World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

In-vitro antioxidant and antidiabetic activity of *Crinum latifolium*

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World Journal of Biology Pharmacy and Health Sciences, 2024, 19(03), 295–303

Publication history: Received on 16 June 2024; revised on 22 July 2024; accepted on 24 July 2024

Article DOI: https://doi.org/10.30574/wjbphs.2024.19.3.0319

Abstract

The present study was done to investigate the *In-Vitro* antioxidant and antidiabetic activity of ethanolic extract of *Crinum latifolium* leaves. Phytochemicals of *Crinum latifolium* leaf extract was analysed by using qualitative methods. *In-Vitro* antioxidant and antidiabetic study were done by Hydrogen peroxide (H_2O_2) assay and Glucose uptake in Yeast cells method respectively. Phytochemical screening of ethanolic extract of leaves of *Crinum latifolium* revealed that it is rich in alkaloids, glycosides, tannins, flavonoids, gums and mucilage's.

The results for Hydrogen peroxide assay showed significant inhibitory potential in graded dose responsive against H_2O_2 free radicals at different concentrations of ascorbic acid (0.2 to 1.2 mg/ml). The standard ascorbic acid showed better inhibitory activity compared to sample, however 1.2 mg/ml ethanolic extract showed 71.94% inhibition and found to be equivalent to 76.82% of ascorbic acid.

The results for Glucose uptake in Yeast cells method showed significant uptake of glucose across the plasma membrane of yeast cells at concentrations of (0.2 to 1.2 mg/ml) at 5mM glucose concentration. The standard Metronidazole showed better activity compared to sample, however 1.2 mg/ml ethanolic extract showed 83.07 % of glucose uptake and found to be equivalent to 87.98 % of Metronidazole.

Overall, the present study results suggested that ethanolic extract of *Crinum latifolium* leaves has potential antioxidant and antidiabetic activities. However, further preclinical studies have to be done along with in vivo studies for confirming the results.

Keywords: Antidiabetic; Antioxidants; Ascorbic acid; *Crinum latifolium* Metronidazole

1. Introduction

Most humans have been studying nature, especially plants, in an effort to discover new drugs since ancient times. Significantly, a large number of therapeutically useful medicinal plants are at present are being used to treat a wide variety of diseases and disorders. At present 40 % of the world's population relies solely on plant-based medicine for their health care.[1]

Various ancient Indian medicinal practises like Unani, Ayurveda, homoeopathy, and Siddha are practised.[2] These pharmaceuticals are sourced from a single plant or a group of plants, the effectiveness of these drugs is depending on the choice of an efficient plant component and the availability of sufficient secondary metabolites in the raw material determining the medicine's efficacy.[3] Over 80 % of the world's population, or 4.3 billion people, rely on traditional plant-based systems of medicine as their main health care, as reported by the World Health Organization. In traditional systems in India, plant-based medicines made up over 95 % of all prescriptions. In response, there is a growing interest in evaluating herbal remedies, which are seen to be less toxic and to have negligible side effects.[4][5]

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The incidence of diabetes is increasing in the developing world, with an increase in the number of diabetes patients in younger age groups. The therapeutic management of diabetes without any side effects remains a challenge. The oxidative stress, increases due to several factors including hyperglycaemia, insulin resistance, and altered mitochondrial function, is playing a substantial role for causing type 2 diabetes. Therefore, largescale research is conducted on medicinal plants with antioxidant potential, as an alternative to the current treatment for diabetes. [6,7]

Sudarshna (*Crinum latifolium*) is a historical plant has a valuable importance because of its medicinal properties. *Crinum latifolium* Linn (Sudarshana) is a herbaceous flowering plant belongs to family Amarylidaceae is widely used in ayurveda mainly for painful swellings, fevers of unexplained origin, poisoning and skin ailments. Crinum is a genus of about 180 species comprising family of various beautiful perennial plants.[8] They are good for decoration, garden, bouquets and also known as various types of lilies like spider lily, Trumpetlily, and Swamp lily and so on. Crinum is basically a tropical plant growing in Asia, South east, Australia, Pacific Island and spread up to Caribbean, Florida and Louisiana. Sudarshana is a small plant that grows up to 3 feet contain big green leaves to a length 2-4 inch with 3–4-inch width. It grows all over India.[9] The main chemical constituents of Sudarshana are Crinamine, Lycoricidine, Lycoriside, Cirnasiatin, Hippadine, Crinine, Crinasiatine, Methyl linoleate, Cridnidine Glucans A & B, Alkoloids-zeylamine crinofoline, crinofolidine,tazetine,flexinine,harmenthamine,ambelline, galanthamine.The leaves contain alkaloids latifine, cherilline, 3-0-acetalamine, crinomine and crinine. Thus, the plant has diverse pharmacological actions. [10,11,12,13]

C.latifolium exhibits various pharmacological effects like antiinflammatory, anti-diarrhoeal, hypoglycemic, antioxidant, hepatoprotective, antipyretic, and antimicrobial activities anti-bacterial, anticancer, anti-ulcer, antisecretory, hepatoprotective, hypoglycaemic, sore throat and wound healing etc. [14,15,16,17]

2. Plant Collection and Authentication

The leaves of *Crinum latifolium* was collected from local places of Medchal, Telangana. *Crinum latifolium* was authenticated with Voucher Number. 0908, by Dr. K. Madhava Chetty (Retd.) M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Plant Taxonomist (IAAT: 337), Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh.

Figure 1 Shade drying of *C. latifolium* leaves

Figure 2 Coarse powder of *C. latifolium* leaves

3. Extraction by Maceration

3.1. Maceration procedure

- Fresh leaves of C. latifolium was washed with water to get rid of contaminants like dirt and other impurities and were shade dried.
- These dried leaves were ground into coarse powder.
- 50g of weighed leaves of C. latifolium was taken in a beaker and 200ml of Ethanol is added.
- The mixture was kept for Maceration for 7 days and stirred occasionally.
- The mixture was filtered with a Filter paper and the filtrate was collected by pressing the marc.
- The filtrate was allowed for Evaporation at room temperature to obtain the Ethanolic extract.
- Filtrate was collected and extractive value was determined after evaporating the solvent using formula:

Extractive Value = (Weight of ethanolic extract / Weight of powder of *C. latifolium*) × 100

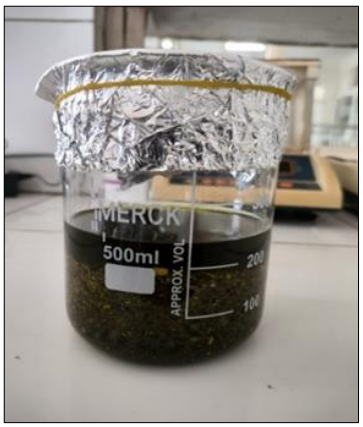

Figure 3 Maceration

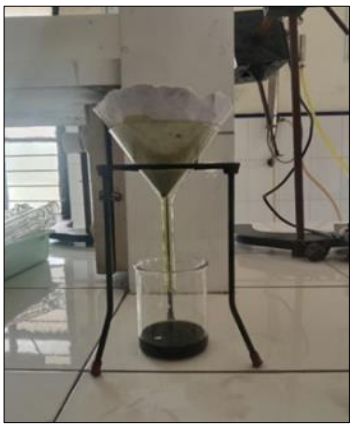

Figure 4 Filtration

Figure 5 Filtrate and Marc

Figure 6 Ethanolic Extract

3.2. Qualitative evaluation of Phytoconstituents

The sample was screened for the presence of various phytoconstituents like carbohydrates, flavonoids, polyphenolic compounds, saponins, tannins, triterpenoids, etc

3.2.1. Test for Carbohydrates

- Fehling's test: 0.5ml of Fehling's A was added to 0.5ml of Fehling's B solution and to this mixture, 2ml of sample was added. The mixture was heated in boiling water for 5-10 minutes and a yellow colour followed by a brick-red precipitate shows the presence of carbohydrates.
- Benedict's test: 0.5ml of sample is taken in a test tube and to this 0.5ml of Benedict's reagent was added. The mixture was boiled for 5 minutes and a brick red precipitate shows the presence of carbohydrates.
- Molisch's test: 2-3ml of sample was taken in a test tube and to this, few drops of α-naphthol solution were added. The test tube was shaken and conc. H2SO4 was added from the walls. Violet ring at the junction of two liquids indicates the presence of carbohydrates.

3.2.2. Test for Amino acids

- Millions test: A small quantity of sample was treated with few drops of Millon’s reagent and observed for the formation of white precipitate which indicates the presence of amino acids.
- Ninhydrin test for Amino acids: 3ml of sample was heated and to it 2-3 drops of 5% Ninhydrin solution was added. The mixture was kept in a boiling water bath for 10 minutes. The purple or bluish colour indicates the presence of amino acids.

3.2.3. *Test for Proteins*

- Biuret's test: 1ml of hot sample was taken in a test tube, and to it 0.5ml of NaOH and 0.1ml of 3 percent copper sulphate solution was added. A red or violet colour shows the presence of proteins.

3.2.4. Test for Fats and fixed oils

The sample was taken on a filter paper and permanent stain on filter paper indicates the presence of fats and oils.

3.2.5. Test for Gums and mucilage's

Sample was hydrolysed using dilute HCl. The solution was subjected to Fehling's test and red colour indicates the presence of gums.

3.2.6. Test for Alkaloids

- Dragendorff's test: Sample was treated with Dragendorff's reagent (potassium bismuth iodide) and formation of reddish-brown precipitate indicates the presence of alkaloids.
- Mayer's test: Sample was treated with Mayer's reagent (potassium mercuric iodide) and the formation of cream colour precipitate indicates the presence of alkaloids.
- Wagner's test: Sample was treated with Wagner's reagent (iodine in potassium iodide) and formation of brown precipitate indicates the presence of alkaloids.
- Hager's test: Sample was treated with Hager's reagent (saturated solution of picric acid) and formation of yellow colour precipitate indicates the presence of alkaloids.

3.2.7. Test for Glycosides

- Legal's Test for glycosides
- To sample, 1ml pyridine and 1ml sodium nitroprusside were added. Blood red colour appears which indicates the presence of Cardiac glycosides.
- Keller kiliani test: extract the drug with chloroform and evaporate it to dryness. Add 0.4 ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube, add carefully 0.5 ml of concentrated sulphuric by the side of the test tube. Acetic acid layer shows blue colour indicates presence of Cardiac glycosides.
- Froth formation test: Place 2ml of solution drug in water in a test tube, shake well, stable froth is formed which indicates the presence of saponin glycosides.

3.2.8. Test for Flavonoids

- Shinoda test: 0.5ml of sample is taken in a test tube, and to it few pieces of magnesium turnings were added followed by the addition of 0.5ml of concentrated hydrochloric acid was added dropwise and a pink scarlet or crimson red or occasionally green to blue colour appears after few minutes.
- Lead acetate test: To 1ml of sample 1ml of lead acetate solution was added and a yellow colour precipitate indicates the presence of flavonoids.
- Alkaline reagent test: To the test solution add few drops of sodium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicate presence of flavonoids.

3.2.9. Test for Tannins (Polyphenols)

- Ferric chloride test: Treat the extract with ferric chloride solution. Blue or green colour appears indicates the presence of tannins.

3.2.10. *Test for Phytosterols*

- Salkowski test: To 2ml of sample, 2ml of chloroform and 2ml conc. H_2SO_4 was added. Shake well, then a red chloroform layer appears and acid layer shows greenish yellow fluoresce, which indicates presence of steroids.
- Liebermann-Burchard test: To the 2ml of sample add chloroform, then 1-2ml of acetic anhydride was added and 2 droops of conc. H_2SO_4 was added from the sides of the test tube, then a red colour followed by blue colour and green colour appears.

3.2.11. Test for Naphthoquinones

- Juglone test: Treat 2ml of chloroform extract and 2ml of ethyl ether with dilute ammonia solution. Appearance of pink colour indicates naphthoquinones. [18]

3.3. *Invitro* antioxidant activity of Ethanolic extract of *C. latifolium*

3.3.1. Hydrogen peroxide method

Materials/Chemicals required: Hydrogen peroxide

Phosphate buffer (0.1M, pH 7.4)

Ascorbic acid (Standard).

Preparation of Phosphate buffer: 1.361 g of Potassium dihydrogen phosphate was taken and dissolved in water and diluted to 100ml with the same solvent. The pH was adjusted by using a 35g/l solution of Disodium hydrogen phosphate.

3.4. Method

- The obtained extract should be made into different concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2mg/ml).
- To the extract, add 0.6ml of 4mM H2O2 solution prepared in phosphate buffer, vortex them and incubate for 10 mins.
- Same should be performed with the standard solution containing ascorbic acid.
- Measure the absorbance at 230 nm.
- Ascorbic acid was used as standard/positive control.
- All tests were conducted in triplicates. [19]
- The ability to scavenge the hydrogen peroxide should be calculated using the equation below:

$$\% \text{ scavenged } (H_2O_2) = (Ao - A1)/Ao \times 100$$

Where., Ao is the absorbance of the control

A1 the absorbance of the sample.

3.5. *Invitro* antidiabetic activity Ethanolic extract of C. latifolium

Glucose uptake in yeast cells:

- Materials/Chemicals required: Yeast
- Plant extract
- Glucose solution
- Metronidazole

3.5.1. Procedure

- The yeast was added in distilled water 1% (w/v) and kept for overnight.
- It was subjected to repeated centrifugation (2500rpm, 5mins) until clear supernatant fluid were obtained.
- Various concentrations of plant extract (0.2, 0.4, 0.6, 0.8, 1.0, 1.2mg/ml) were added to 1 ml of glucose solution (5mM) and incubated together for 10 minutes at 37° Celsius.
- Reaction was started by adding 0.1ml of yeast suspension followed by vortexing and further incubating at 37° C for 60 minutes.
- After 60 minutes the tubes were centrifuged at 2500rpm for 5 mins and amount of glucose was estimated in supernatant.
- Metronidazole was used as standard drug.
- All the experiments were carried out in triplicates. [20]
- The percentage increase in glucose uptake by yeast cells was calculated using following formula:

$$\text{Increase in glucose uptake } (\%) = \frac{\text{Absorbancecontrol} - \text{Absorbancesample}}{\text{Absorbancecontrol}} \text{X } 100$$

Where; Abs sample is the absorbance of test samples and

Abs control is the absorbance of control reaction (having all reagents except the test sample.)

4. Results

4.1. Results of Extractive value

- The extractive value was found to be 4.8% (2.4gm / 50gm) x 100

4.2. Results of Qualitative evaluation of phytochemical constituents

Table 1 Results of Qualitative evaluation of phytochemical constituents

Phytoconstituents	**Inference**
Carbohydrates	Present
Amino acids and Proteins	Present
Alkaloids	Present
Glycosides	Present
Tannins	Present
Phytosterols	Absent
Flavonoids	Present
Saponins	Absent
Fats and fixed oils	Absent
Gums and mucilage's	Present
Naphthoquinones	Present

The preliminary Phytochemical Screening showed the presence of various phytoconstituents like Alkaloids, Glycosides, Tannins, Flavonoids, Carbohydrates, Amino acids and proteins, Gums and mucilages, and Naphthoquinones in Ethanolic extract of *C. latifolium*. Results were shown in **Table 1.**

4.3. Results of Hydrogen Peroxide (H_2O_2) assay.

The antioxidant activity of ethanolic extract of *C. latifolium* was done against H_2O_2 assay. The results showed significant inhibitory potential in graded dose responsive against H_2O_2 free radicals at different concentrations of ascorbic acid (0.2 to 1.2 mg/ml). The standard ascorbic acid showed better inhibitory activity compared to sample, however 1.2 mg/ml ethanolic extract showed 71.94% inhibition and found to be equivalent to 76.82% of ascorbic acid.

Table 2 Results of H_2O_2 assay

Concentration	**Ascorbic acid**	**Ethanolic extract**
0.2	38.96	29.32
0.4	42.38	36.12
0.6	51.60	43.65
0.8	62.63	51.06
1.0	69.23	59.31
1.2	76.82	71.94

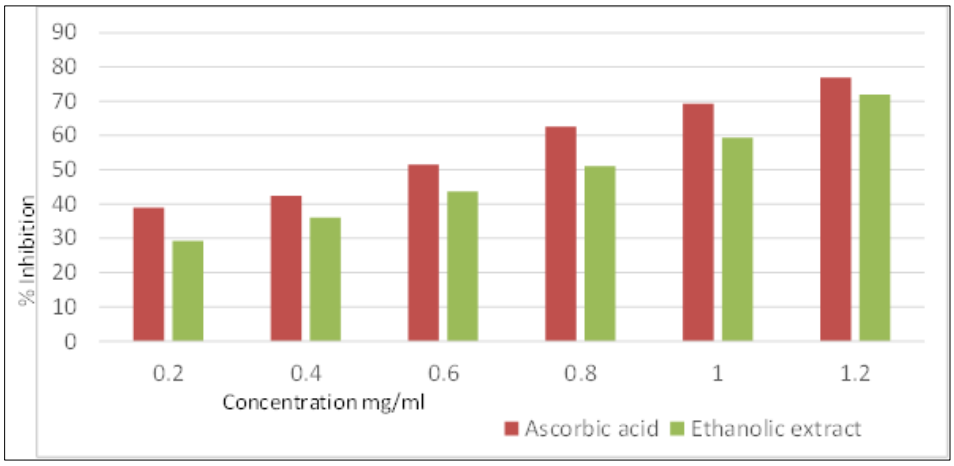

Figure 7 In- vitro antioxidant activity ethanolic extract of C. latifolium

4.4. Results for Glucose uptake in yeast cells method

The antidiabetic activity of ethanolic extract of *C. latifolium* was done against glucose uptake in yeast cells method. The results showed significant uptake of glucose across the plasma membrane of yeast cells at concentrations of (0.2 to 1.2 mg/ml) at 5mM glucose concentration. The standard Metronidazole showed better activity compared to sample, however 1.2 mg/ml ethanolic extract showed 83.07 % of glucose uptake and found to be equivalent to 87.98 % of Metronidazole.

Table 3 Results of Glucose uptake in yeast cells

Concentration	Metronidazole	Ethanolic extract
0.2	59.23	48.98
0.4	62.79	53.46
0.6	67.98	60.77
0.8	76.58	69.23
1.0	81.34	77.89
1.2	87.98	83.07

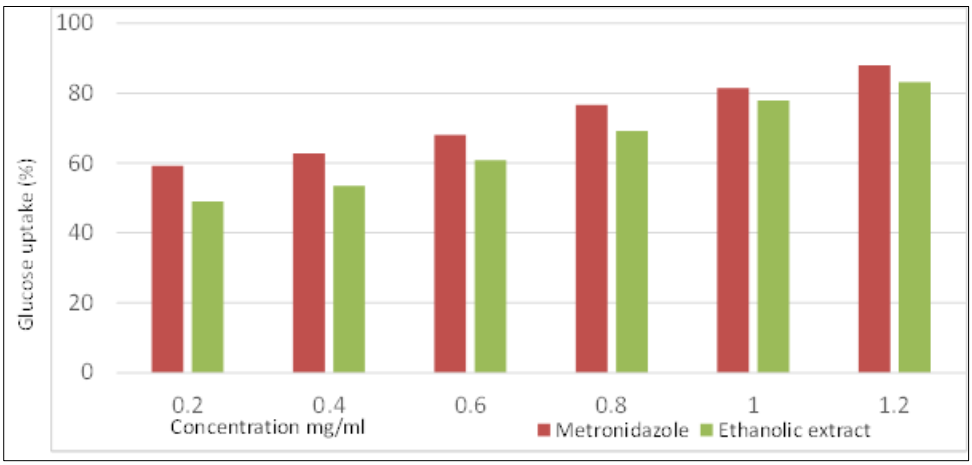

Figure 8 *In-Vitro* antidiabetic potential of ethanolic extract of C. latifolium

5. Conclusion

The findings of this study showed the *Crinum latifolium* leaf extracts are highly concentrated in alkaloids, glycosides, tannins, flavonoids, gums and mucilages and other phytochemicals. By evaluating the effectiveness of *C. latifolium* against that of common medications, its *In-Vitro* antioxidant and antidiabetic potential has been validated. The ethanolic extract of *C. latifolium* leaves showed potential antioxidant action as indicated by its % inhibition in Hydrogen peroxide (H_2O_2) assay. *C. latifolium* showed potential antidiabetic activity by glucose uptake in yeast cells.

The antioxidant activity of ethanolic extract of *C. latifolium* was done against H_2O_2 assay. The results showed significant inhibitory potential in graded dose responsive against H_2O_2 free radicals at different concentrations of ascorbic acid (0.2 to 1.2 mg/ml). The standard ascorbic acid showed better inhibitory activity compared to sample, however 1.2 mg/ml ethanolic extract showed 71.94% inhibition and found to be equivalent to 76.82% of ascorbic acid.

The antidiabetic activity of ethanolic extract of *C. latifolium* was done against glucose uptake in yeast cells method. The results showed significant uptake of glucose across the plasma membrane of yeast cells at concentrations of (0.2 to 1.2 mg/ml) at 5mM glucose concentration. The standard Metronidazole showed better activity compared to sample, however 1.2 mg/ml ethanolic extract showed 83.07 % of glucose uptake and found to be equivalent to 87.98 % of Metronidazole.

Overall, the present study results suggested that *Crinum latifolium* ethanolic leaf extract has potential antioxidant and antidiabetic activities. However, further preclinical studies have to be done along with *in vivo* studies for confirming the results.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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