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ORIGINAL ARTICLE **OPEN ACCESS**

Preliminary Pharmacognostic and Phytochemical Screening of *Crinum solapurense*

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ABSTRACT
Pharmacognostic research primarily aids in plant identification and establishes standard principles that not only aid but also prevent adulteration. Pharmacognostic research will aid in the authentication of medicinal plants and assure reproducible quality of plant products, resulting in plant efficacy and safety. The primary concern with synthetic medications is the occurrence of unfavorable side effects that are bad for the environment as well as people. Plant-based medications have the advantages of being widely available, having few or no adverse effects, and being affordable. The fact that there are numerous sources of adulteration is a drawback. Based on their efficacy, plant-based drugs are in higher demand. Plant-based drugs are increasingly in demand; hence they are frequently contaminated with low-quality plant ingredients to suit that demand. As a result, an effort was made to investigate the pharmacognostic parameters and physiochemical parameters like total ash, water soluble ash, acid insoluble ash, water soluble extractive value, and alcohol soluble extractive value etc. of Crinum solapurense.
Keywords: *Physico-chemical investigation, phytochemical investigation, Pharmacognostical, Crinum solapurense.*

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INTRODUCTION

The name of the Solapur district in India is thought to have originated from the two terms "sola," which means "sixteen," and "pur," which means "village." The area is primarily an agricultural region, and the plains of the Bhima, Sina, and Man Rivers are home to a variety of natural resources. The district generally has a dry and harsh environment. Tropical dry deciduous woods, open scrub forests, and large grasslands make up the vegetation.[1] The medications derived from natural sources are once again receiving attention because they are largely regarded as safe and devoid of negative side effects. Recent years have seen a rise in the importance of studying medicinal plants from a pharmacognostic perspective. Pharmacognostic research primarily aids in the identification of plants and establishes the standards parameters that not only aid but also guard against adulterations. Described from a marsh along the Bhima River in the Solapur district of Maharashtra State, India, *Crinum solapurense* is a new species of plant. It resembles C. lorifolium Roxb. ex Ker Gawl and C. viviparum (Lam.) R. Ansari & V. J. Nair var. viviparum. However, it varies in possessing 1–10 bulblets on the mother bulb, solid, canaliculate 12-27 leaves, 10–30 flowering umbels, an undivided stigma, and 3–12 seeded fruits that are members of the Amaryllidaceae family.[2]

Fig: 1 *Crinum solapurense* plant

MATERIAL AND METHODS

Collection of plant materials

In the Solapur district of Maharashtra, India, the entire plant *Crinum solapurense* was harvested from the Bhima River between the Machnur villages.

Plant identification

The stage of plant collecting and plant authentication is finished, which is crucial for subsequent work. Therefore, Dr. D. L. Shirodkar, Botanist, BSI, WRC, Pune-1, recognized and verified the species *Crinum solapurense* from the Pune branch of the Botanical Survey of India. [Botanical Survey of India, Western Regional Center, 7- Koregaon Road, Pune-411001 (Government of India, Ministry of Environment, Forests & Climate Change).]

Collection of the leaves

Dr. D. L. Shirodkar, a plant taxonomist, identified and verified the plants before separating the leaves from the *Crinum solapurense* plants. Selected medicinal plant components (Crude medicine) were broken up into little pieces, washed, and allowed to dry out in the shade at room temperature before being tested physically using various metrics. The plant material is manually screened for impurities and dried in the shade. Then, these chosen medicinal plant parts were subjected to size reduction to obtain coarse powder, separately, in a mechanical grinder and then passed through sieve no. 40 to obtain desired particle size. The homogeneous powder was then standardized using several factors. Until its use, these tiny leaf powders were kept apart in a cold, dry location.

Extraction of leaf material

250 g of powdered plant material will be treated to Soxhlet extraction using aqueous, ethanolic, or methanolic solvents, or any other appropriate solvents, utilizing a Soxhlet extractor at a temperature not exceeding 50°C in order to prepare different leaf extracts. Prior to usage, the extracts were concentrated, dried using a rotary evaporator, and kept in a refrigerator at 4°C.

PHARMACOGNOSTIC STUDIES

Morphological characters: [2, 3]

It is the simplest way to determine a drug's identity and guarantee its quality. Using a magnifying glass or the naked eye, one can see the macroscopic characteristics of medicinal plants. The habit, bract, and leaf base, shape, flower, stamen, gynoecium, papillate stigma, fruiting inflorescence, seeds, size, shape, color, surfaces, venation, presence or absence of petiole, apex, margin, base, odor, and taste are all observed in detail in fresh leaves of Crinum solapurense. Table 1 lists the outcomes.

Microscopic evaluation of the leaf [4]

Transverse section (T.S.) of Leaf

Fresh leaves were submerged in water while pieces were cut at random angles via the midrib. Glycerine was used to glue fine sections to glass slides without the use of any staining agents, and the slides were examined under a microscope. The sections were stained with the staining agent Phloroglucinol so that cellular components and other distinguishing features, such as stomata, could be seen.

PHARMACOGNOSTIC STUDIES FOR LEAVES [3, 4, 5, 6]

Leaf Quantitative Microscopy:

In epidermal strips, it contains the palisade ratio, stomata number, stomata index, vein-islet number, and vein termination number. Table 2 presents the findings.

Estimation of the stomatal number and stomatal index

i. Stomatal number

The average number of stomata per square millimeter is referred to as the stomatal number of the epidermis of a leaf. The ratio of a leaf's total number of stomata to its total number of epidermal cells is known as the stomatal index. It refers to the typical number of stomata per square mm of the leaf's epidermis.

ii. Stomatal index

Stomatal fraction is the ratio of stomata to total epidermal cells (including stomata) in a unit area of leaf.
It is calculated by using this formula:

S. I = S/E + S x 100

Where,
S. I = Stomatal Index,
S = No. of stomata per unit area,
E = No. of epidermal cells in the same unit area.

iii. Estimation of palisade ratio

Each epidermal cell on a leaf fragment was examined for the presence of palisade cells. The palisade layer beneath the epidermal cells was then marked off on drawing paper using a camera lucida. From various leaf slices, four epidermal cells with five groups each were counted, and the average number was determined as the palisade ratio. Each epidermal cell has an average of this many palisade cells beneath it.

iv. Estimation of vein termination and vein-islet number

Leaf constants such veinlet termination and vein-islet number were seen in Per mm^2 of leaf surface between the edge and the midrib, Black paper and Lucida were set up for the drawing camera. The field's center was created as a 4 mm square. Traces of the square's veins, including overlapping islets between neighboring sides, were made. The four adjacent squares' average vein islet count and veinlet termination were noted.

Vein-islet number

It is calculated by counting the vein-islets in a 1 square mm area of the leaf's center, which is located between the midrib and the margin.

Vein termination number

The number of vein terminations per square millimeter of the leaf surface, halfway between the midrib and the margin, is known as the vein termination number. The T. S. of the leaf (Fig. 2 and 3 a, b, c, d, e, f) shows that the upper and lower surfaces of the leaf each have a single layer of epidermis, which is followed by a thick cuticular layer. The upper and lower epidermises are both thick. There is a layer of parenchyma below this. Following this layer, the vascular bundles are encircled by lignified reticulate parenchyma. The adaxial surface of the mid rib area has cells that are about circular in form but somewhat columnar elsewhere. Paracytic stomata exist. Lower mesophyll cells are 12–15 cells thick, moderately chlorophyllous, thin-walled, iso-diametric in shape, and spongy in arrangement. Upper mesophyll cells are single layered, thin-walled columnar in shape, arranged with no intercellular spaces (that is, palisade), and have many chloroplast. Phloem surrounds the xylem in a concentrated vascular bundle of the amphicribal type that is distributed along the breadth of the leaf blade. In studies of the leaf epidermal surface, emphasis was placed on features like the morphology of the stomata, the epidermal cells, and the type of stomatal complex. These findings were documented in a schematic showing guard cells and stomata (Fig. 4). Abaxial and adaxial epidermal stomata layers are shown in Figure 5(a) (Figure 5(b) and (c)). Leaf surfaces are glabrous (i.e., hairless) on both the abaxial and adaxial epidermis, and the stomata are of the paracytic type, that is, they are bordered by one or more pairs of lateral subsidiary cells that are parallel to the guard cells. The stomatal shape is elliptic with mean length about twice the mean breadth on both surfaces; classified as generally small (that is, mean length less than 15 m); density, significantly higher on Adaxial than the Abaxial leaf surface and a corresponding significantly larger guard cell area on the adaxial surface, but equal stomatal indices on both. Epidermal cells are tabular in shape with 6 or 7 sides; density/mm^2, significantly higher on the Adaxial surface.

Physico-chemical analysis

Foreign Organic Matter Extractive Values, Water-Soluble Extractive Values, Alcohol-Soluble Extractive Values, Ash Values, Total Ash Content, Acid Insoluble Ash, Moisture Content (Loss on Drying), etc. are some of the characteristics that are evaluated. Table 3 presents the findings.

Ash value determination:

Ash value is a crucial standardized indicator for assessing the quality and purity of unprocessed plant material. After incineration, total ash is made up of leftover materials, including inorganic salts such silicates, carbonates, and phosphates of calcium, potassium, sodium, and magnesium. Two grams of air-dried *Crinum solapurense* leaves were precisely weighed and burned at 450^0C in six tarred silica crucibles to determine the total amount of ash. Separately, water (25 ml) was heated with the ash in the first three crucibles. Water-insoluble ash was collected using ashless filter paper and burned at 450°C after being washed in hot water to a consistent weight. Separately, 25 ml of 2M HCl were used to boil the ash in the remaining three silica crucibles. The acid-insoluble ash was similarly collected using ashless filter paper and burned at 450°C after being washed in warm water to a consistent weight. The mean Ash value for *Crinum solapurense* is displayed in Table 3.

Preliminary phytochemical analysis [3, 4, 7, 8]

Standard techniques are used to test the various *Crinum solapurense* extracts for various phyto components. Alkaloids, flavonoids, tannins, phenols, cardiac glycosides, steroids, and saponins are among the substances that are typically examined for in them. Table 4 presents the findings.

Powder microscopic characters of *Crinum solapurense* [3, 4]

Crinum solapurense powder is typically green in color, contains calcium oxalate crystals of the acicular type, cell inclusions, trichomes, stomata, diacytic stomata, stomata carrying epidermal cells, and starch grains. Fig. 6 displays the powder properties of *Crinum solapurense* from (a to l).

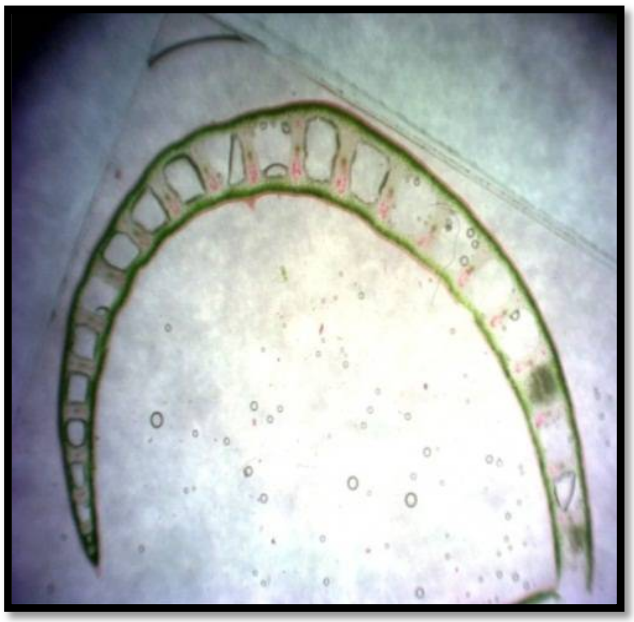
RESULTS AND DISCUSSION

Crinum solapurense

Crinum solapurense S. P. Gaikwad, K. U. Garad & R. D. Gore sp. nov. observes the following characters and it reports exactly like Crino viviparo similis, bulbis principalibus surculis 1 to 10, foliis, canaliculatis firmis, umbelli 10 to 30 floris and fructibus 3 to 12 spermis differ. Type: Solapur, Maharashtra, and India. Woody, conical, 5–7 cm long; roots thick, 1 cm or less in diameter; basal plate. Conical or elongated, laterally compressed or globular ellipsoid bulbs, measuring 10 to 20 8 to 15 cm, are either white or pale pink before turning deep pink when exposed to light; the neck, which is cylindrical and up to 30 cm long, contains 1 to 10 bulblets. Leaves are newer than the flowers, radical, strong, 12–27, lanceolate, 40–85 5–7 cm, broad at the base, The cross section of the leaf shows chlorophyllous cells in rings around the air channels, vascular bundles alternating with fiber bundles, and deeply channeled, canaliculate or U-shaped edges. Scapes growing laterally from bulbs are compressed, 50–95 cm long, and greenish purple. Bracteoles are linear, 4 to 6 cm long, and pale white in color. Involucres bracts are membranous, 6 to 8 4 to 5 cm, deltoid, scarious, and weakly ribbed. 10 to 30 flowering umbels. Flowers are pedicellate, radially symmetric, and have a pedicel up to 1 cm long. The perianth tube is 9 to 14 cm long, bluntly angled, and straight at anthesis. There are six lance-shaped perianth segments, each measuring 4 to 7.5 cm long and 1.3 to 1.6 cm wide. Stamens six, upright at anthesis; filaments four to five and a half to five centimeters long, shorter than the lobes of the perianth; anthers deep purple, flexible, linear, up to 1.5 centimeters long; pollen yellow, ellipsoidal, echinulate. Styles can be up to 9 cm long, with white below the style's purple apex. Three to twenty-two capsules per infructescence, irregularly globose, membranous, juvenile green, dark purple at maturity, beaked by the persistent perianth tube base of seven to nine centimeters in length. 3 to 12 irregularly shaped, chlorophyllous seeds.

Table 1: Morphological characteristic of *Crinum solapurense* leaves

Leaves	12 – 27, sturdy, lanceolate, narrow towards apex, broad at base, 40 – 85 × 5 – 7 cm, deeply channelled, canaliculate or U–shaped, dull green
Vegetative propagation	1 – 10 lateral offsets (daughter shoots) on the mother bulb
Bulbs	conical or globular-ellipsoid, 10 –20 × 8 – 15 cm, white – pale pink, turning into deep pink when exposed to light
Seeds	3 – 12 per capsule, chlorophyllous
Style	much longer than the stamens
Perianth segments	lanceolate, spreading or recurved
Stamens	erect at anthesis
Umbels	10 – 30-flowered
Stigma	undivided



Microscopic evaluation of the leaf

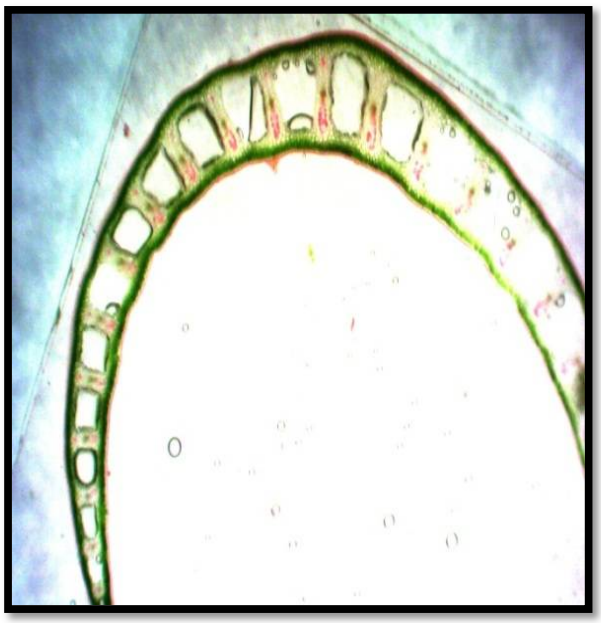


Fig.2. Transverse section of leaf

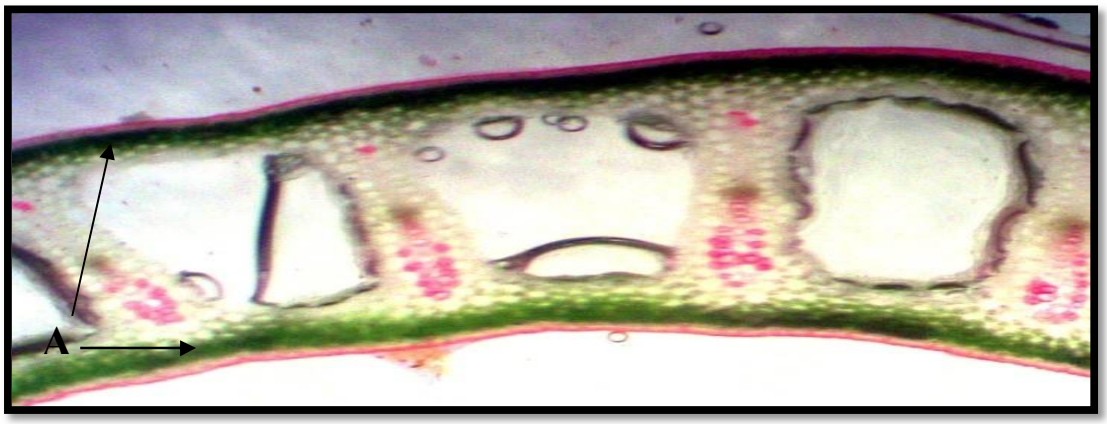


Fig.3 (a)

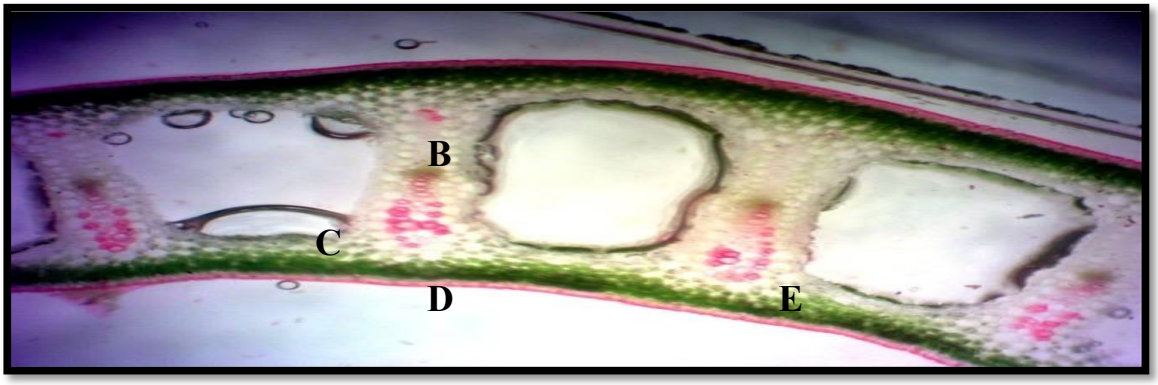


Fig.3 (b)

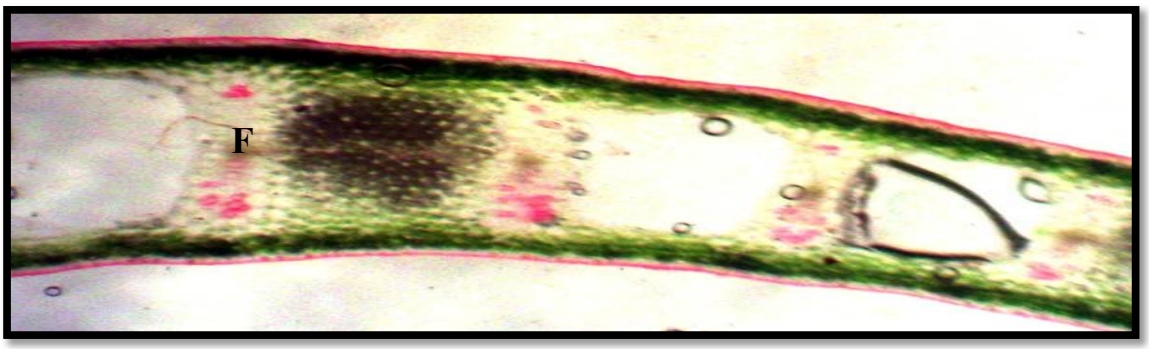


Fig.3 (c)

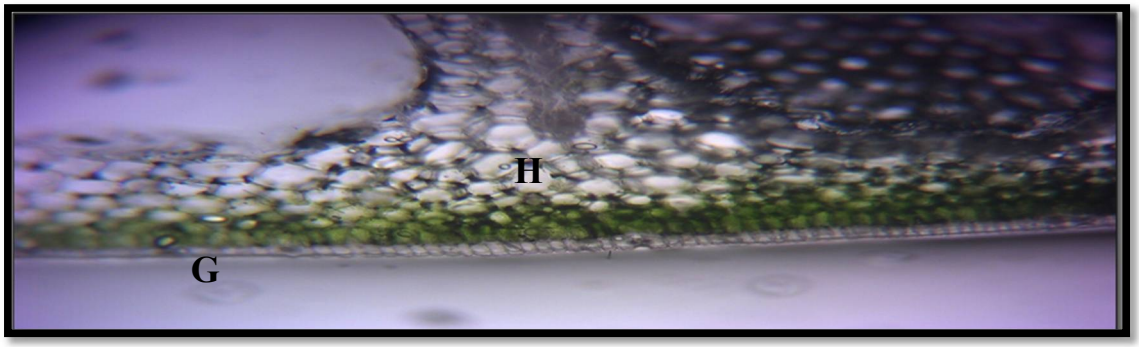


Fig.3 (d)

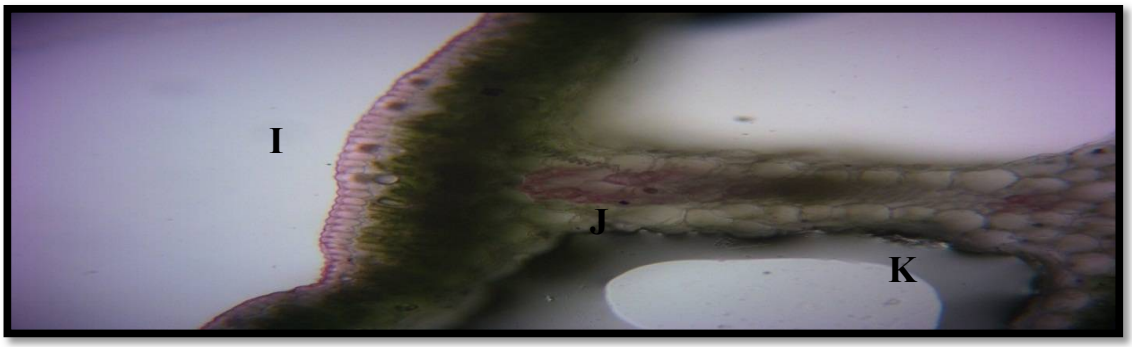


Fig.3 (e)

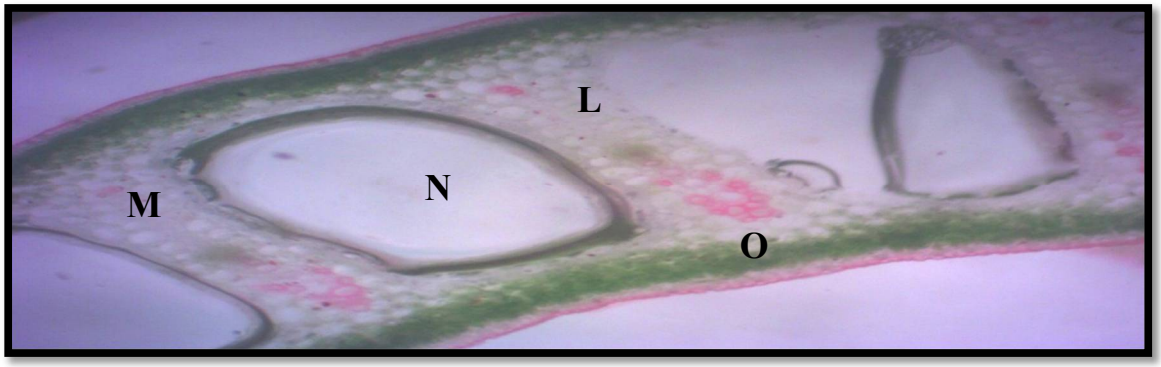


Fig.3 (f)

Fig:3 – (a) A-Epidermal Layer (Upper &Lower-Thick cutical), (b) B–phloem, C–metaxylem, D–protoxylem, E-xylem, (c) F–vascular bundle, (d) G–cuticle, H–palisade mesophyll, (e) I: Epidermis, Rectangular Cells With Distinct Cuticle; J: Stomata; K: Spongy Mesophyll, (f) L: Palisade Cell M: Spongy Parenchyma; N: Air Space; O: Vascular Bundle, More Towards Ventral Surface

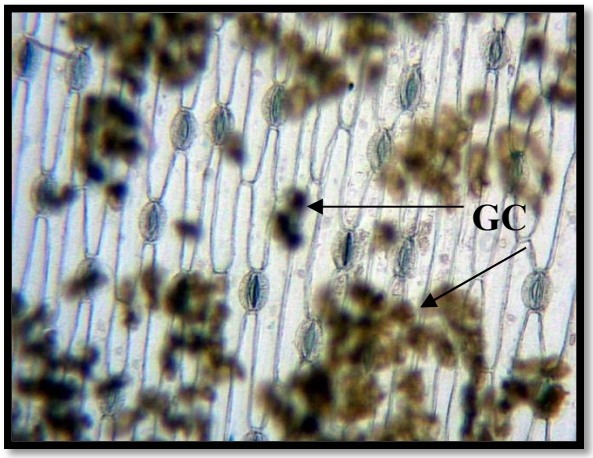


Fig 4.Stomata

Fig.5 (a) Guard Cells (gc)

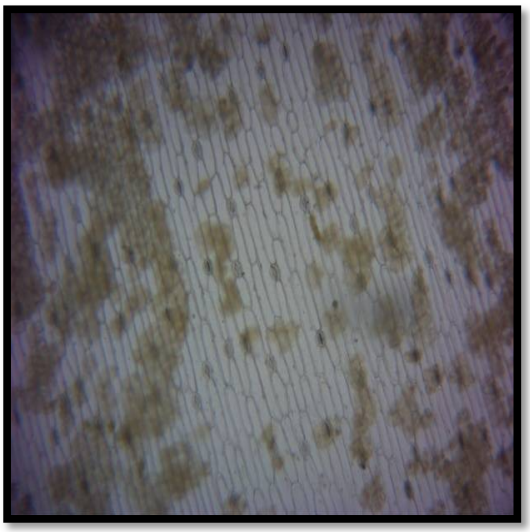


Fig.5 (b) Stomata on Abaxial epidermis

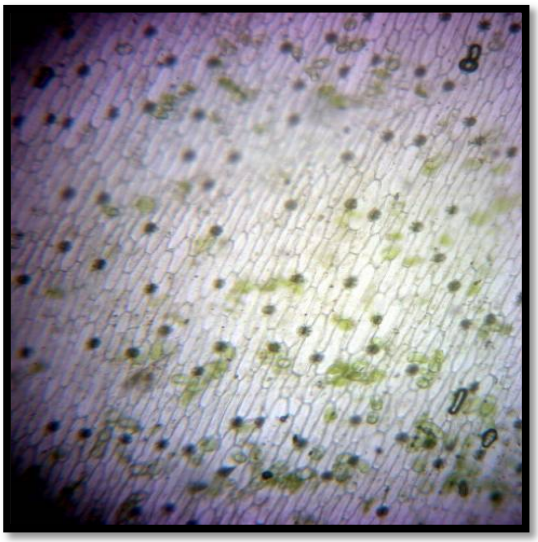


Fig.5 (c) Stomata on Adaxial epidermis

Table 2: Determination of leaf constants of Crinum solapurense.

Sr. No.	Leaf constants	Crinum Solapurense
1	Stomatal number/sq mm (Upper epidermis)	4.3
2	Stomatal number/sq mm (Lower epidermis)	5.1
3	Stomatal index (Upper epidermis)	9.03
4	Stomatal index (Lower epidermis)	8.1
5	Palisade ratio	4.1
6	Vein islet number/sq mm	2.1
7	Vein termination number/sq mm	4.2

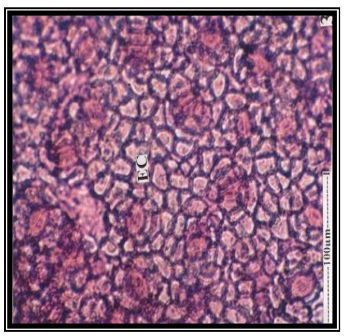
Table 3: Physico-chemical investigational Parameters of *Crinum solapurense* Leaves Extract.

Sr. No.	Standardization Parameter	*Crinum solapurense* Leaves Extract
1	% Foreign Organic Matter (w/w)	<1.5
2	% Extractive Values (w/w)	1.20
3	Water Soluble	39.31%
4	Alcohol Soluble	24.73%
5	% Total Ash (w/w)	8.29
6	% Acid Insoluble Ash (w/w)	0.25
7	%Water Soluble Ash (w/w)	1.41
8	Sulphated Ash Value (%)	0.968
9	Moisture Content (LOD) (w/w)	1.372

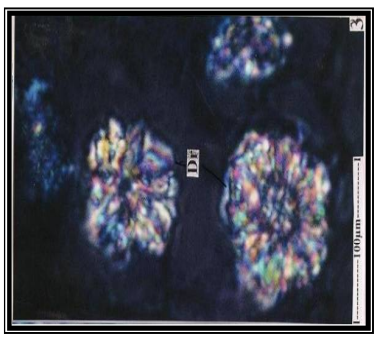
Table 4: Preliminary phytochemical investigational Qualitative Chemical Tests of *Crinum solapurense* Leaves Extract.

Phytochemical constituents	Types of tests	Pet. Ether Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract
Carbohydrate	Molisch's test	+	-	+	+
	Fehling's test	+	+	-	-
	Benedict's test	-	-	+	+
	Barfoed's test	-	+	-	-
	Iodine test	+	-	+	-
Protein	Biuret test	+	-	+	-
	Precipitation test	+	-	+	-
	Millions test	-	-	-	+
	Xanthoproteic test	-	+	+	-
	Test for sulphur	-	-	+	+
	Test for free amino acids	+	+	+	-
Fats And Oils	Stain test	-	-	+	+
Steroids	Salkowaski test	-	-	+	-
Saponin Glycosides	NaOH Soaked Paper test	-	-	-	+
Alkaloids	Dragendroff's test	+	+	+	+
	Mayer's test	+	+	+	+
	Wagner's test	+	+	+	+
	Hager's test	+	+	+	+
Flavonoids	Shinoda test	+	+	+	+
Tannins and Phenolic Compounds	5% Ferric-chloride solution test	+	-	+	+
	Lead acetate solution test	-	-	+	-
	Gelatin test	-	+	+	-
	Bromine water test	-	-	+	-
	Acetic acid solution test	+	+	+	+
	Potassium dichromate solution test	+	+	+	+
	Dilute Iodine solution test	-	-	+	-
	Dilute HNO_3 test	+	-	+	-
	Dilute Potassium Permanganate solution test	-	+	+	+

[Note: **+** sign indicate Presence whereas **–** sign indicate Absence]



(a)



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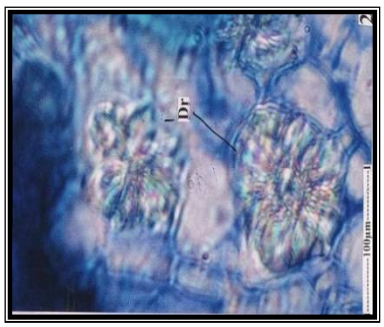

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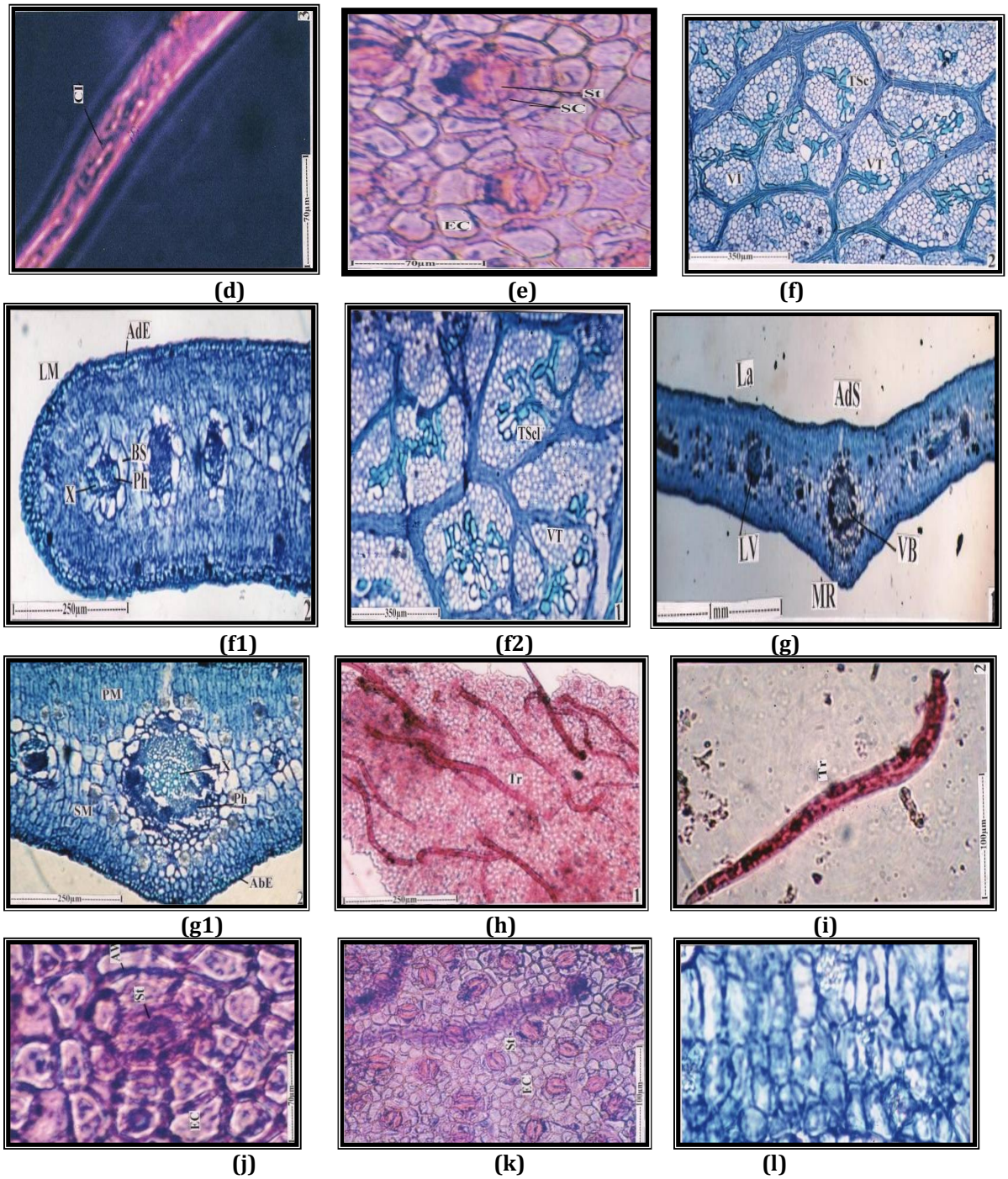

Fig:6 : (a) Adaxial epidermis, (b) Calcium oxalate druses under bright field light (Under polarized), (c) Calcium oxalate druses under bright field light, (d) cell inclusions, (e) Cyclocytic stomata, (f) lamina (350µm), (f1) lamina (250µm), (f2) Lamina showing vein terminations and terminal sclereids, (g) midrib (1mm), (g1) midrib (250µm), (h) non-glandular trichomes, (i) Single trichomes, (j) stoma, (k) stomata, (l) Phloem fibers.

CONCLUSION

The primary benefits of *Crinum solapurense* pharmacognostic investigations include identification and purity evaluation. Therefore, the plants are assessed using conventional procedure for macroscopic, powder microscopic, physicochemical, florescence, and phytochemical investigation. The results of *Crinum solapurense* aid in developing quality assurance guidelines and criteria for raw material purity. In a nutshell, the findings presented here can be regarded as crucial traits for identifying and authenticating Crinum solapurense.

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