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PHARMACOGNOSTICAL STANDARDIZATION OF PLANT *CRYPTOLEPIS BUCHANANI* ROEM. & SCHULT. AERIAL PART

Ashutosh Meher ^{*1}, Biswaranjan Behera ² and Bikash Kumar Nanda ³

The Pharmaceutical College ¹, Barpali, Bargarh - 768029, Odisha, India.

Kanak Manjari Institute of Pharmaceutical Sciences ², Rourkela - 769015, Odisha, India.

Jayapur College of Pharmacy ³, Jayapur - 764002, Odisha, India.

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Correspondence to Author:

Mr. Ashutosh Meher

Associate Professor,
Department of Pharmacognosy,
The Pharmaceutical College,
Samaleswari Vihar, Tingipali,
Barpali, Bargarh - 768029, Odisha,
India.

E-mail: mashumeher@gmail.com

ABSTRACT: *Cryptolepis buchanani* Roem. & Schult. (Family: Asclepiadaceae) commonly-known as Wax leaved climber, Kali Sariva in English and Sanskrit. It is broadly used in Ayurveda as a remedy of ailment. Though, its diverse medicinal aspects, no detail pharmacognostical study is available till date. The present study is to investigate the pharmacognostical characteristics of *C. buchanani* aerial part. The investigation was carried out in terms of macroscopical, microscopical, leaf constant, powder behavioral with chemical reagents and fluorescence analysis by using yardstick methods. Sensory and macroscopic studies found that leaves are lanceolate, thin, spirally arranged, dark green in colour, with an astringent taste and acute apex. In transverse section, cuticularised epidermis having polygonal cells was found. Mesophyll cells were differentiated into single-layered palisade cells on each surface and 2 to 3 layered spongy parenchyma. Bowl-shaped vascular bundle in mid rib portion containing xylem and phloem tissues. The quantitative microscopy of leaves showed the presence of anomocytic stomata with cicatrix which is an identifying character. The stomatal number and stomatal indexes of the lower surface of leaves were found as 33.33 and 16.99. Leaves venation were parallel, vein islet and vein termination were calculated as 16.16 and 33.66. Powder microscopy of the plant showed the presence of unicellular and uniseriate hollow trichomes, stellate trichomes, crystal, stone cells, brown masses, pollen grain, starch grain, xylem vessels, fibres and fragment endosperms. The present investigations reveal that the presence of key diagnostic characteristics may serve to establish identity, purity and quality control standards of drugs.

INTRODUCTION: In Ayurvedic system of medicine, Sariva is a renowned and significant plant that has been broadly used since the primeval time for the treatment of different diseases and disorders of a human being.

In Ayurvedic text as well as other texts several plants are known by the name 'Sariva', that are *Hemidesmus indicus* (Linn.) R. Br. (Asclepiadaceae), *Cryptolepis buchanani* (Linn.) Roem. & Schult. (Asclepiadaceae), *Ichnocarpus frutescens* R. Br. (Apocynaceae) and *Decalepis hamiltonii* Wight and Am. (Asclepiadaceae). *Hemidesmus indicus* is known as Sweeta Sariva and *Ichnocarpus frutescens*, *Cryptolepis buchanani* are known as Krishna Sariva ¹.

Cryptolepis buchanani Roem. & Schult. (Asclepiadaceae), commonly well-known as Jambu

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Patra, Krishna Sariva in Sanskrit, Karanta in Hindi and as Wax leaved climber in English. It is a perennial, much-branched creeper with milky juice, found all over the country from Western Kashmir to Assam, ascending to 1200 m in the Himalayas and South up to Kerala^{1, 2}. Globally distributed from north of Pakistan, Nepal and Bhutan through India to Sri Lanka and Burma^{2, 3}. The plant is used as conventional medicine as anti-ulcerative, anti-inflammatory, anti-diarrhoeal, antibacterial, anti-cough, blood purifier, lactation in women³, bone fracture⁴ and also curing rickets in children⁵.

C. buchanani has been reported for the presence of phytochemicals such as cryptosin⁶, sarverogenin, isosarverogenin glycosides⁷, new nicotinoyl glucoside⁸, *cryptolepin*⁹, buchanin¹⁰ and possess antioxidant, hepatoprotective¹¹, analgesic, anti-inflammatory, chondroprotective¹², immunomodulatory¹³, cardioprotective activities¹⁴. The plant has amazing restorative potential, with this background; the present work was aimed to develop a quality standard for the aerial part to investigate macroscopical, microscopical, leaf constant, the behaviour of chemical reagents with powder and fluorescence analysis of *C. buchanani*. This would be a milestone to access the quality of the crude drug for further development.

MATERIALS AND METHODS:

Chemicals: Formalin, absolute alcohol, safranin, fast green, phloroglucinol, toluidine blue, iodine, acetic acid, Canada balsam, beeswax, H₂SO₄, NaOH, FeCl₃, KOH, Chloroform, etc. of analytical grade chemicals (E. Merck, Germany, Hi-Media Laboratories) were used for this study.

Collection and Authentication of Plant: The fresh plants were collected from the adjoining area of Barpali (Dist-Bargarh, Odisha, India) in October and authenticated as *Cryptolepis buchanani* Roem. & Schult. (Asclepiadaceae) by Botanical Survey of India, Central National Herbarium, Howrah, Kolkata, India. A voucher specimen No.CNH/I-I/17/2010/Tech.II/197 is retained in the Department of Pharmacognosy, The Pharmaceutical College, Barpali, Odisha.

Drying and Size Reduction of Plant: The aerial part of the plant was dried under shade and powdered by the help of the mechanical process.

The powder was stored in airtight container for further studies.

Sensory Evaluations: Aerial part viz. leaves, stem, flower, seed and fruit of *C. buchanani* were evaluated by the help of sensory organs for organoleptic properties. Its color, odor, taste, shape, size and other diagnostic parameters were observed and recorded^{15, 16, 17, 18}.

Microscopical Evaluations: Microscopical evaluations were done on both qualitative and quantitative basis. All evaluations were performed on trinocular compound microscope^{15, 16, 17, 18}.

Qualitative Microscopy:

Study of Transverse Sections: For qualitative microscopical evaluation, transverse section of leaf, petiole and stem were made by freehand using a razor blade. The thin sections were stained as per standard procedure and identifying characters were studied under the microscope. Different magnifications of photographs were taken with a Sony digital camera.

Powdered Microscopy: For powder microscopy, the dried powdered material was cleared with sodium hydroxide and treated with different staining reagents such as safranin, toluidine blue, fast green, phloroglucinol and iodine separately. The treated powdered materials were mounted on a glass slide in a glycerine medium. Different cellular structures and inclusions were studied under the microscope and photographs of different magnifications were taken.

Quantitative Analysis: For quantitative microscopical analysis, different leaf constants viz. stomatal number and stomatal index, vein islet and veinlet termination numbers and palisade ratio were analyzed by using standards methods^{15, 16, 17}.

Stomatal Number and Stomatal Index: Stomatal number is the average number of stomata per square mm of the epidermis and the number on each surface of a leaf. Stomatal index is the percentage of stomata from the total number of epidermal cells. Six readings of the stomatal number along with stomatal index were taken and the average values were counted.

Stomatal index can be explained as:

$$I = S/S+E \times 100$$

Where I = Stomatal index, E = Total number of epidermal cells in the same unit area, S = Number of stomata per unit area.

Vein Islet Numbers and Veinlet Termination:

Vein-islet number is the average number of vein-islet per square mm of the leaf surface midway between the central part of the lamina and margin. Veinlet-termination is the number of veinlet-termination per sq. mm of the leaf surface, midway between midrib of the leaf and its margin.

Six countings of vein-islets numbers, as well as vein terminations, were taken,

Palisade Ratio: The average number of palisade cells beneath each upper epidermal cell in the leaf, which is determined by counting the palisade cells beneath six continuous epidermal cells.

Six countings were taken and the average value was calculated.

The behaviour of Powder with Different Chemical Reagents: Aerial part of fine plant powder was placed in the watch glass having a white background, treated with several chemical reagents and changes in behavior were noted.

Fluorescence Analysis: Different reagents were treated with plant aerial part powder and observed characteristics of fluorescent colors under visible light, short (254 nm) and long (365nm) UV wavelength region^{15, 16, 17, 18}.

RESULTS:

Sensory Evaluations: The fresh leaves of *C. buchanani* are 6.2 to 15.4cm long, 2.6 to 8.1 cm wide and petiole 0.5 to 1.2 cm in length, simple, opposite, glabrous, exstipulate, elliptic-oblong to broadly lanceolate in shape, apiculate apex with entire margin and parallel venations of main nerves 26 - 30 pairs, secondary nerves numerous. The upper surface is smooth, shining deep green and the lower surface is rough, greenish-white with a characteristic odor and slightly pungent in taste.

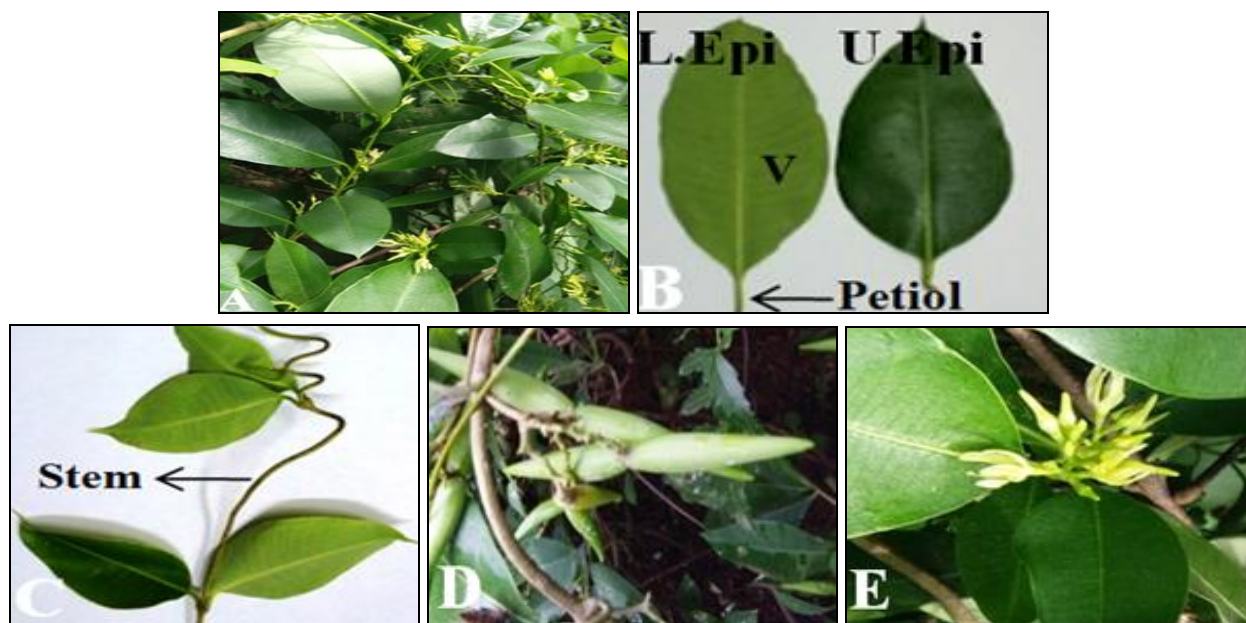


FIG. 1: A: HABIT OF *C. BUCHANANI*, B: LEAVES, C: STEM, D: FRUITS, E: FLOWERS U. EPI: UPPER EPIDERMIS, L.EPI: LOWER EPIDERMIS, V: VENATION

The stem is a green woody cylindrical climber with characteristic odor and astringent taste. Flowers are greenish-white, fragrant, tasteless in color, odour, taste and 1.0 - 1.4 cm across. Fruits of the plant are darkish green in color with slightly odour, tasteless, 4.5 - 10 cm in long, 1.2 - 1.9 cm diameter, poniard shaped, follicles 2, divaricated, somewhat compressed and glabrous **Fig. 1**.

Microscopic Evaluations:

Qualitative Microscopic:

Transverse Section:

Leaves: Transverse section through mid-rib is planoconcave covered with upper and lower epidermis. The upper epidermis consists of a single layer of thin wall rectangular to polygonal parenchymatous cells and is covered with a cuticle.

The lower epidermal layer consists of a single layer of parenchymatous cells, which are comparatively smaller in size than the upper epidermis. Both the epidermal layer devoid of any external outgrowth. Few layers of collenchymatous cells is present upper and below the epidermis. Ground tissue covers the major area of the midrib. The vascular bundle is arc shape consists of lignified xylem and non-lignified phloem. The vascular bundle is conjoint bicollateral as phloem is present on both

sides of the xylem. Lamina is dorsiventral as palisade cells are present below the upper epidermis and absent at lower epidermis. These palisade cells are small, elongated compactly arranged. Mesophyll region consists of spongy parenchyma (lose arrangement of parenchymatous cells). Few vascular bundles are seen in mesophyll region. The lower epidermis is present below the spongy parenchyma.

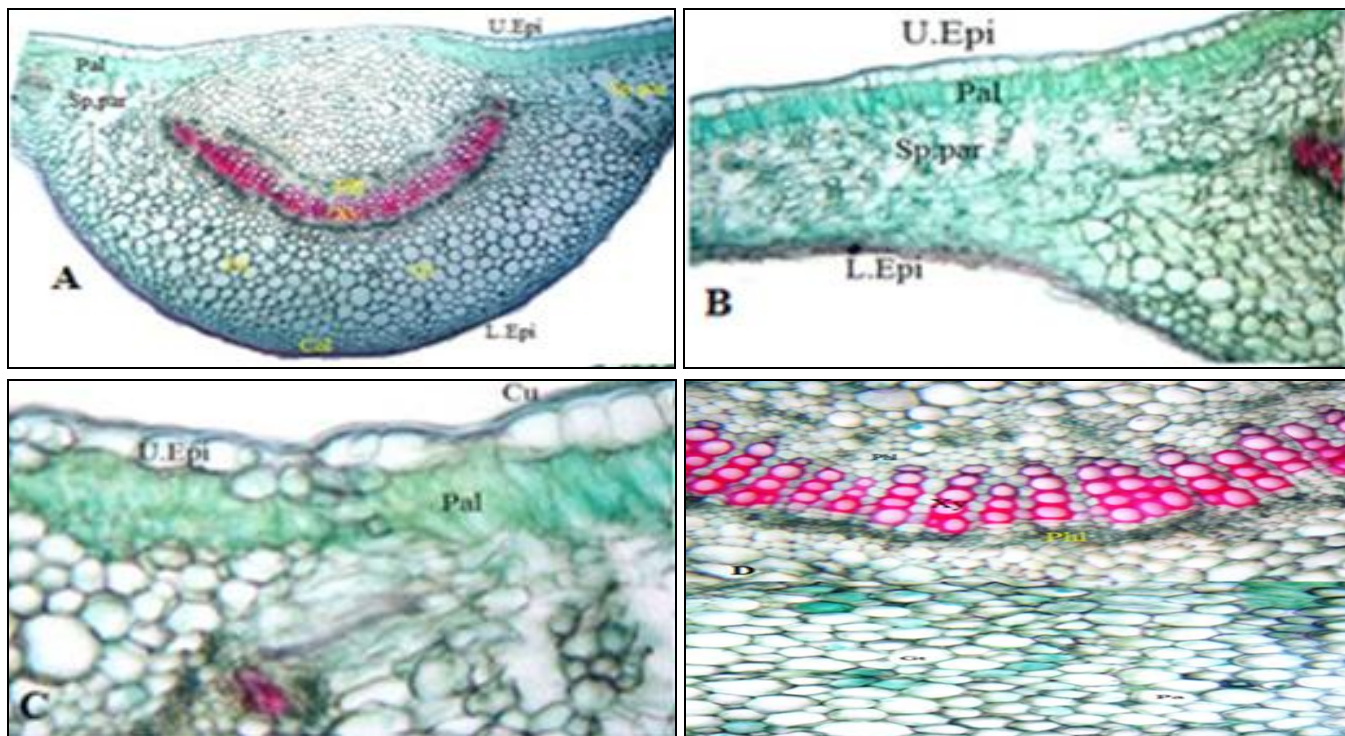


FIG. 2: A: T.S OF LEAF, B: LAMINA (5X10X), C: LAMINA (5X40X), D: MID RIB (5X40X). U. Epi: Upper Epidermis, L.Epi: Lower Epidermis, Pal: Palisade cell, Sp. par: Spongy parenchyma, Gt: Ground tissue, Col: Collenchyma, Cu: Cuticle, Pa: Parenchyma, Phl: phloem, Xy: Xylem

Petiole: Transverse section of the petiole is circular in outline with two wings at dorsal side. A single layer of epidermal layer covers the entire outline. Ground tissue occupies a major portion under the epidermal cells. Ground tissue consists of varying size of thin-walled polygonal cells without intercellular spaces. The vascular bundle is an arch shape consists of xylem and phloem. The vascular bundle is conjoint bicollateral as phloem is visible on both sides of xylem. Few calcium oxalate crystals are found within the xylem.

Stem: Transverse section of the stem is circular in outline. The outer layer shows a single epidermal layer consists of rectangular parenchymatous cells. The epidermal layer is followed by 6-7 layers of cork region. It consists of thin wall polygonal

parenchymatous cells. The vascular bundle is surrounded by a single ring of an endodermal layer. Many patches of pericyclic fiber are present below the endodermal layer. These pericyclic fibers are consists of sclerenchymatous cells which are grouped in a number of about 25-40. The vascular bundle is conjoint collateral. Xylem vessels are highly lignified due to the presence of lignin. Phloem tissue comprises 6-7 layers above xylem vessels. The central portion of the section is largely covered by pith, which contains aerenchyma and loosely arranged parenchymatous cells **Fig. 4**.

Powder Microscopic:

Leaves: Powder microscopic of *C. buchmanii* leaves revealed the presence of epidermal cells, stomata, trichomes, fibers, calcium oxalate and

starch grain. Epidermal cells found as a polygonal fragment of rectangular parenchymatous cells. Stomata are anomocytic. Trichomes are unicellular,

uniseriate covering trichomes and stellate trichomes.

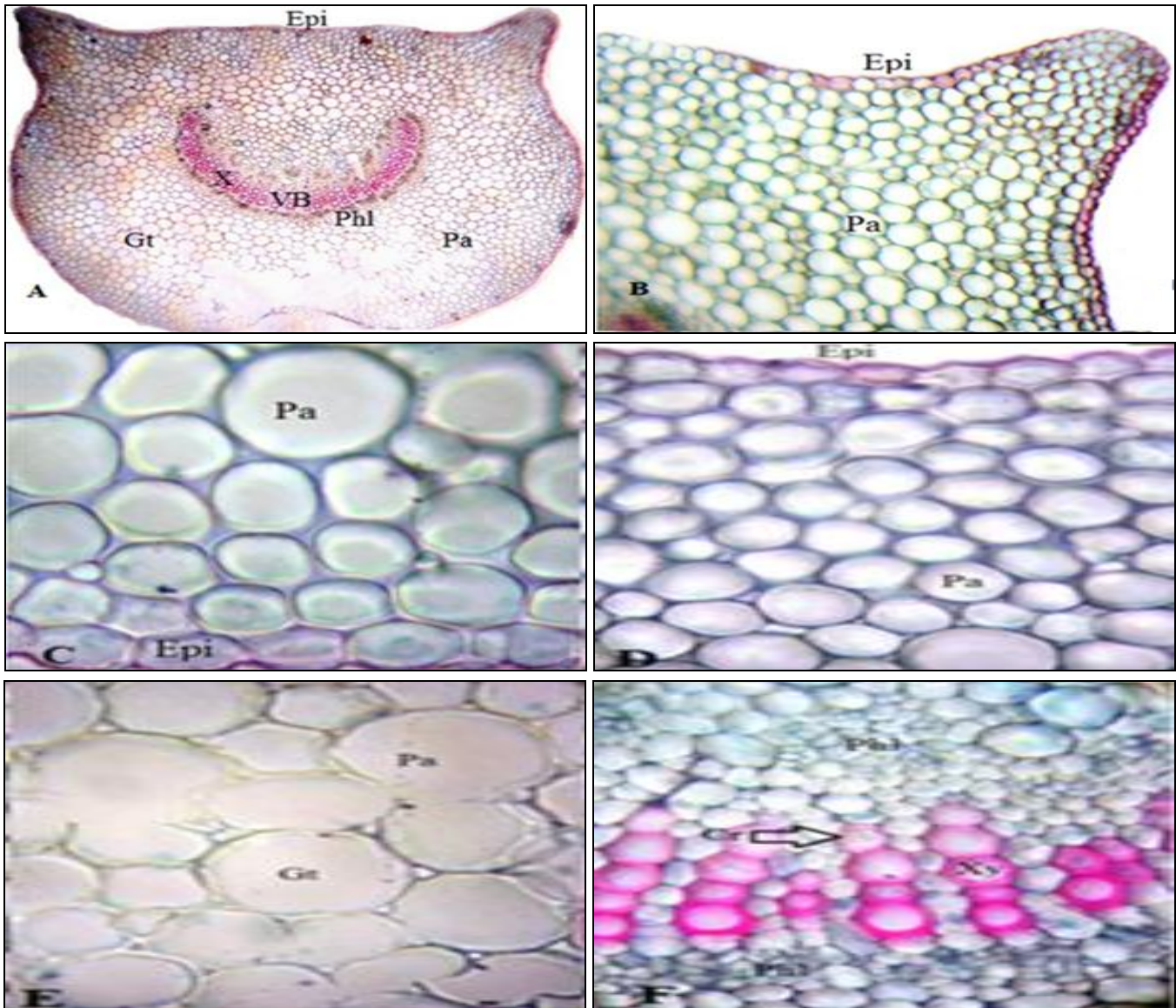


FIG. 3: A: T.S OF PETIOLE, B: T.S OF PETIOLE (5X10X), C: AND D: PARENCHYMA (5X40X). E: GROUND TISSUE (5X40X), F: VASCULAR BUNDLE (5X40X), Epi: Epidermis, VB: Vascular Bundle, Xy: Xylem, Phl: Phloem, Cr: Crystal, Gt: Ground Tissue

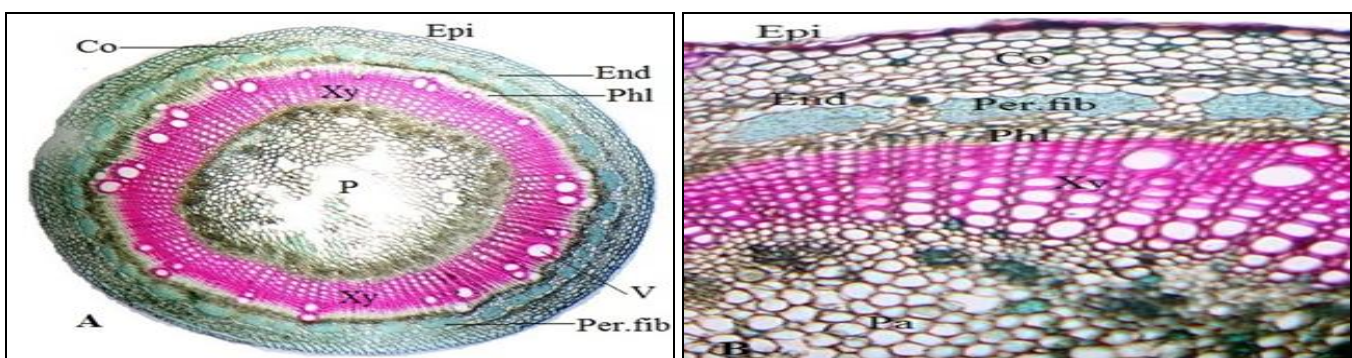


FIG. 4: A: T.S OF STEM, B: T.S OF STEM (5X10X). Epi: Epidermis, Co: Collenchyma Xy: Xylem, Phl: Phloem, P: Pith, End: Endodermis, Per. fib: Pericyclic fiber, Pa: Parenchyma, V: Vessels

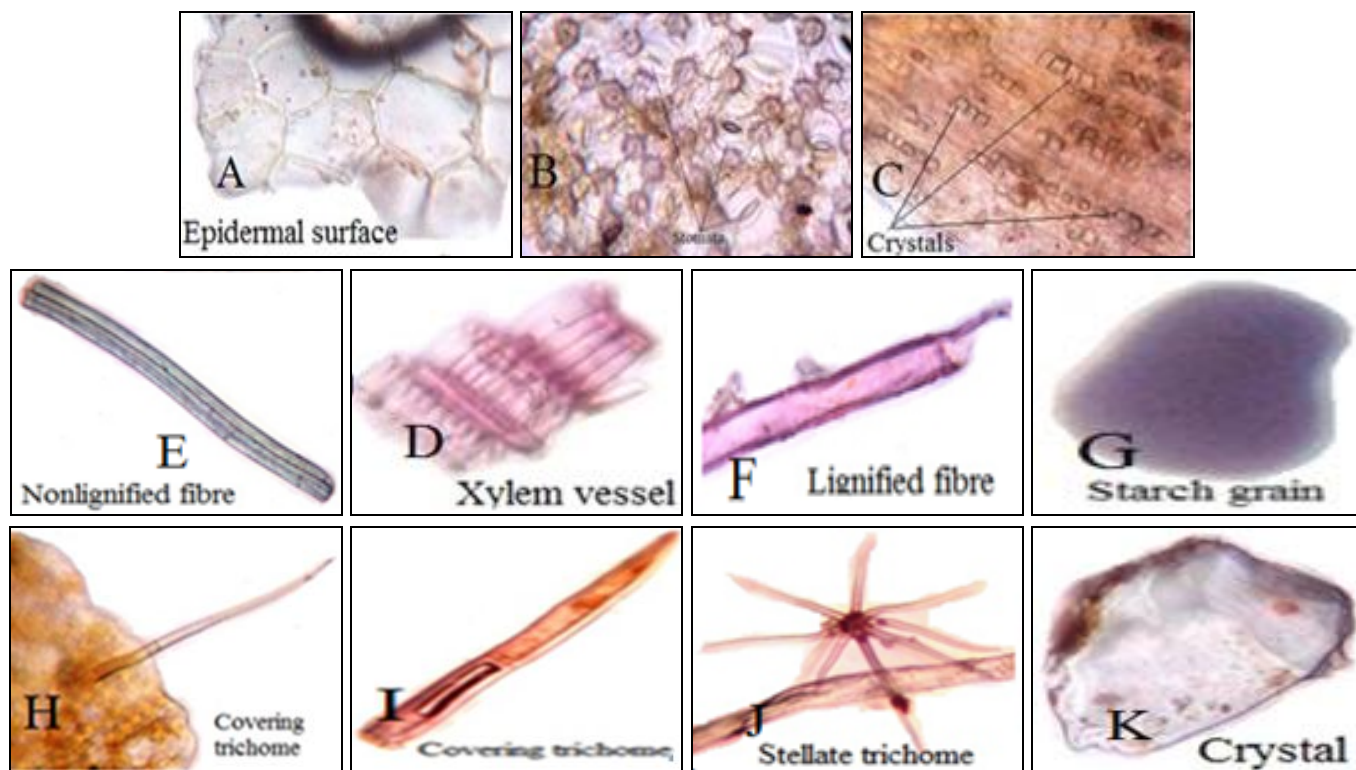


FIG. 5: POWDER MICROSCOPY OF *C. BUCHANANI* LEAVES. A: Epidermal surface, B: Stomata, C: Crystal, D: Xylem vessels, E: Non-lignified fiber, F: Lignified fiber G: Starch grain, H & I: Covering trichome, J: Stellate trichome, K: Calcium oxalate crystal

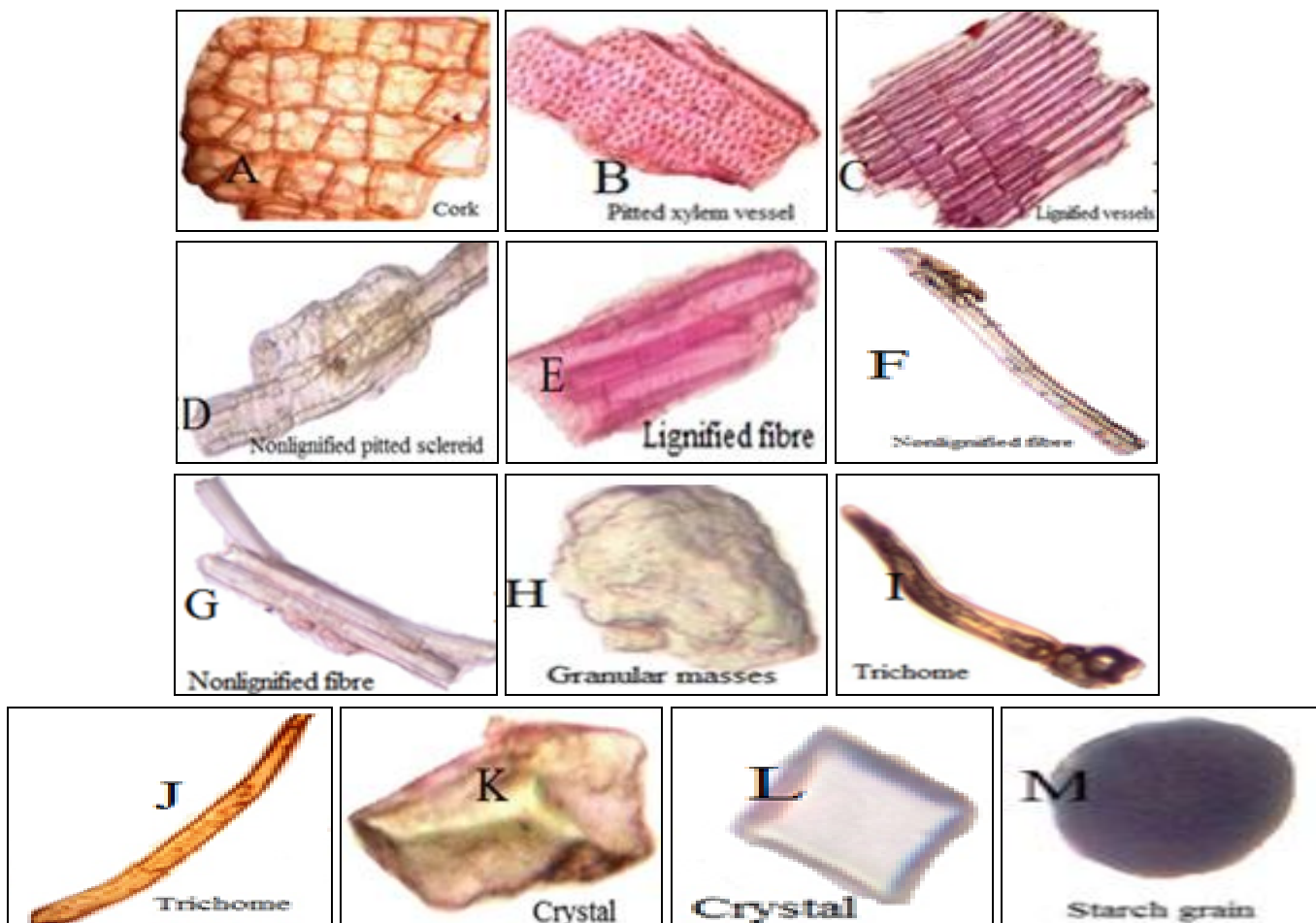


FIG. 6: POWDER MICROSCOPY OF *C. BUCHANANI* STEMS, A: Cork, B: Pitted xylem vessels, C: Lignified vessels, D: Non lignified pitted sclereid, E: Lignified fiber, F & G: Non-lignified fiber, H: Granular masses, I & J: Trichome, K & L: Crystal, M: Starch grain

Lignified fibers with thick wall, wider lumen and non-lignified fibers with thick-walled with narrow lumen and spiral or annular xylem vessels were observed. Microscopic powder features also revealed the presence of starch granules and prismatic calcium oxalate crystals embedded within the epidermal cells **Fig. 5**.

Stems: Powder microscopic of *C. buchanani* stem showed the presence of cork cells, pitted xylem vessels fibres, trichomes, calcium oxalate, granular mass and starch grain. Fragment cork cells found as brownish to red rectangular. Trichomes were unicellular, uniseriate covering trichomes with thick-walled and blunt apex. Also lignified pitted

xylem vessels, non-lignified pitted sclereid, lignified fibers (single and in groups), non-lignified phloem fibers (single and group), calcium oxalate crystals, few granular masses, spherical shape Starch grains were found in powder **Fig. 6**.

Flowers: Powder microscopic of *C. buchanani* flowers showed the presence of few pollen grains, trichomes, brownish granular mass, crystal, lignified fibers, non-lignified fibers, fragment epidermal surface, xylem vessels and starch grain. Trichomes of varying size, prismatic calcium oxalate crystals, lignified xylem vessels, the spherical shape of starch grains embedded within parenchymatous cells were found in powder **Fig. 7**.

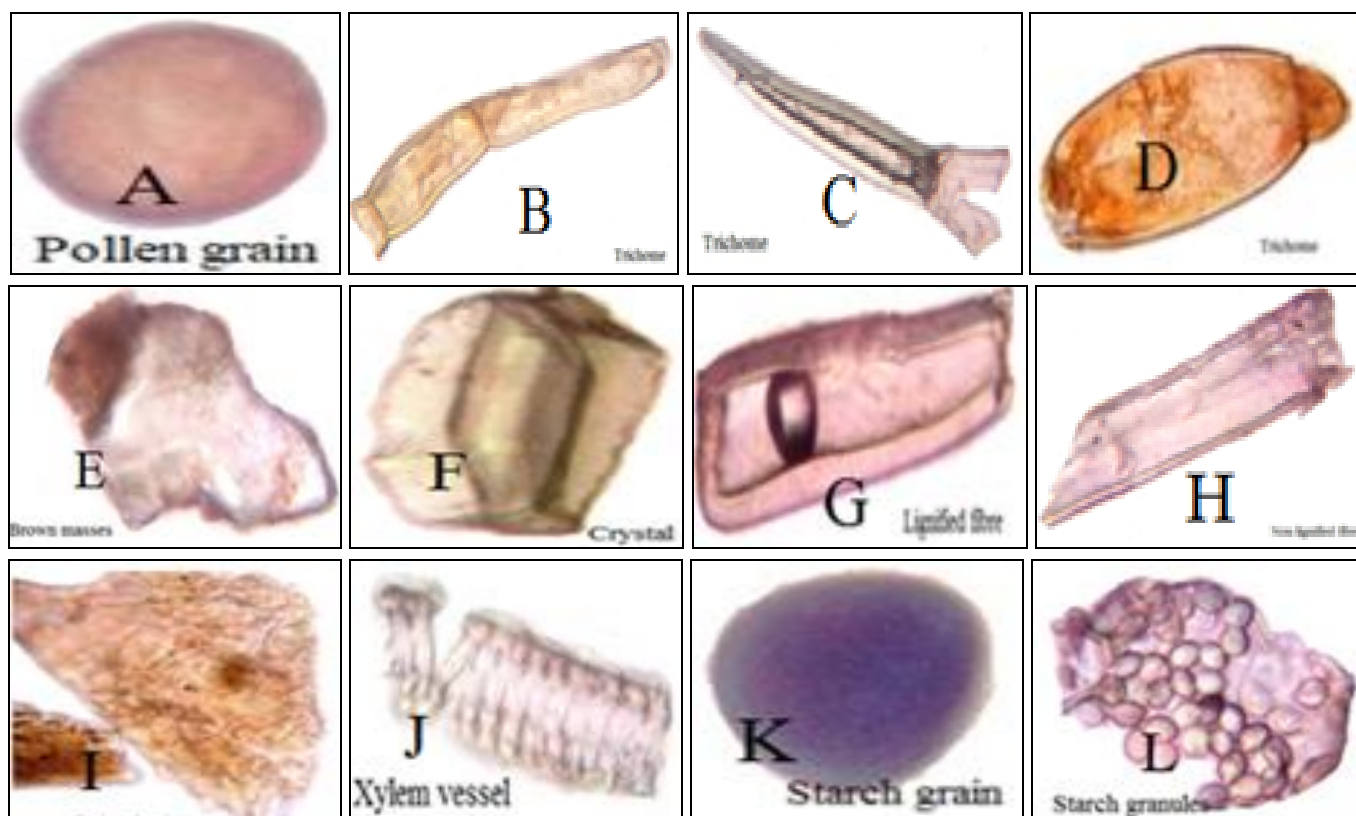


FIG. 7: POWDER MICROSCOPY OF *C. BUCHANANI* FLOWERS. A: Pollen Grain, B & C: Trichomes, D: Trichomes Fragment, E: Brown Masses, F: Crystal, G: Lignified Fibre, H: Non-lignified Fibre, I: Epidermal Surface, J: Xylem Vessels, K: Starch Grain, L: Starch Granules

Fruits: Powder microscopic of *C. buchanani* fruits showed the presence of sclereids, yellow epidermal cells, fragments of epicarp and mesocarp, xylem vessels, fibers, calcium oxalate crystal, granular mass and starch grain. Pitted wall with the narrow lumen of sclereids, spiral and pitted xylem vessels, lignified and non-lignified phloem fibers, brownish granular masses, spherical shape starch grains and fragments of endosperm were found in powder **Fig. 8**.

Quantitative Microscopy: Anomocytic stomata with cicatrix (identifying character) were present on the lower surface of leaves. The stomatal number was found as 33.33.

The stomatal indexes of the lower surface were found 16.99. Leaves venation were parallel, vein islet and vein termination were calculated as 16.16 and 33.66. The palisade ratio was found to be 1: 9.43 **Fig. 9**.

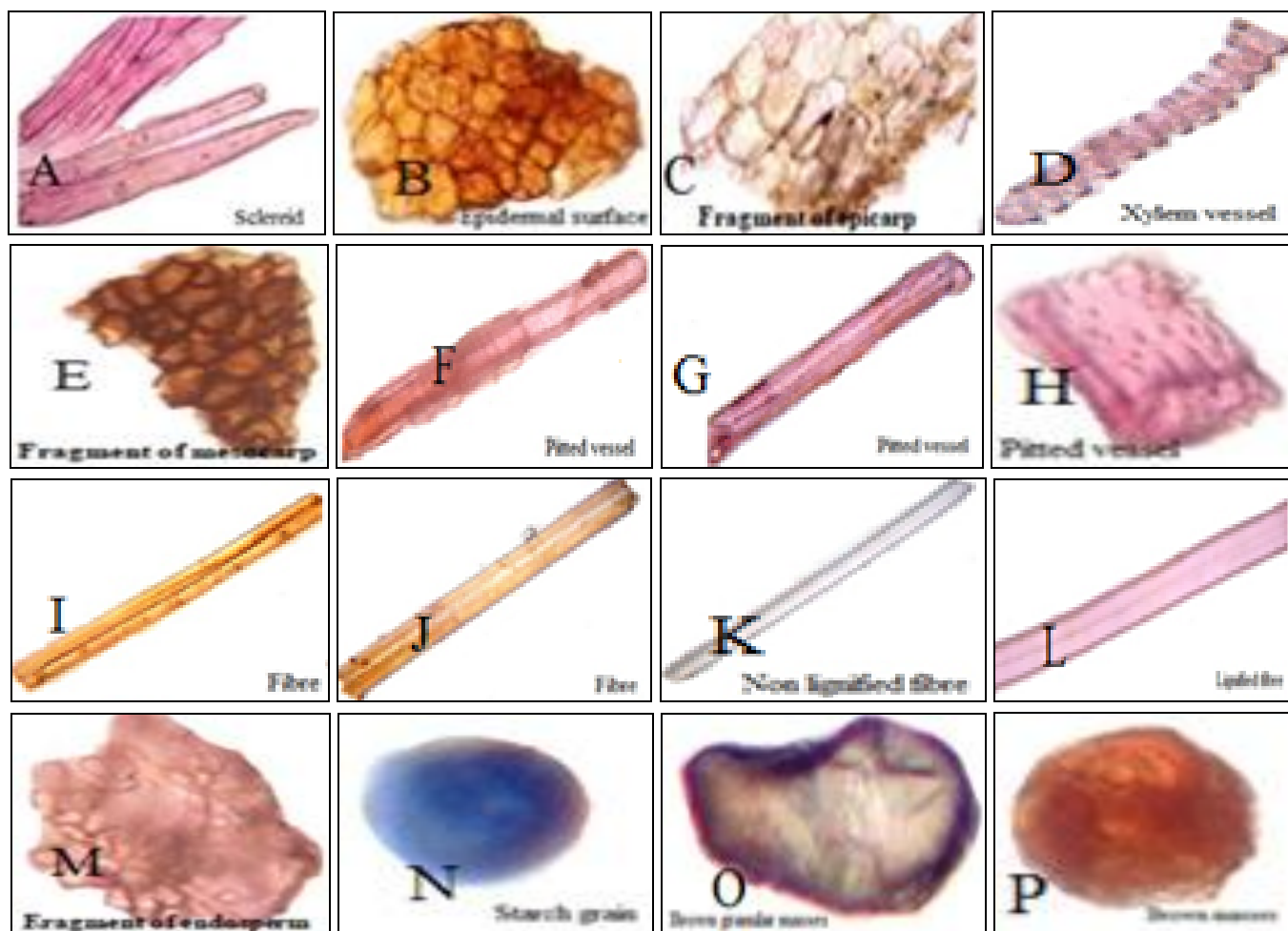


FIG. 8: POWDER MICROSCOPY OF *C. BUCHANANI* FRUITS. A: Sclereid, B: Epidermal Surface, C: Fragment of Epicarp, D: Xylem Vessels, E: Fragment of Mericarp, F, G & H: Pitted Vessels, I & J: Fibre, K: Non-lignified Fibre, L: Lignified Fibre, M: Fragment of Endosperm, N: Starch Grain, O: Brown Granular Mass, P: Brown Masses

Behavior of Powder with Different Chemical Reagents: Behavior of *C. buchanani* aerial part powder with different chemical reagents was performed to detect the phytoconstituents. The observed color changes under ordinary daylight were tabulated in **Table 1**, **Fig. 10**.

Fluorescence Characteristics: Fluorescence characteristics of *C. buchanani* aerial part powder were treated with some chemical reagents and observed in daylight and under ultraviolet light. The results were recorded in **Table 2**.

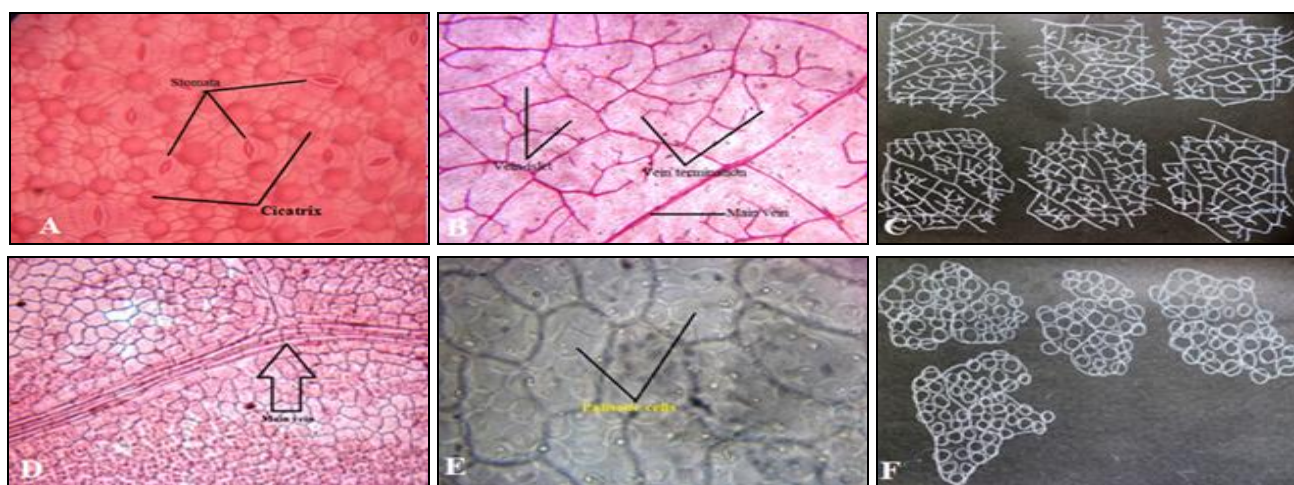


FIG. 9: LEAVES CONSTANT OF *C. BUCHANANI*. A: Stomata, B: Vein Islet and Veinlet Termination, C: Hand Sketch of Vein Islet and Veinlet Termination, D: Epidermal Surface, E: Palisade Cells, F: Hand Sketch of Palisade Cells

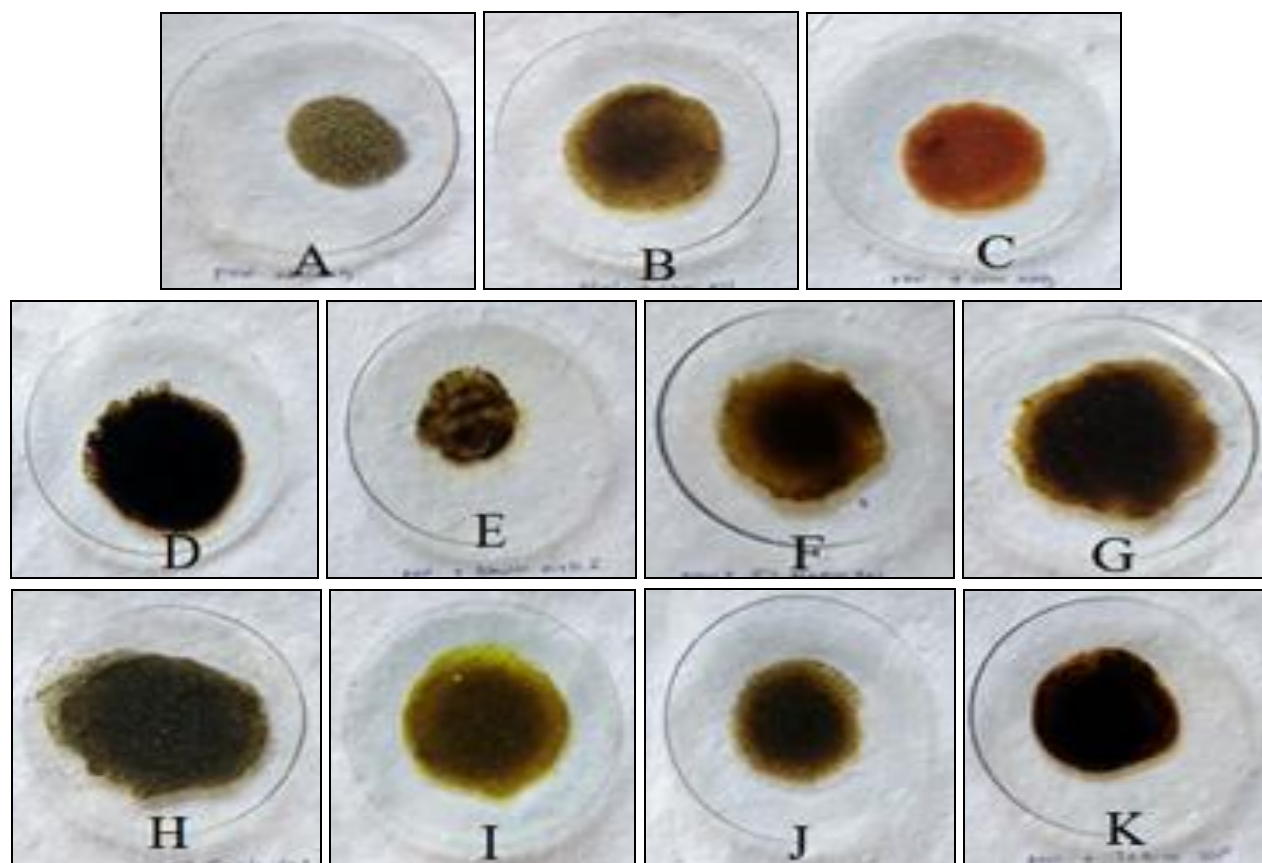


FIG. 10: BEHAVIOUR OF C. BUCHANANI AERIAL POWDER WITH DIFFERENT CHEMICAL REAGENTS. A: Powder as such, B: Powder with conc. HCl, C: Powder with conc. HNO₃, D: Powder with conc. H₂SO₄, E: Powder with Glacial CH₃COOH, F: Powder with 5% NaOH solution, G: Powder with 5% KOH solution, H: Powder with 5% FeCl₃ solution, I: Powder with Picric acid, J: Powder with Ammonia, K: Powder with Iodine sol.

TABLE 1: BEHAVIOUR OF POWDER OF C. BUCHANANI AERIAL PART WITH DIFFERENT CHEMICAL REAGENTS

Reagents	Color observed
Powder as such	Grayish green
Powder + Conc. HCl	Yellowish Green
Powder + Conc. HNO ₃	Orange
Powder + Conc. H ₂ SO ₄	Blackish brown
Powder + Glacial CH ₃ COOH	Light green
Powder + 5% NaOH sol.	Golden yellow
Powder + 5% KOH sol.	Yellowish-brown
Powder + 5% FeCl ₃ sol.	Deep green
Powder + Picric acid	Fluorescence yellow
Powder + Ammonia	Yellowish Green
Powder + Iodine sol.	Reddish-brown

TABLE 2: FLUORESCENCE ANALYSIS OF POWDER OF C. BUCHANANI AERIAL PART

Reagents	Daylight	Fluorescence Observed	
		Short wavelength	Long-wavelength
Powder as such	Grayish green	Green	Deep green
Powder + 1N NaOH in methanol	Greenish gray	Green	Greenish black
Powder + 1N NaOH in water	Golden yellow	Fluorescence green	Deep green
Powder + 50% HCl	Light gray	Fluorescence green	Deep green
Powder + 50% Sulphuric acid	Brownish gray	Green	Black
Powder + 50% Nitric acid	Orange	Fluorescence green	Dark brown
Powder + Petroleum ether	Green	Deep green	Deep brown
Powder + Chloroform	Green	Green	Deep brown
Powder + Picric acid	Fluorescence green	Fluorescence green	Greenish black
Powder + 5% FeCl ₃ sol.	Deep green	Blackish grey	Black
Powder + 5% Iodine sol.	Deep brown	Dark brown	Black
Powder + Methanol	Deep grey	Deep green	Brownish black
Powder + (HNO ₃ + NH ₃)	Brown	Fluorescence green	Coffee

DISCUSSION: In the traditional system of medicine, different parts of *C. buehanani* are used to cure diverse ailments, but there are no such reported pharmacopoeial standards for its identification and evaluation. So the standardization of this plant is necessary to assure identity, purity, quality and also safety uses of a human being. Hence, in the present study, the aerial part of *C. buehanani* was investigated for its pharmacognostical aspect. The study deals with sensory, macroscopy, qualitative microscopy, quantitative microscopy, the behavior of powder with different chemical reagents and fluorescence analysis. The sensory characters and macroscopical characters of the aerial part of the plant can provide as a diagnostic parameter. Qualitative analysis is one of the important methods to identify the plants or drugs correctly and the indemnity of raw material. Leaf constants like a stomatal number, stomatal index, vein islet number, veinlet termination number and palisade ratio are helpful to identify as well as establish a quality standard of the plant. The behavior of powder with different chemical reagents and fluorescence characteristics of powder is vital diagnostic parameters for identification of the plant, detection of adulterants and also the presence of phytoconstituents, which are important in drug evaluation tool along with the development of quality parameters of the species.

CONCLUSION: The present investigation may serve as distinguishing parameters to identify, decide the authentication and standardization.

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CONFLICTS OF INTEREST: None

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