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## ORIGINAL ARTICLE

# Preliminary Pharmacognostical and Phytochemical Evaluation of Leaf of *Crinum solapurense*

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**ABSTRACT**
*A herb Crinum solapurense belongs to the Amaryllidaceae family well known as pankanda has long been used in conventional medical practices to cure a variety of ailments. For many hundreds of years, people have been used Crinum species as tonics, laxatives, fever reducers, antimalarials, antitumors, antilymphocytic, and anti-asthmatics. This study aimed to examine its preliminary pharmacognostical properties and phytochemical investigations. Physicochemical criteria such as ash value, extractive value, and phytochemical investigation of the leaves extract of Crinum solapurense revealed the presence of alkaloids, phenols, steroids, glycosides, and flavonoids. Calcium oxalate crystals, glandular trichomes, covering trichomes, and anomocytic stomata were all confirmed by powder microscopy. The current study's findings may help validate the safety and efficacy of plant materials by providing information about their physicochemical properties.*
***Keywords:*** *Crinum solapurense, Amaryllidaceae, Alkaloid, Standardization, Pharmacognostical.*

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## INTRODUCTION

From the beginning of recorded history, people worldwide have made medicinal uses of plants. A wide variety of medicinal plants and a wealth of traditional knowledge have contributed to India's rich cultural legacy [1]. Natural medicines are all the rage these days for good reason: they are plentiful, safe, and do not have any of the side effects that synthetic pharmaceuticals are known to have. So, it is need for today's researchers to study taxonomical and phytochemical investigation of medicinally useful plants [2]. The objective of the present study was to investigate the possible uses of the medicinal herb *Crinum solapurense* in the Solapur district. The banks of the Bhima, Sina, and Man Rivers are fertile agricultural regions rich in a variety of resources. The district's grasslands are home to a wide variety of birds. The Indian Bastard inhabits in these grasslands.125 families of flowering plants are available in the Solapur district. Majorly Fabaceae, Poaceae, Euphorbiaceae, Apocynaceae, Solanaceae and Amaryllidaceae families are reported in taxonomic work [3]. There are 15 distinct species of Crinum in India, each of which has a variety of biologically active alkaloids. There is a long history of using Crinum species as medicinal plants to treat a wide range of conditions. The ground-dwelling bulbous flowering plants of the Amaryllidaceae family are monocotyledonous and have been the subject of much research due to the medicinal alkaloids they generate [4]. The plants belonging to the Amaryllidaceae family can be utilized in the drug development assay [5].

A new species from a wetland that borders the Bhima River in the Solapur district of Maharashtra State, India is described as *Crinum solapurense* by botanist S. P. Gaikwad, K. U. Garad, & R. D. Gore and the following characteristics set it apart from *C. viviparum* (Lam.) R. Ansari & V. J. Nair var. *viviparum* and *C. lorifolium* Roxb. ex Ker Gawl is as having 1–10 bulblets on the mother bulb; 12–27 strong and canaliculate leaves; 10–30 umbels of flowers; undivided stigma and 3–12 seeded fruits [6]. The physicochemical characteristics, preliminary phytochemical constants, and thin layer chromatography are performed as

there is no comprehensive standardization study reported on leaves. The investigation highlighted distinct identities for the specific crude drug, which will be valuable in the detection and prevention of adulterations of the natural drug.

## MATERIAL AND METHODS

### Collection of plant material

The plant specimens for the study were collected from a swamp bordering the Bhima river in Solapur district (Maharashtra, India) 17.5661° N and 75.5619° E in the month of January and were positively identified and authenticated by the Dr. S.P. Gaikwad, Professor & Head of the Department, Department of Botany, Walchand College of Arts & Science, Solapur, Maharashtra, India and by Botanical Survey of India, Western Regional Centre, Pune. The Specimen Number is ASJCS-1. The plants were picked with care based on age and overall health. After being trimmed to size, the leaves were gently taken off the plant, cleaned with water to get rid of any last bits of dirt, and allowed to dry in the sun. The microscopical identification was performed on the fresh leaf sample, which had been shade dried and mechanically pulverized to reduce size. To prepare it for the powder microscopy experiment, it was put through a #60 filter and the resulting fine powder was collected.

### Pharmacognostical studies

### Taxonomic Classification

**Table 1: Taxonomical classification of *Crinum solapurense***

| | |
|---|---|
| Kingdom | Plantae |
| Phylum | Tracheophyta |
| Class | Equisetopsida |
| Subclass | Magnoliidae |
| Order | Asparagales |
| Family | Amaryllidaceae |
| Genus | *Crinum* |
| Species | *Crinum solapurense* |

### Macroscopical characterization

The leaves were examined under a microscope and their size, color, texture, flavor, and aroma were recorded [7,8].

### Extraction methods

### Soxhlet extraction or hot continuous extraction

A total of 100 g of plant materials were extracted using ethanol, acetone, and water-based solvents in a Soxhlet apparatus maintained at a temperature of 45°C or below in order to prepare leaf extracts. A rotary evaporator was used to dry and concentrate the extracts before they were used. Subsequently, they were chilled to 4°C and kept in a fridge [9].

### Phytochemical screening

A wide range of phytochemicals, including tannins, flavonoids, saponins, phenols, glycosides, steroids, alkaloids, and terpenoids, were detected in plant extracts. Alkaloids have been tested, by performing lead acetate solution, ferric chloride, Mayer's, Shinoda, Dragendroff's, and Wagner's test. . A pink tint was used to identify flavonoids, a brilliant green color for ferric chloride, and a yellow precipitate for alkaline reagent. A foamy froth signaled the presence of saponins. A white precipitate indicated lead acetate, a $Fecl_3$ solution indicated tannins, and a gelatin solution indicated gelatin. To ensure the plant specimen contained concentrated tannins, bromine water was utilized for confirmation. The presence of cardiac glycosides may be identified using the Keller-Killiani test, which turned blue in colour. These tests were conducted according to recognized protocols in order to detect the presence of phytochemicals in plant extracts [10].

### Physico-chemical analysis

The drug's quality and purity are primarily assessed using the physico-chemical characteristics. All three types of ash as well as the extractive values (alcohol soluble, water soluble, and water soluble) and the amount lost during drying were calculated as part of the physico-chemical analysis. The weight-to-weight ratios were determined using the air-dried medication as a reference [11,12].

### 1. Determination of total ash value

Ten grams of drug powder burned in a silica crucible over an open flame. We used a furnace to heat the charred material to 500 to 600°C for six hours. When a sample is burned, the byproduct that remains is

called ash. Desiccators chill the crucible. Using the air-dried medicine as a reference, the total ash content percentage was determined.

**Determination of acid insoluble ash value**

Using 25 milliliters of diluted HCl, the ash was heated to a boil for five minutes. After removing insoluble substances from the crucible, they were rinsed with hot water, set on fire, and finally weighed. Using the air-dried medicine as a reference, the percentage of insoluble ash in acid was determined.

**Determination of water soluble ash value**

After combining the ash with 25 milliliters of distilled water, keep it for a period of five minutes. The insoluble materials were collected on filter paper, burnt, cleaned in hot water, and quantified. This has been done by calculating the weight of the ash and subtracting the weight of the solid substance. Using the drug's weight after air drying as a reference, we determined the water-soluble percentage of ash. To find it, just substitute 25 milliliters of water for the diluted hydrochloric acid, just like acid insoluble ash.

**Determination of moisture content**

In a delicate porcelain plate, the powdered medicine weighed around 1.5 g. We weighed it after cooling it in desiccators after drying it in an oven set to 100–105°C. Its dry weight (DW) was measured. We used the following formula to find the moisture content:

**Moisture content (IW) = IW-DW XI00**

**Powder Microscopy**

The plant powder is analyzed by boiling a little amount of fine powder in a chloral hydrate solution for a few minutes in a test tube. After mounting the slide with phloroglucinol, a few drops of powder were applied, then few drops of concentrated HCl. Next step was to photograph the prepared slides after observing them under a microscope [13].

**Fluorescence analysis of powder material**

The material was mixed with various reagents before being examined at short wavelengths of 254 nm and long wavelengths of 365 nm under visible and UV light. These reagents included distilled water, methanol, iodine, petroleum ether, chloroform, glacial acetic acid, 1N NaOH, 1N HCl, FeCl3, and ethyl acetate [14].

**Total Phenolic Content**

The Folin-Ciocalteu reagent technique was used to calculate the quantity of phenol in the ethanolic extract with a few modifications. 2.5 milliliters of Folin-Ciocalteu reagent (10%) and 2 milliliters of a Na2CO3 solution (2%) were added to 1 milliliter of plant extract. A 15-minute incubation period was given to the resultant combination at room temperature. A wavelength of 765 nm was used to measure the sample's absorbance. The standard used was gallic acid (1 mg/ml). We ran each test three times to ensure accuracy [15].

**Thin Layer Chromatography (TLC) Test**

The TLC plate was made of aluminum silica gel and had a 0.2 mm layer thickness. Eluent used for TLC was a mixture of benzene and methanol at a ratio of 20:3. Spots were applied using a capillary tube, with a line drawn with a pin 1.5 cm from the bottom serving as a guide. After drying, the sample was subsequently covered and placed within the chromatographic tank. Once the entire plate was coated with solvent, it was delicately removed, labeled, dried, and then placed in an iodine chamber [16].

## RESULTS AND DISCUSSION

WHO states both macroscopic and microscopic descriptions are necessary in order to verify the authenticity of crude pharmaceuticals and ascertain their quality. Pharmacognostic parameters are essential to determine identification and purity of a medicinal plant. [17]

**Macroscopical study**

**Leaves:** The leaves of *C. solapurense* were 12 - 27 cm long, 12 - 85 × 5 - 7 cm wide, with a dull green hue and a form that was lanceolate, sturdy, thin at the top and broad at the bottom. It had characteristic odor, and slightly astringent taste (Fig.1).

**Bulb:** Conical to elongated, compressed laterally or globular-ellipsoid, with dimensions of 10–20 × 8–15 cm; white or pale pink hue that deepens to pink in the presence of light; up to 30 cm in length, with a cylindrical neck that is accompanied by one to ten bulblets.

**Table 2: Macroscopical study**

| Plant Parts | Organoleptic characters | | | |
|---|---|---|---|---|
| | **Odor** | **Texture** | **Taste** | **Color** |
| Leaves | Characteristic | Soft | Astringent | Dull green |
| Bulb | Characteristic | Soft | Bitter | White or pale pink |

**Figure.1: *Crinum solapurense***

**Preliminary Phytochemical Studies**

Initial phytochemical analyses revealed that the alkaloids, flavonoids, saponin, and cardiac glycosides were present. (Table.3). Research of its phytochemical composition revealed the presence of alkaloids, flavonoids, glycosides, saponins and secondary metabolites suggests that the plant might be possessing medicinal importance. The polyphenolic chemicals known as flavonoids possess strong antioxidant, antibacterial, anti-inflammatory, and anti-cancer capabilities. There have been reports of alkaloids having analgesic, antispasmodic, and antimicrobial effects.

**Table 3: Preliminary Phytochemical screening of leaf of *Crinum solapurense***

| S. No | Phytochemical tests | | *Crinum solapurense leaves* | |
|---|---|---|---|---|
| | | | **Ethanolic Extract** | **Aqueous Extract** |
| 1 | Alkaloids | Mayer'stest | + | + |
| | | Dragendroff's test | + | + |
| | | Wagner's test | + | + |
| 2 | Flavonoids | Shinoda's test | + | + |
| | | $FeCl_3$ test | + | + |
| | | Aqueous NaOH test | + | + |
| | | Lead acetate test | + | + |
| 3 | Saponin | Foam test | + | + |
| 4 | Tannin | $FeCl_3$ test | - | - |
| | | Lead acetate test | - | - |
| | | Gelatin test | - | - |
| | | Bromine water test | - | - |
| 5 | Cardiac glycoside | Kellar- killiani test | + | + |

**Physico-chemical analysis**

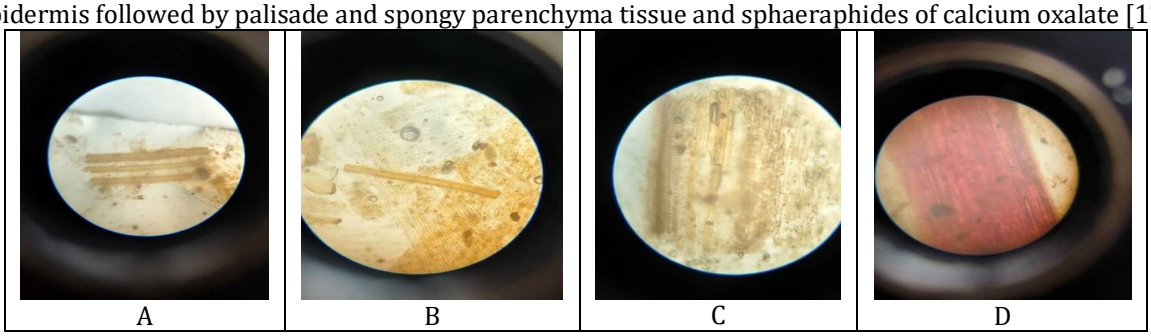
Amount of minerals and earthy material linked to plant material was determined to be 9.12%w/w, as shown by the total ash value of the plant material. There was 6.37 percent weight of acid insoluble siliceous material in the plant. It was determined that there were 6.26% w/w polar components based on the alcohol soluble extractive values. The drying loss value was determined to be 6.8% w/w (Table 4). Physico-chemical factors are crucial and it is an indication of the amount of inorganic materials, earthy substances, or other contaminants in a medicine, it can be found by looking at its ash value. The physico-chemical evaluation is a useful source of information that establishes the quality of this plant material for further study or application and it shows that the amount of moisture in the extracts can be determined by its loss on drying.

**Table 4: Physico-chemical parameters**

| Parameters | Value (w/w) |
|---|---|
| Loss on drying | 6.8% |
| Total ash | 9.12% |
| Acid insoluble ash | 6.37% |
| Water insoluble ash | 6.26% |

**Powder Microscopy**

The observations recorded from powder microscopic studies are shown in [Fig. 2 (A - D)] powdered drug observed under microscope, showed clumps of rounded to squarish thin walled parenchymatous cells. Several pieces of epidermal cells were noted in surface view. Fragment of the powder show upper epidermis followed by palisade and spongy parenchyma tissue and sphaeraphides of calcium oxalate [17].



A B C D

**Figure.2: Powder microscopy of leaves of *Crinum solapurense***

**Fluorescence analysis of powder material:**

The Fluorescence study results showed modifications in the behavior of the powdered drug when exposed to specific chemical reagents under both UV and Visible light. These findings are summarized in Table 5.

**Table 5: Fluorescence analysis of *Crinum solapurense* leaves**

| S. No. | Reagents | Visible light | UV 254 nm | UV 365 nm |
|---|---|---|---|---|
| 1 | Powder drug | Light brown | Light orange | Olive green |
| 2 | Powder drug + distilled water | Chocolate brown | Dark orange | Dark olive green |
| 3 | Powder drug + 10% aq. sodium hydroxide | Moderate brown | Blackish orange | Green |
| 4 | Powder drug + ammonia | Dark brown | Dark orange | Moderate olive green |
| 5 | Powder drug + conc. sulfuric acid | Black | Blackish orange | Black |
| 6 | Powder drug + sulfuric acid + water | Chocolate brown | Moderate orange | Dark olive green |
| 7 | Powder drug + conc. hydrochloric acid | Dark brown | Light black | Greenish black |
| 8 | Powder drug + hydrochloric acid + water | Moderate brown | Moderate orange | Olive green |
| 9 | Powder drug + iodine | Light brown | Light black | Greenish black |
| 10 | Powder drug + glacial acetic acid | Chocolate brown | Blackish orange | Bluish green |
| 11 | Powder drug + petroleum ether | Moderate brown | Light orange | Light olive green |
| 12 | Powder drug + chloroform | Moderate brown | Blackish orange | Moderate olive green |
| 13 | Powder drug + ethyl acetate | Light brown | Moderate orange | Olive green |
| 14 | Powder drug + methanol | Chocolate brown | Light orange | Dark olive green |
| 15 | Powder drug + 5% ferric chloride | Moderate brown | Blackish orange | Greenish black |

**Total Phenolic content**

Plant extracts have the ability to scavenge free radicals because they contain hydroxyl groups and extract's phenolic content was determined using the Folin-Ciocalteu reagent. The sample's absorbance was measured at 765 nm. (Table 6)

**Table 6: Total Phenolic Content**

| Test | Ethanolic extract of *Crinum solapurense* |
|---|---|
| Total phenolic content | 209.13 ± 0.98 |

**TLC Test**

Cold extracts were loaded on precoated silica gel plate using Benzene: Methanol (20:3) as solvent system. The C4 fraction showed 5 separate clear colour bands viz yellow, orange, blue, brown and pink. Observations of color bonds were shown in Figure. 3. The TLC profile of the extract yields indicating the existence of several phytochemicals. This information supports in determining the suitable solvent system for the subsequent isolation of compounds from these plant extracts [18].

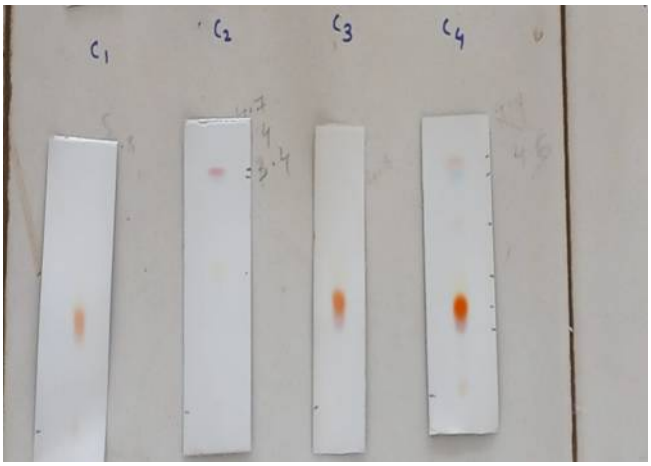



**Figure.3: Thin layer chromatography study**

**CONCLUSION**

Standardization of herbs becomes one of the most indispensable steps to determine the plant material's quality. The current study's findings may help validate the safety and efficacy of plant materials by providing information about their physicochemical properties. The pharmacognostic and phytochemical results of *Crinum solapurense* can be employed in the preparation of official monographs and standardization of the plant.

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