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(RESEARCH ARTICLE)

Phytochemical and pharmacological investigation of *Crinum solapurense* Lam

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# Abstract

The widely used indigenous medicinal plant *Crinum solapurense* Lam is the subject of the current inquiry. According to the study and pertinent literature, the plant *Crinum solapurense* Lam. It is most well-known for its use in traditional medicines, while different portions of the plant have received less attention. The current research program aims to assess the phytochemical analysis and antioxidant, anthelmintic efficacy of *Crinum solapurense* bulb ethanolic extract with the following objectives: authentication and storage of plant materials, successive extraction of different solvents from powdered drug, preliminary phytochemical screening review, assessment of the above extracts for action in vitro as an anthelmintic and antioxidant using DPPH and ABTS process. *Crinum solapurense* Lam, a drug, has been discovered during this inquiry. could be used as a more effective medicine for anthelmintic treatment. The plant *Crinum solapurense* might be a brand-new source of biologically active, stable antioxidants that could provide a solid scientific foundation for its usage in contemporary medicine.

Keywords: Crinum solapurense Lam; Phytochemical Screening Review; DPPH method; Iin vitro Anthelmintic; Antioxidant

# 1. Introduction

The pan tropical genus Crinum L. comprises about 112 species distributed in tropical Africa, America, Asia and Australia. The genus is most diverse in Southern Africa. In India, the first detailed taxonomic treatment of Crinum is that of William Roxburgh's Flora Indica, in which he recorded 14 species from British India. Subsequently, in his classical work on Amaryllidaceae included six species and four varieties of Crinum from British India. in the Handbook of the Amaryllidaceae gave detailed insight into the genus. Out of 79 species of Crinum listed by him, 12 species and 2 varieties were from British India. At the beginning, he gives a subgeneric classification scheme of Stenaster, Platyaster and Codonocrinum in reduced form. Hooke recognized 17 species from British India of which 7 species were treated as imperfectly known.

# 1.1. Taxonomy

# 1.1.1. Crinum solapurens

Crinoviviparosimilis, bulb is principal ibussurculis 1-10, foliiscanaliculatisfirmis, umbellis 10-30 floris, fructibus 3-12 spermisdiffert. Typus: India. Basal plate woody, conical, 5-7 cm long; roots thick, c.1 cm in diam. Bulbs conical or elongated, laterally compressed or globular-ellipsoid, 10-20 × 8-15 cm, white or pale pink, turning into deep pink when exposed to light; neck to 30 cm long, cylindrical along with 1-10 bulblets. Leaves contemporary with flowers, radical, sturdy, 12-27, lanceolate, 40-85 × 5-7 cm, narrow towards apex, broad at base, deeply channeled, canaliculate or U-shaped; margins entire, minutely spinulate; leaf in cross-section with chloro-phyllous cells in rings around air channels and vascular bundles alternating with fibre bundles.

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Figure 1 Crinum solapurense (Amaryllidaceae), a new species from Maharashtra, India



Figure 2 Photograph of Crinum solapurense plant

## 1.1.2. In Vitro Antioxidant Activity

The *in vitro* antioxidant activity was evaluated by following method.

## **DPPH** Assay

DPPH assay is based on the measurement of the scavenging ability of antioxidant towards the stable DPPH radical. The free radical DPPH is purple in color in methanol and is reduced to the corresponding hydrazine, which is yellow in color, when it reacts with hydrogen donor. It is a decoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in the absorbance is measured at 517 nm.

# 2. Materials and methods

Collection of plant materials: The whole plant *Crinum solapurense* Lam. Was collected from the Bhima River between Machnur village in Solapur district of Maharashtra, India.

## 2.1. Preparation of Extract of bulbs of Crinum solapurense

The bulbs of *Crinum solapurense* Lam. were shade dried and coarsely powdered by blender. 500 gm of dry powdered drug was extracted with 1.5 liters of ethanol at room temperature for 10 days for Maceration. The extract was filtered by using muslin cloth and whatman filter paper No.1. The filtrate was concentrated to dryness to obtain crude extract. The dried extract of *Crinum solapurnse* was stored in desicators under good condition till it is used for experimental use. The dried extract was subjected to screening of Phytochemical, Anthelmintic and Antioxidant activity.

## 2.2. Method

Ascorbic acid, DPPH (1,1-Diphenyl-2-Picryl-hydrazyl), Methanol, UV-visible, Shimadzu 1601 spectrophotometer. The scavenging of 2,2-diphenyl-1- picrylhydrazyl (DPPH) radicals was assayed as follows. A solution of DPPH was prepared by dissolving 25 mg of DPPH radical in 10 mL of methyl alcohol and kept in the dark at  $4 \pm 1^{\circ}$ C.

Stock solutions of *Crinum solapurense* bulb extract of different concentration of (20, 40, 60, 80  $\mu$ g/mL) were prepared. A volume of 10 ml of stock solution was added into the amber coloured volumetric flask. Vitamin C was used as positive control and pure methanol were used as negative control. Stock solutions of Vitamin C of different concentration of (20, 40, 60, 80  $\mu$ g/mL) were prepared. About 0.5 mL of methanolic solution of DPPH was added into each volumetric flask and reaction was continued for 30 min in dark. The absorbance was recorded at 517 nm by UV spectrometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH radical scavenging effect was calculated using the formula. Tests were carried out in triplicate. The amount of extract needed to inhibit free radicals concentration by 50 %, IC<sub>50</sub>, was graphically estimated using a non-linear regression algorithm.

## 2.3. ABTS assay

Preparation of ABTS radical was done by reacting equal volumes of 1.1 mg/mL aqueous ABTS and 0.68 mg/mL potassium per sulfate ( $K_2S_2O_8$ ). Solution was stored in dark for 6 h at room temperature. Stock solutions of *Crinum solapurense* bulb extract of different concentration of (20, 40, 60, 80 µg/mL) were prepared. A volume of 10 ml of stock solution was added into the amber coloured volumetric flask. Vitamin C was used as positive control and pure methanol were used as negative control. Stock solutions of Vitamin C of different concentration of (20, 40, 60, 80µg/mL) were prepared. About 2.5 mL of ABTS radical was added into each volumetric flask and reaction was continued for 30 min in dark. The absorbance was recorded at 734 nm by UV spectrometer. The ABTS radical scavenging activity was calculated using the formula. Tests were carried out in triplicate. The amount of extract needed to inhibit free radicals concentration by 50 %, IC<sub>50</sub>, was graphically estimated using a non-linear regression algorithm.

## 2.4. Preliminary Phytochemical Screening [1-5]

The preliminary phytochemical screening helps us in identifying the type of secondary metabolites present in plants. The screening was carried out with aqueous and methanolic extract of *Crinum solapurense* Lam bulb powder. The various chemical tests carried out are described.

## 2.5. Detection of Alkaloids

About 2g of the powdered material was mixed with 1g of calcium hydroxide and 5mL of water into a smooth paste and set aside for 5min. It was then evaporated to dryness in a porcelain dish on a water bath. To the residue, 20mL of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To the residue, 5mL of dilute hydrochloric acid was added. The solution was divided into four parts and 2mL of each of the following reagents were added and the colour noted below indicates the presence of alkaloids.

## 2.5.1. Mayer's Test

The extracts were treated with a few drops of Mayer's reagent. Formation of a creamy white precipitate indicated the presence of alkaloids.

## 2.5.2. Dragendroff's Test

Extracts were treated with a few drops of Dragendroff's reagent. Formation of orange red precipitate indicated the presence of alkaloids.

## 2.5.3. Wagner's Test

Extracts were treated with Wagner's reagent. Formation of brown/reddish brown precipitate indicated the presence of alkaloid

#### 2.5.4. Hager's Test

Extracts were treated with Hager's reagent. Formation of yellow coloured precipitate indicated the presence of alkaloids.

## 2.6. Detection of Carbohydrates

### 2.6.1. Molisch's Test

The aqueous extract of the powdered bulb when treated with alcoholic solution of  $\alpha$ -naphthol in the presence of sulphuric acid. Purple colour indicates the presence of carbohydrates.

#### 2.6.2. Benedict's test

The aqueous extract of the powdered was treated with Benedict's reagent and boiled on water bath and cooled. An orange colour precipitate indicates the presence of carbohydrates.

#### 2.6.3. Fehling's Test

The aqueous extract of the powdered was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. A red precipitate indicates the presence of free-reducing sugars.

## 2.6.4. Drugs and Chemicals used

Albendazole (Glasko Smith Kline) was used as reference standard purchased from local medical shop, Vijayapura. Chemicals: Ethanol (95% V/V) (S.D.Fine chemicals, Mumbai).

#### 2.6.5. Preparation of test sample

Samples for experiments were prepared by dissolving extract to obtain a stock solution of 100 mg/ml, from the stock solution, different working dilutions were prepared to get concentration range of 25, 50 and 75 mg/ml of Ethanolic extract of *Crinum solapurense* (ECS). For the present study Albendazole had taken as standard drug. The concentration of the standard drug was prepared in 1 % gum acacia to give 20 mg/ml concentration.

#### 2.6.6. Storage of Drug Solution

Fresh drug solutions were prepared. The solutions were store in air-tight amber-colored bottles at room temperature till use.

## 2.6.7. Experimental Animals Group Dividing

The Indian adult earthworms can be divided into five groups. Each group consists six earthworms.

- Group- I: is contain vehicle (1% gum acacia in normal saline)
- Group- II: Contains Albendazole as a reference standard (20 mg/ml)
- Group- III: containing Ethanolic extract (25 mg/ml)
- Group- IV: containing Ethanolic extract (50 mg/ml) Group- V: containing Ethanolic extract (75 mg/ml)

## 2.7. Evaluation of Anthelmintic Activity [6-11]

Experimental Animals: Anthelmintic activity was evaluated on adult Indian earthworms (*Pheretima posthuma*) due to its anatomical and physiological resemblance with the intestinal round worms of human beings. The Indian earthworms *Pheretima posthuma* were collected from moist soil of the field and washed with normal water and saline solution to remove soil and fecal matter. The Earth worms of 4-8 cm in length and 0.2-0.3 cm in width were used for all experimental parameters.

The evaluation of anthelmintic activity was followed by *Ajaiyeoba et al* method. Three different concentrations (as given earlier) were prepared and the group of six earth worms which having equal size were released into 10 ml of sample with desired concentration in Petri dish. Observations were made for the time taken to cause paralysis and death of the individual worms. Mean time for the paralysis in any sort could be observed. Time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worm neither moved when shaken vigorously nor when dipped in warm water (50 °C) followed with fading away of their body colors. Potency is inversely proportional to time taken for paralysis and / or death of worm.

## 2.8. Statistical analysis

The results were expressed as mean ± SEM and statistically analysed by ANOVA followed by the Tukey test.

# 3. Results

In present study, Ethanolic extract of bulb of *Crinum solapurense* were subjected to preliminary phytochemical and pharmacological investigation (Anthelmintic and Antioxidant activity)

## 3.1. Extraction of Plant material

Table 1 Percentage yield of the Ethanolic extract of Crinum solapurense bulb

Sr. No.	Extract	Color and Consistency	% Yield
1.	Ethanolic extract of Crinum solapurense	Brownish and Semisolid	6.8 % w/w

## 3.2. Preliminary Phytochemical investigation

The phytochemical analysis revealed the presence of alkaloids, carbohydrates, flavonoids, tannins and polyphenols in the *Crinum solapurense* ethanolic extracts.

### 3.3. Preliminary phytochemical screening

Results obtained from the preliminary phytochemical screening are presented in Table no. II.

Table 2 Preliminary phytochemical investigation of Ethanolic extract of Crinum solapurense bulb

Sr. No.	Chemical constituent	Test	Observations
1	Alkaloids	Mayers test	+
		Dragendorff's test	+
1.		Wageners test	+
		Hagers test	+
	Carbohydrates	a. Molisch test	+
2.		b. Benedicts test	+
		c. Fehling's test	+
	Glycosides	Liberman Burchad test	-
3.		Modified Borntragers test	-
		Legal's test	-
4.	Saponins	a. Foam test	-
-	Phytosterols	Salkowaski test	-
5.		Libermann Burchard's test	-
6.	Fixed oils	a. Stain test	-
7.	Phenols	a. Ferric chloride test	+
0	Proteins	Xanthoproteic test	-
0.		Ninhydrin test	-
9	Flavonoids	Lead acetate test	+
· ·		Shinoda test	+
10	Tannins	a. Gelatin test	+

(+) indicates detected, (-) Indicates absent

# 4. Discussion

The preliminary phytochemical screening procedure of the Ethanolic extract of *Crinum solapurense* bulb showed the presence of carbohydrates, alkaloids, Phenols, Flavonoids, Tannins. Glycosides, Phytosterols, protein, Saponins and Fixed oils were absent.

## 4.1. Pharmacological Investigation

Anthelmintic activity: In the present work, ECS bulbs extract was used to evaluate anthelmintic activity against Indian earthworms (*Pheretima posthuma*). Each extract containing 25, 50, 75 mg/ml concentration produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death of worms. As shown in table No.6.3.

**Table 3** Anthelmintic activity of Ethanolic extract of Crinum solapurense on Indian earthworms (Pheretima posthuma)

Name of Group	Name of extract and concentration	Time taken for paralysis in minute (Mean ± SEM)	Time taken for death in minute (Mean±SEM)
Group -I	Gum acacia in normal saline (1%)	Not paralysed till 48 hr	No death till 48 hr
Group -II	Albendazole (20 mg/ml)	31.8 ± 0.66	37.04 ± 0.61
Group -III	ECS (25 mg/ml)	61.1 ± 0.85	68.13 ± 0.36
Group -IV	ECS (50 mg/ml)	49.8 ± 1.01	62.31 ± 0.65
Group -V	ECS (75 mg/ml)	27.8 ± 0.42	48.49 ± 0.64

ECS-Ethanolic extract of Crinum solapurense Values are expressed as mean ± SEM, (n=6) ANOVA followed by Tukey test. when ECS 25, ECS 50, ECS 75 group were compared with standard group –II.



Figure 3 Anthelmintic activity of Crinum solapurense bulb

Antioxidant activity: An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by- products of cell metabolism.

Free radicals may be either oxygen derived (ROS, reactive oxygen species) or nitrogen derived (RNS, reactive nitrogen species). The oxygen derived molecules are  $O^{2-}$  [Superoxide], HO [hydroxyl], HO<sub>2</sub> [hydroperoxyl], ROO [peroxyl], RO [alkoxyl] as free radical and H<sub>2</sub>O<sub>2</sub> oxygen as non-radical. Nitrogen derived oxidant species are mainly NO [nitric oxide], ONOO [peroxy nitrate], NO<sub>2</sub> [nitrogen dioxide] and N<sub>2</sub>O<sub>3</sub> [dinitrogen trioxide].

## 4.2. DPPH Assay

The free radical scavenging activity of ethanolic extract of *Crinum solapurense* was determined according to the DPPH radical scavenging method and is shown in **Table 4**. According to this method, a compound with high antioxidant activity effectively reacts with the radical; hence prevent its reactivity and the resultant chain reaction. Ethanolic extract of *Crinum solapurense* in different concentrations were analyzed for DPPH radical scavenging effect. The results are presented as IC<sub>50</sub> values.

S.No.	Concentrations (µg/mL)	% DPPH radical scavenging activity of Ethanolic extract	% DPPH radical scavenging activity of Vitamin C
1	20	31.25 ± 0.106	56.06 ± 0.085
2	40	57.04 ± 0.173	66.64 ± 0.526
3	60	63.04 ± 0.276	71.76 ± 0.387
4	80	73.41 ± 0.110	88.54 ± 0.576
5	IC <sub>50</sub>	27.29 μg/ml	37.12 μg/ml

**Table 4** Percentage DPPH radical scavenging activity of Ethanolic extract of Crinum solapurense bulb



Figure 4 Comparison of % DPPH Scavenging Activity of ECS and Vitamin C

The free radical scavenging activity of the extracts is evaluated by assessing their ability to reduce the colour of DPPH in ethanol according to Brand Williams. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts.

A simple method developed to determine plants' antioxidant activity utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolonization is stoichiometric with respect to number of electrons captured.

# 4.3. ABTS Assay

The free radical scavenging activity of Ethanolic extract of a bulb of *Crinum solapurense* was determined according to the ABTS radical scavenging method and is shown in Table no-V. Ethanolic extract of *Crinum solapurense* in different concentrations were analyzed for ABTS radical scavenging activity. The results were expressed as IC<sub>50</sub> values and obtained from regression lines as shown intable.

S. No.	Concentrations (µg/mL)	% ABTS radical scavenging activity of Ethanolic extract	% ABTS radical scavenging activity of Vitamin C
1	20	30.87 ± 0.183	41.81 ± 0.211
2	40	54.08 ± 0.249	60.94 ± 0.107
3	60	60.08 ± 0.250	71.50 ± 0.247
4	80	72.07 ± 0.207	82.15 ± 0.164
	IC <sub>50</sub>	43.410 μg/mL	28.614 μg/mL

Table 5 Percentage ABTS radical scavenging activity of Ethanolic extract of Crinum solapurense bulb



Figure 5 Comparison of % ABTS Scavenging Activity of ECS and Vitamin C



Figure 6 Calculation of IC<sub>50</sub> value of ECS by regression analysis



Figure 7 Calculation of IC<sub>50</sub> value of Vitamin C by regression analysis

# 5. Conclusion

The plant *Crinum solapurense* was selected in the present study to explore the scientific information on Pharmacognosy, Phytochemical, and Pharmacological aspects. The parameters which are reported under pharmacognostical and phytochemical studies could be used for botanical identification of the drug in the crude form and preparation of monograph of the plant *Crinum solapurense* Lam.

The present investigation has brought the drug *Crinum solapurense* Lam. could serve as a better drug for effective treatment as Anthelmintic. Also, the plant *Crinum solapurense* may represent a new source of antioxidants with stable, biologically active compounds that can establish a scientific base for use in modern medicine.

# **Compliance with ethical standards**

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## Disclosure of conflict of interest

The authors state that there were no connections between them and any companies or organizations that may be seen as generating a conflict of interest during the research.

# Author Contribution

C.V. Nagathan, Suresh Gunaki, and Shivanand Kolageri performed an appropriate literature review, gathered data, designed the study, produced a section of the paper, and gave their all during the editing process. Shivanand Kolageri designed and created the analysis. All the authors reviewed and edited the final version.

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