



PHYTOCHEMICAL SCREENING AND MICROBIOLOGICAL ASSAYS OF *CRINUM LATIFOLIUM* –LEAF

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ABSTRACT

Sudarshan *Crinum latifolium* Ker-Gawl. family-Amaryllidaceae is an important medicinal plant. Its leaf is commonly used to cure ear pain fever, skin diseases, leprosy, asthma, arthritis, joint pain, and anti bacterial agents and preparation of the Ayurvedic formulations. The present paper deals with phytochemical screening and microbiological assays of leaf which mainly included physicochemical study, preliminary phyto-chemical analysis, HPTLC finger printing and microbial screening. The study will help identification and quality control of drugs.

KEYWORDS: Microbiological assays, *Crinum latifolium*, Phyto-chemical analysis, HPTLC fingerprint profile, Physico chemical parameter.

INTRODUCTION

Crinum latifolium Linn. (family-Amaryllidaceae) (Sudarshan) is an important medicinal plant. It is a herbs, bulb globose, 120-150cm long. Leaves- numerous, broadly oblong, lorate, 15-40 cm long, bright green, obtuse at apex, glabrous, coriaceous. Flowers white with pinkish tinge, fragrant, in 8-10, rarely 20 flowered umbels, on 15-20 cm long stalk. Perianth infundibuliform, lobes oblong-lanceolate, up to 10 cm long, tube 7-10 cm long. Fruits bulbous 4.5 cm across. And found along streams in open places, also introduced in the gardens and parks.^[1,2] The study provides physicochemical parameters, preliminary phytochemical screening HTLC finger printing and microbiological details helped in laying down standardization and pharmacopoeial parameters.

Since no enough record on the pharmacognostical works on the leaf of this drug plant. The same has been attempted. The present paper deal with mainly botanical identification, macroscopic, microscopic study of the leaf along with other important Physico - chemical parameters such as loss on drying at 105°C, total ash value, acid soluble ash value, water soluble ash value, alcohol soluble extractive values and water soluble extractive value. Important phyto-chemical tests, microbial screening and thin layer chromatographic findings from the standardization point of view. These data will be helpful in identification, quality control and pharmacognostic studies of the drugs.

MATERIALS AND METHOD

Authentic samples were collected from Chitrakoot (Bagdara ghati) forest. The standardization parameters were determined according to the methods detailed in the Ayurvedic Pharmacopoeia of India.^[3,5] Organoleptic characters and particle size of the both samples were recorded. Quantitative analysis for loss on drying at 105°C, ethanol soluble extractive value, water soluble extractive value, total ash value, acid insoluble ash values and water soluble ash values were carried out in triplicate samples of *Crinum latifolium* leaf curna. Preliminary phytochemical analysis and HPTLC finger printing profile were also determined in the sample. Preliminary tests were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavanoids, tannins, resins, carbohydrates, proteins and saponins.

For HPTLC, the powdered leaves 5 gm of sample was extracted with 100 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F₂₅₄ (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of *Toluene: Ethyl acetate* (7:3 v/v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was

30 min. at room temperature. The length of chromatogram run was 10 cm. 20 ml of the mobile phase. Subsequent to the development, TLC plates were dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with 5% methanolic –sulphuric acid reagent) at 254nm, 366nm and day light with Win cat software and R_f values noted.

For microbial screening tests were done for determination of Total aerobic microbial count, Yeast and Moulds, Enumeration of *E.coli*, *Salmonella spp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.^[6]

RESULTS AND DISCUSSION

Macroscopic Characters

Sudarshan leaves taste is bitter, odour, astringent, and greenish yellow colour.

Physico-chemical study: Detailed study of the powder of the leaf pertaining to physico-chemical parameters such as moisture content (loss on drying at 105°C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and water soluble ash value have been given in (Table 1). Drug was tested qualitatively for presence or absence of various chemical constituent and results are given in (Table 2).

High Performance Thin Layer Chromatography (HPTLC)

Ethanol extract of sample showed major spots before derivatization under UV light R_f Values are

0.54(yellowish green), 0.72(light yellow), 0.77(light black), 0.88(light green); under 366nm R_f Values are 0.08(pink), 0.19(reddish pink), 0.26(sky blue), 0.74(light blue), 0.83(dark pink), 0.90(yellowish pink) and after derivatization under UV light R_f Values are 0.05(light black), 0.57(brownish black), 0.66(light black), 0.82(greenish blue), 0.94(yellowish green) and 366nm R_f Values are 0.05(pink), 0.57(white), 0.66(white), 0.82(red) and 0.96(red). (Fig.1 to 4).

Determination of microbial load

Microbial tests were carried out to determine the microbes in *Crinum latifolium* leaf powder. The results are given in (Table 3 & Fig. 7 to 12).

Table 1: Physico-chemical Analysis of Sudarshan leaf.

S. No.	Name of Test	Results
1	Foreign matter	2.18%
2	Loss on drying (LOD)	6.08%
3	Alcohol Soluble extractive	15.48%
4	Water Soluble extractive	19.20%
5	Total ash	8.35%
6	Acid in soluble ash	1.26%
7	Water soluble ash	4.59%

Table-2: Qualitative Analysis of Sudarshan leaf.

S. No.	Name of Test	Result
1	Resin	Present
2	Saponin	Present
3	Tannin	Present

Table 3: Determination of Microbial Load assays.

S. No	Parameters	Result	Permissible Limits
1.	<i>Staphylococcus aureus</i> /g	Absent	Absent
2.	<i>Salmonella spp.</i> /g	Absent	Absent
3.	<i>Pseudomonas aeruginosa</i> /g	Absent	Absent
4.	<i>E.coli</i>	Absent	Absent
5.	Total Aerobic microbial count. (AMC)	132cfu/g	10 ³ /gm
6.	Total Yeast & mould.	16 cfu/g	10 ³ /gm

DISCUSSION AND CONCLUSION

Crude drug is the base material for manufacturing herbal medicines. Efficacy of any drug depends on the genuineness of the raw material used for its preparation. Adulteration of the genuine raw material is the main cause for deterioration of the desired therapeutic effect of a particular drug. The present study of *Crinum latifolium* leaves following a series of physico chemical parameters such as total ash, water soluble ash, acid in-soluble Ash, ethanol soluble extractive value, water soluble extractive value and loss on drying on 105°C, preliminary phytochemical characteristics, HPTLC profile, Microbial screening and microbiological assays. So it can be concluded that these parameters can be used for quality

evaluation of single drug and for manufacturing authentic polyherbal medicines with correct identity.

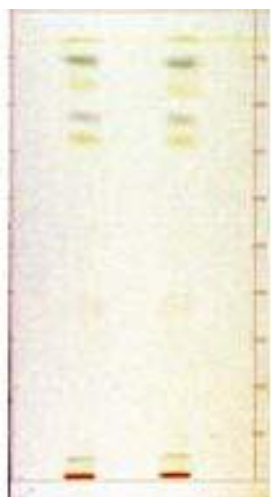


Fig. 1: HPTLC at UV light before derivatization

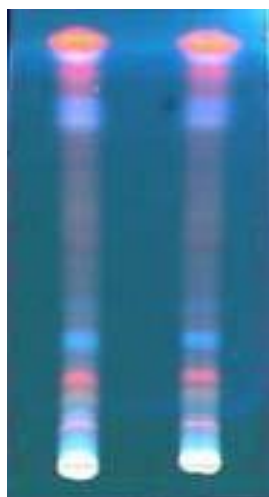


Fig. 2: HPTLC at 366nm Before derivatization

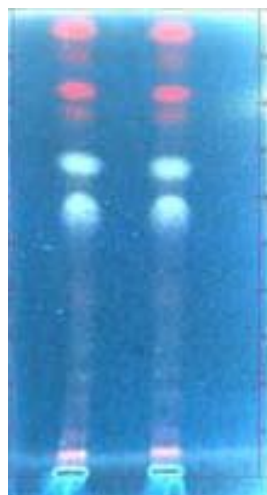


Fig. 3: HPTLC at 366nm after derivatization



Fig. 4: HPTLC at UV light after derivatization



Fig. 7 *staphylococcus aureus*



Fig.8 *pseudomonas aeruginosa*



Fig.9 *E.Coli.spp.*

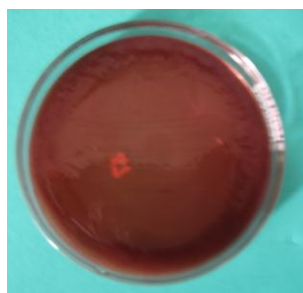


Fig.10 *Salmonella spp.*

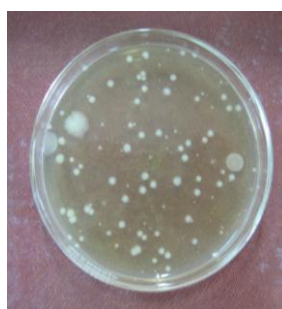


Fig.11 Total bacteria count



Fig.12 yeast & moulds

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