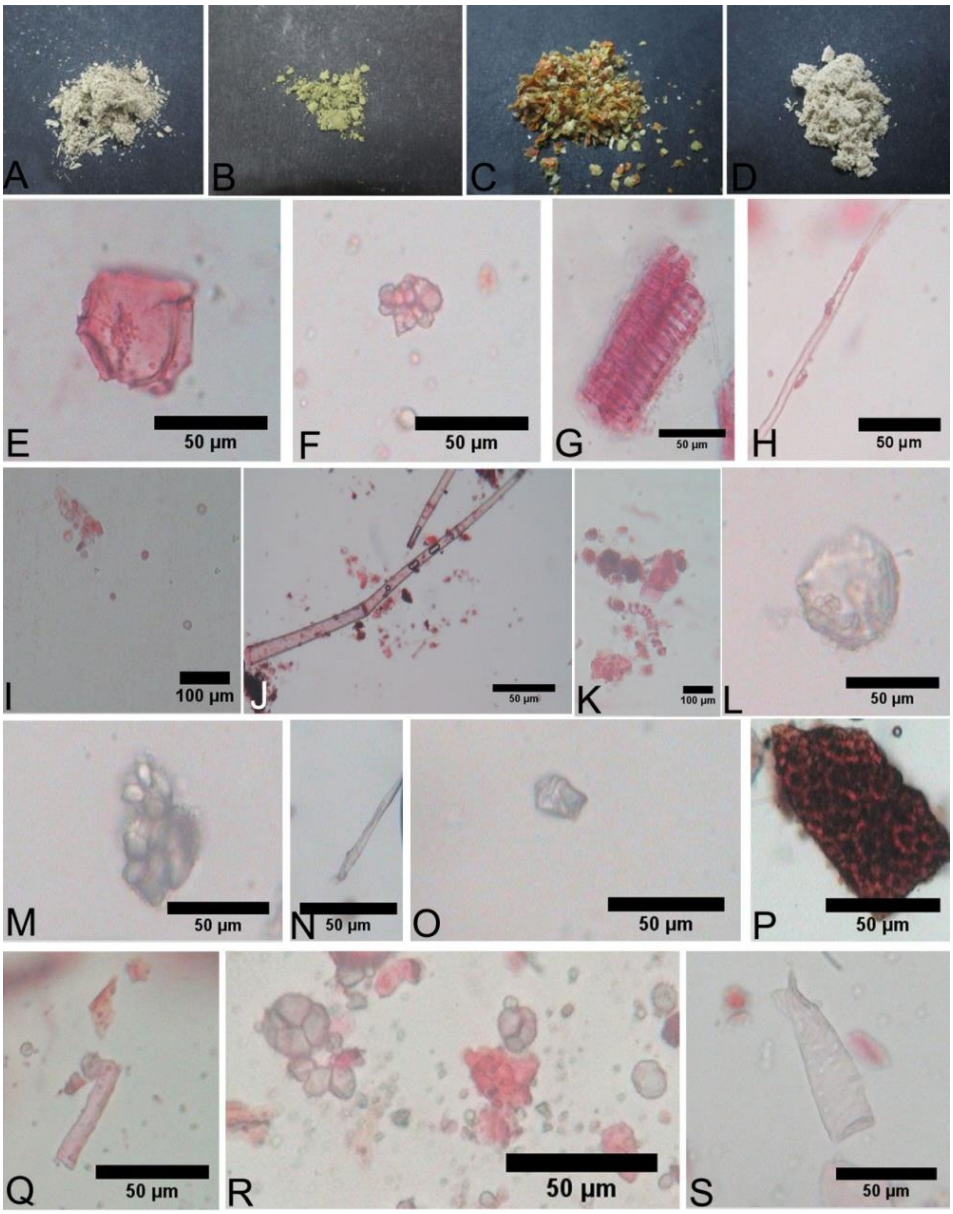

Fig 14: Powder Analysis: (A-D) Drug powder as such: (A) Stem; (B) Leaf; (C) Fruit; (D) Root; (E-H) Powder analysis of stem: (E) ruptured cell with crystals; (F) starch grains; (G) xylem vessel with spiral thickening; (H) fiber; (I-K) Powder analysis of leaf: (I) starch grains; (J) trichome; (K) spiral thickening of xylem vessel; (L-P) Powder analysis of fruit: (L) cell with starch grain; (M) starch grains; (N) fiber; (O)endosperm cell; (P)fruit wall; (Q-S) Powder analysis of root: (Q) fiber; (R) starch grains; (S) pitted vessel

Table II: Reaction to chemicals

Reagents	Stem	Leaf	Fruit	Root
Powder as such	Cream	Dark green	Brown Red	Cream
Conc. H_2SO_4	Brown	Pale green	Deep red	Brown
Conc. HCl	Brownish Yellow	Light green	Yellow	Flesh colour
Glacial acetic acid	Cream	Yellow	No colour change	Cream
Iodine solution	Brown	Reddish brown	Reddish brown	Reddish brown
Aq. Ferric chloride	Orange	Orange	Deep yellow	Brown
Aq. NaOH (5%)	Cream	Pale green	Yellow	Pale yellow
Aq. KOH (5%)	Cream	No colour change	Yellow	Pale yellow
Ammonia solution	Cream	Pale green	Deep yellow	Pale yellow
Distilled water	Cream	No colour change	Light yellow	No colour change
Acetone	White	Green	No colour change	White
Ethyl acetate	No colour change	Pale green	No colour change	White

Abbreviations: Conc. - Concentration; Aq. – Aqueous

Physicochemical Parameters

The pH value of drug solution ranges between 6-6.8. The moisture content was high in leaf (86.75%) followed by other parts. In leaf, stem and root the extractive value was highest for water soluble matters where as in fruit, ethanol soluble extractive was highest. In allthe four parts the total ash content was highest than acid insoluble ash content (Table III).

Table III: Physicochemical parameters

Parameters		Stem	Leaf	Fruit	Root
pH value	1% solution	6.8	6.2	6.3	6.4
	10% solution	6	6	6	6
Moisture content		83.66%	86.75%	79.1%	76.66%
Extractive values	Water soluble	15%	16%	7%	8%
	Ethanol soluble	3%	5%	18%	3%
	Methanol soluble	6.25%	11.25%	16.72%	3.37%
	Chloroform soluble	0.5%	0.12%	0.08%	0.25%
	Ethyl acetate soluble	4.37%	3.12%	0.17%	1%
Ash Value	Total ash	68%	49%	87%	80%
	Acid insoluble ash	49%	34%	84%	31%

Preliminary phytochemical screening

Phytochemical screening of different extracts revealed that among the stem extracts ethyl acetate extract contains steroids and protein where as methanolic extract has shown the presence of coumarin, steroids, resin, protein and carbohydrate. Chloroform extracts has not revealed the presence of any phtyoconstituents. In leaf, methanolic extract is rich in phytochemicals such as coumarin, alkaloids, steroids, saponin, resin and carbohydrate. Only coumarin is present chloroform extract while ethyl acetate extract contains coumarins and resin. Among the fruit extracts ethyl acetate extract contains alkaloid, steroid and resin. The chloroform extract has shown the presence of alkaloids, steroids and protein. Steroids, tannin, phenol, glycoside and carbohydrate are present in methanolic extract. The phtyoconstituents present in ethyl acetate root extract are steroids, resin, protein and carbohydrate. Chloroform extract contain only protein and methanolic extract have shown the presence of steroid, resin and carbohydrate (Table IV).

Table IV: Preliminary phytochemical screening of stem, leaf, fruit and root

Tests		Stem			Leaf			Fruit			Root		
		E	C	M	E	C	M	E	C	M	E	C	M
Flavonoids		-	-	-	-	-	-	-	-	-	-	-	-
Coumarins		-	-	+	+	+	+	-	-	-	-	-	-
Alkaloids	Mayer's test	-	-	-	-	-	+	+	+	-	-	-	-
	Wagner's test	-	-	-	-	-	+	+	+	-	-	-	-
Tannin		-	-	-	-	-	-	-	-	+	-	-	-
Steroids / Terpenoids		+	-	+	-	-	+	+	+	+	+	-	+
Saponins		-	-	-	-	-	+	-	-	-	-	-	-
Quinines		-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones		-	-	-	-	-	-	-	-	-	-	-	-
Phenol		-	-	-	-	-	-	-	-	+	-	-	-
Resin		-	-	+	+	-	+	+	-	-	+	-	+
Glycoside		-	-	-	-	-	-	-	-	+	-	-	-
Protein	Xanthoprotein test	+	-	+	-	-	-	-	+	-	+	+	-
	Biuret test	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrate		-	-	+	-	-	+	-	-	+	+	-	+

Discussion

Pharmacognostic parameters are widely used for the identification of medicinal plants and also to detect adulteration in drugs [26]. Morphological features of *S.capsicoides* are similar to other related species of *Solanum* but it can be distinguished by its orange red fruit and straw coloured winged seeds [27]. The fruit type in many other *Solanum* species is berry [28] as that of *S. capsicoides*. In Solanaceae the use of fruit type as taxonomic character have proved to be systematically helpful [29]. The winged nature of the seeds enable them to disperse by wind [30] or by floating through water [31]. Seed morphology, especially the sculpturing of outer seed coat, is a powerful tool for analyzing the taxonomic relationship among plant families [32] as it has been proved to be different among the species of same genus [33-34]. Seed surface of *S.capsicoides* resembles *S. torvum* as both have convoluted cells with sinuous pattern and lack fibrils [35]. The extra axillary position of inflorescence can be found in many species of *Solanum* including *S.capsicoides*. According to Anup and Singh (1984), the inflorescence in *Solanum* shifts from axillary to extra axillary position by the activation of intercalary meristem which maintains the main axis always vegetative and the flowers are produced laterally [36].

The morpho-anatomical features of the leaf agree with the report of Ferreira *et al* (2013) [37]. The presence of leaf trichomes can be correlated with water control mechanism and defense function [38]. Non glandular trichomes act as a mechanical barrier that deters the insect movement and feeding [39]. Glandular trichomes have heads that contain various compounds which includes terpenes, flavonoids, alkaloids, acyl-sugars and defense related proteins [40] that provide protection against herbivores and pathogens [41]. The presence of glandular trichomes is a prominent character of genus *Solanum* [42]. Types of trichomes can be used as an important taxonomic tool in the intrageneric classification [43-44]. Stomatal characters are important distinguishing feature in Solanaceae [45-46]. The distribution, stomatal size and stomatal index can be used for species delimitation as it is found to be constant for certain species [47-48]. Amphistomatic leaves and the presence of anisocytic and anomocytic stomata are common in Solanaceae [42]. Leaf architectural features are powerful systematic indicators [49]. Leaf venation pattern is species specific suggesting that it is under strict genetic control [50]. The minor veins as well as the vein endings inside the areole help in the transportation of water and photosynthates [51-52]. According to Mohan and Inamdar (1994), the dilated tracheids are involved in mechanical support [53]. The presence of sandy crystals and the bicollateral vascular bundles were reported in various other *Solanum* species [54]. The functions of calcium oxalate crystals include deterring herbivore and aluminium detoxification [55]. It has been used widely for solving taxonomic problems [56-57]. The cuticle deposition pattern in the fruit wall is a changeable feature in *Solanum* [58]. The collenchymatous hypodermis which is commonly found in fruit wall of various *Solanum* species [58-59] is absent in *S.capsicoides*. The hypodermal cells are usually concerned with mechanical support and sometimes the dehiscence mechanism [60-61]. The secondary metabolites present in *S.capsicoides* can be attributed to its medicinal properties. Histochemical screening helps to easily recognize the cell compartment in which the metabolites accumulate [8, 11, 62]. The site of synthesis and accumulation differ in secondary compounds. It has been reported that the lipophilic compounds accumulate in membranes, vesicles,

extracellular sites or dead cells where as hydrophilic compounds accumulates in vacuoles [63-64]. Presence of alkaloids in members of Solanaceae has been reported earlier [42]. Studies indicate that tropane alkaloids are present in Solanaceae. It has anticholinergic activity and is used to treat smooth muscle spasms, hypersecretion and pain.Other properties of alkaloids are that they inhibit increase in blood glucose level [65] and act as precursors in the synthesis of corticosteroid drugs which have anti inflammatory property [66]. In the present study flavonoids were not detected in phytochemical screening but were shown positive in histochemical test. This may be due to the lower concentration of flavonoids. In histochemical localization transverse sections were treated with specific stain so that the components reacts directly with the stain and impart characteristic colour. Phenolic compounds have significant medicinal properties including prevention and curing of various skin disorders [67-68] and tannins are used in the treatment of kidney inflammation, diarrhoea, skin bleeding and transudates [69-70]. Coumarin compounds are very effective in prevention and curing of diseases. Its activities includes anti inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, anti-hypertensive, antitubercular, anticonvulsant, anti- adipogenic, Cytochrome P450 inhibiting, anti-hyperglycemic and neuroprotective [71]. Medicinally and agrochemically beneficial properties have been attributed to plant steroids which comprises anti tumor, immune suppressive, hepatoprotective, antibacterial, plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic and cardiotonic activity [72]. Saponin, an important glycosides has features like red blood cell coagulation, precipitation, haemolytic activity, cholesterol binding properties etc to its credit [73-75]. Resins also have antimicrobial and wound healing activity though their action confined in their chemical composition. Plant proteins form an important source of food for all living organism [69]. Singh *et al*. (2015) hypothesized that the bioactive proteins from Solanaceae have quorum quenching properties that can be utilized to establish a therapeutic strategy against virulence [76].

Conclusion

Due to diminishing supply and overuse the availability of raw drug has now become a serious problem in the Ayurvedic scenario. The pharmacognostic standards derived from this study can be used as powerful tool for the detection of adulteration and authentication of the raw drug *Solanum capsicoides* All. This will also shed light into the new areas where researchers can intervene in developing new therapeutic drugs for future use.

Conflict Of Interest

The authors have no conflict of interest.

Acknowledgements

The First author wish to thank UGC for the Junior Research Fellowship; Dr.Nesy E.A. and Dr. Jaseela F., K.K.T.M. Govt. College, Pullut for providing the trilacunar microscope facility; Dr. M.G. Sanilkumar and Ms. Nithya, SN College, Maliankara for helping in stereozoom microscopy and Mr. Jishnu, Maharajas College, Ernakulam for electron microscopic photography.

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