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PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL EVALUATION OF THE LEAVES OF *CRINUM LATIFOLIUM* L.

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ABSTRACT

Crinum latifolium L. is a rosette-like herb showing promising results as rubefacient, tonic, for treatment of allergic disorders, benign prostatic hyperplasia, cancer and others**.** The present study deals with pharmacognostic and preliminary phytochemical evaluation of leaves of *Crinum latifolium* (Amaryllidaceae). The pharmacognostical studies were carried out as organoleptic, microscopic and physical parameters such as moisture content, ash value, fluorescence analysis and extractive value were determined. For phytochemical evaluation, *Crinum latifolium* leaves were subjected to successive solvent extraction using petroleum ether, chloroform, hydroalcoholic solution and water. These extracts were then screened for presence of different chemical constituents. Thin layer chromatography (TLC) of the tested extracts was also performed to determine the active principles. These studies are useful in identification and chemical characterization of *Crinum latifolium* and to explore its phytochemical and pharmacological potential.

INTRODUCTION: *Crinum latifolium* L. (Amaryllidaceae), popularly known as Sudarshan, has been used in Asian folk and traditional medicine [1] in the treatment of serious health conditions like prostatitis adenoma, benign prostate enlargement [2], uterine fibroids, hypoxia, inflammation, detoxification, tissue regeneration, hormone balancing, to enhance cell-mediated immunity and an effective T-lymphocyte activator [3].

Leaf juice is used for earache, rheumatic pain, and sprain. These activities are attributed to the presence of different chemical principles such as Amaryllidaceae alkaloids [4] lycorine, 2-epilycorine, pyrrolophenan-thridine alkaloids, 2-epilyocorine and 2-epipancrassidine [5], carbohydrates, glycosides, proteins & amino acids etc. and thus imparting significant role in medicines. But insufficient data is found in literature regarding pharmacognostic and physiochemical characteristics which can further help in betterment of its medicinal activity.

Hence, the present study was designed to evaluate the pharmacognostic aspects of leaves including morphology, histology and quantitative microscopy, determination of various physicochemical parameters, fluorescence analysis, phytochemical screening and TLC of different leaf extracts of *Crinum latifolium* L.

MATERIALS AND METHODS: Collection and authentication of plant material: The leaves of *C. latifolium* L. were collected from herbal garden, Maharshi Dayanand University, Rohtak, Haryana, India in december 2010 and authenticated by Dr. J.P. Yadav, Department of Biosciences, M.D. University, Rohtak

and a voucher specimen was deposited in Herbarium of Pharmaceutical Sciences, M.D.University (voucher specimen number DPS 0016).

Pharmacognostical Investigation:

Morphological Features: The morphological features of leaves such as presence of foreign organic matter, color, odor, size, shape and taste were studied [6].

Microscopic features: Microscopic characteristics were studied under the following headings-

a. Microscopy of intact drug as well as powdered drug characteristics
b. Quantitative microscopy

Powder Characteristics: The powder microscopy was performed using standard methods and characteristic features were recorded [7].

Quantitative Microscopy: Various leaf constants such as stomatal number, stomatal index, palisade ratio, vein islet number and vein termination number were determined followed by official methods [8].

Physicochemical Parameters: Physicochemical parameters, i.e., percentage of ash values, extractive values, percentage of moisture content and fluorescence analysis were performed according to recognized methods [9].

Determination of Ash Values: The ash of the leaf powder was prepared by incinerating the powder in muffle furnace and ash value was determined [10].

Determination of Extractive Values: The leaves were shade dried, coarsely powdered and then were extracted successively with petroleum ether, chloroform, hydro alcoholic solution (70:30) and finally with chloroform water using hot extraction technique. The extractive values were determined in percentage w/w [9].

Fluorescence Analysis: Fluorescence analysis of crude drug powder was carried out in various solvents and reported.

Preliminary Phytochemical Screening: Different extracts were then screened for the presence of the various organic chemical constituents in the leaves of *C. latifolium* L [12].

TLC Fingerprint Profile: Thin layer chromatography of different extracts was performed and the R_f values were determined [13].

RESULTS AND DISCUSSION:

Morphological Characteristics: *Crinum latifolium* L. is a rosette-like herb that arises from an underground bulb. It is a stout perennial herb 2 m in height. Leaves are long, linear, acute apex, entire margin, ligulate type [14]. Flowers are white in color and arranged in umbel. The morphological characteristics of *Crinum latifolium* L. are listed in **table 1**.

TABLE 1: MORPHOLOGICAL CHARACTERISTICS OF FRESH *CRINUM LATIFOLIUM* L. *LEAVES*

Color	Green
Odor	Faint
Taste	Characteristic
Shape	Linear
Type	Simple
Margin	Entire
Venation	Parallel
Blade length	> 36 inches

Histological features of *Crinum latifolium* L. leaves.

Microscopy of *Crinum latifolium* L. leaves.

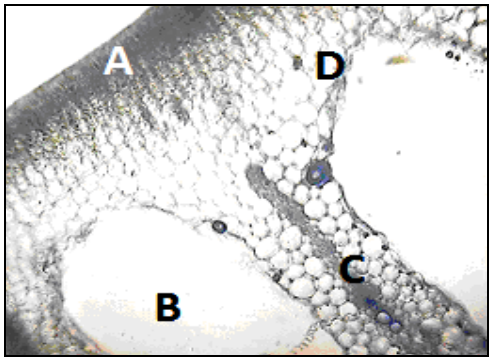

FIG. 1: A: UPPER EPIDERMIS, RECTANGULAR CELLS WITH DISTINCT CUTICLE; B: AIR SPACE; C: VASCULAR BUNDLE, MORE TOWARDS VENTRAL SURFACE; D: PALISADE CELL

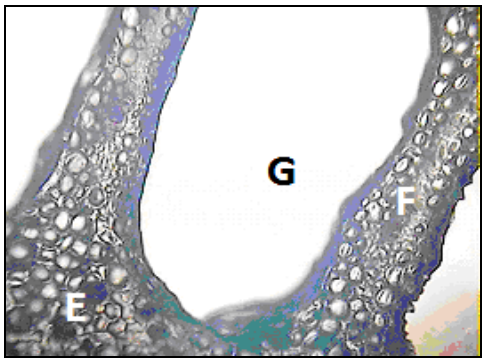

FIG. 2: E: SPONGY PARENCHYMA; F: CRYSTAL SHEATH OF PARENCHYMATOUS LAYER CONTAINING CALCIUM OXALATE PRISM; G: AERENCHYMA

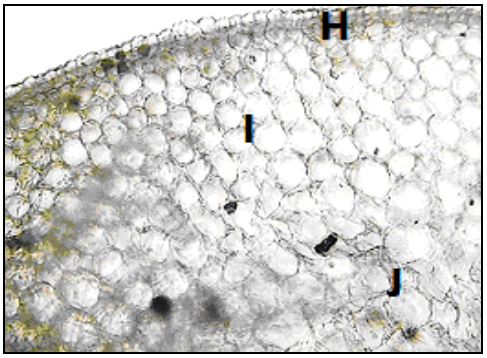

FIG. 3: H: UPPER EPIDERMIS, SINGLE LAYERED, BEADED, COVERED WITH THICK CUTICLE, WAVY AND THICK WALLED CELLS; I: SPONGY PARENCHYMA; J: STARCH GRAINS

Powder characteristics: The powder is green in color and contains diacytic stomata, fibers, epidermal cells with stomata, pitted vessels, acicular calcium oxalate crystals and starch grains (**Figure 4-10**).

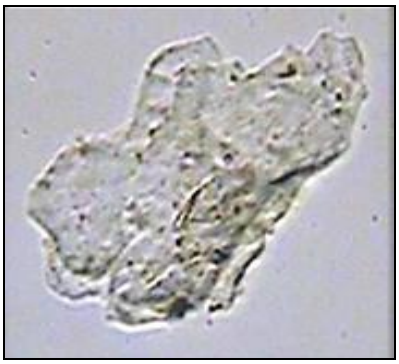

FIG. 4: CALCIUM OXALATE PRISM

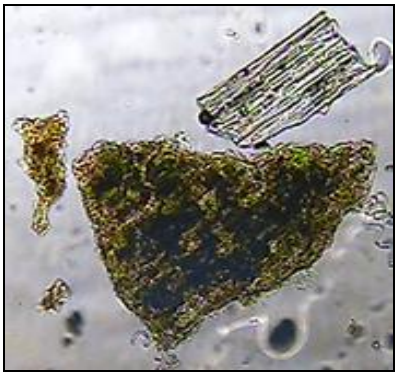

FIG. 5: XYLEM VESSELS

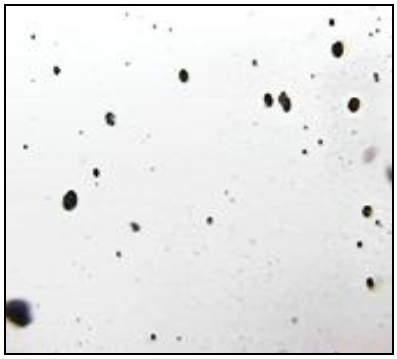

FIG. 6: STARCH GRAINS

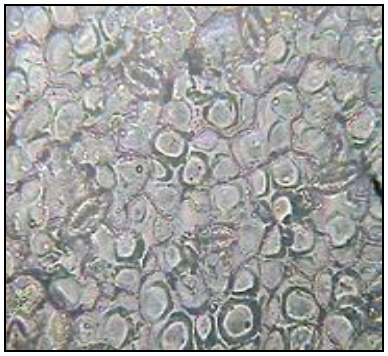

FIG. 7: STOMATA ALONG WITH EPIDERMAL CELLS

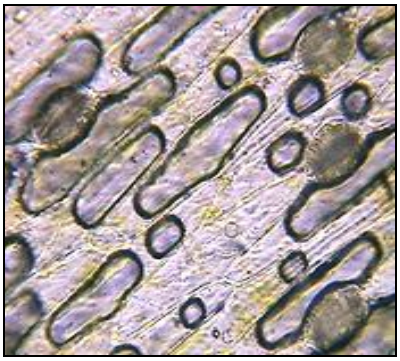

FIG. 8. PALISADE CELLS

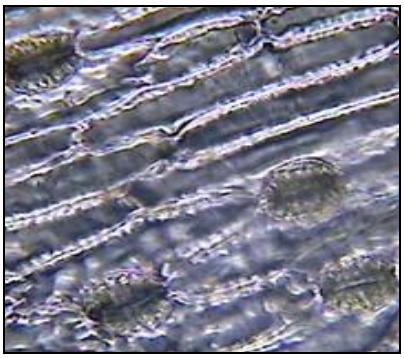

FIG. 9: CYSTOLITHS ALONG WITH EPIDERMAL CELLS AND STOMATA

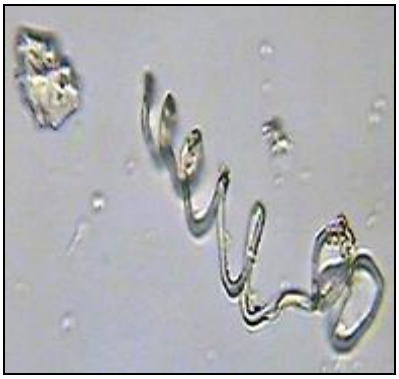

FIG. 10: FIBERS

Quantitative Microscopy: The leaf constants were determined following the standard methods [9] and results have been furnished in **table 2**.

TABLE 2: LEAF CONSTANT VALUES OF *CRINUM LATIFOLIUM* L. LEAVES

Leaf constant	Range	Average
Stomatal Number (upper surface)	3 - 6	4.3
Stomatal Number (lower surface)	4 - 7	5.3
Stomatal index (upper surface)	6.90 - 11.67	9.15
Stomatal index (lower surface)	6.45 - 11.51	8.97
Palisade ratio	3 - 6	4.2
Vein islet number	2 - 4	2.6
Vein termination number	3 - 5	4.3

Physicochemical Parameters: The various physicochemical parameters were determined and are represented in **table 3**.

TABLE 3: PHYSICOCHEMICAL PARAMETERS OF *CRINUM LATIFOLIUM* L. LEAVES

Parameter	%w/w*
Total ash	25.0
Acid insoluble ash	9.0
Water soluble ash	18.0
Sulphated ash	7.5
Petroleum ether soluble extractive	2.5
Chloroform soluble extractive	5.0
Hydroalcoholic extractive	21.75
Water soluble extractive	31.5
Moisture content	89.0

*average of three readings

Fluorescence Analysis: The results of fluorescence analysis are presented in **Table 4**.

TABLE 4: FLUORESCENCE ANALYSIS OF *CRINUM LATIFOLIUM* L. LEAF POWDER

Treatment	Normal light	Under UV light	
		254 nm	366 nm
Dry powder	Brownish green	Dark brown	green
Powder + 5% NaOH	yellowish	Greenish dark brown	Dark brown
Powder + 5% KOH	Light yellowish	Light greenish brown	Light brown
Powder + 5% $FeCl_3$	Yellowish green	Dark brown	Dark black
Powder + conc. H_2SO_4	Reddish brown	Dark black	Dark black
Powder + dil NH_3	Light orange	Dark brown	Yellow
Powder + conc. HCl	Dark brown	Dark black	Dark black
Powder + conc. HNO_3	Orange	yellow	Dark yellow
Powder + iodine solution	Greenish brown	Dark black	Dark brown
Powder + 5% HCl	Yellowish brown	Dark brown	Dark brown
Powder + 5% H_2SO_4	Yellowish brown	Dark brown	Yellowish green
Powder + dil HNO_3	Brownish orange	green	Dark green
Powder + Na_2CO_3	Yellowish brown	Yellowish brown	yellow
Powder + alc. KOH	Light yellow	Dark yellow	Yellowish orange
Powder + NH_4OH	Dark orange	Dark brown	yellow
Powder + 1% $KMnO_4$	Dark brown	Dark brown	Dark black
Powder + $AgNO_3$	Yellow	Light yellow	Light yellow

Preliminary Phytochemical Screening: Preliminary phytochemical screening of different extracts shows the presence of alkaloids, proteins, carbohydrates, anthraquinone glycosides, cardiac glycosides, gums & mucilages and tannins. The results are depicted in Table 5.

TABLE 5: PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF DIFFERENT EXTRACTS OF *CRINUM LATIFOLIUM* L. LEAVES

Group of Phytoconstituents	Extract			
	Petroleum ether	Chloroform	Hydroalcoholic	Aqueous
Alkaloids	+	+	+	+
Carbohydrates	–	+	+	+
Cardiac glycosides	+	+	–	–
Anthraquinone glycosides	+	+	+	+
Steroids	–	–	–	–
Gums & mucilages	–	+	+	+
Fats & oils	–	–	–	–
Flavonoids	–	–	+	–
Tannins & phenolics	–	–	+	+
Saponin glycosides	–	–	–	–
Proteins & amino acids	+	+	+	+

TLC Fingerprint Profile: The thin layer chromatography of different extracts was performed using the following mentioned solvent systems and results are shown in **table 6**. The detection of developed plates was done under UV at 366 nm.

TABLE 6: THIN LAYER CHROMATOGRAPHY OF DIFFERENT EXTRACTS OF *CRINUM LATIFOLIUM* L. LEAVES

Extract	Mobile phase	No. of spots	Color of spot	R_f value
Petroleum ether	Toluene : ethyl acetate (8 : 1)	3	a) orange b) orange c) orange	0.48 0.77 0.95
Chloroform	Toluene : ether : cyclohexane (5 : 2 : 1)	3	a) yellow b) orange c) orange	0.52 0.57 0.94
Hydro-alcoholic	n-butanol : acetic acid: water (4 : 1 : 5)	5	a) light brown b) light yellow c) light blue d) light brown e) very light brown	0.37 0.54 0.72 0.86 0.95
Aqueous	Methanol : chloroform (2 : 3.5)	2	a) yellow F* b) yellow F*	0.27 0.67

*F refers to fluorescence

CONCLUSION: The various morphological, microscopic and physicochemical standards developed in this study will help for botanical identification and standardization of drug in crude form. Further, the authentic plant material can be explored for its pharmacological and phytochemical potential on the basis of its phytochemistry.

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