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In-vitro antioxidant activity of hydroalcoholic extract of *crinum latifolium* using different method

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***Abstract*---**The purpose of this research is to look for phytochemicals and determine the antioxidant potential of *C. latifolium* aerial parts. The qualitative analysis of several phytochemical components was determined using a well-known test methodology. The 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay, hydrogen peroxide, and nitric oxide radical scavenging method were used to investigate the antioxidant activity of a hydro ethanol extract of *Crinum latifolium* bark in vitro. Phytochemical studies discovered the existence of phenols and flavonoids. The extract demonstrated dose-dependent free radical scavenging capabilities in the models studied. For the DPPH method, aerial portions of *Crinum latifolium* extract had an IC50 value of 46.35µg/ml, which was close to that of ascorbic acid (IC50=20.03g/ml). The IC50 value for the Superoxide radical scavenging assay was determined to be 60.94µg/ml, which is comparable to ascorbic acid (IC50= 20.91g/ml). The IC50 value was determined to be 50.30µg/ml in reducing power determination, which was close to that of ascorbic acid (IC50=20.84g/ml). The phytochemical profile and antioxidant activity of *Crinum latifolium*, which will be exploited for medicinal purposes, are described in this paper.

Keywords*---Crinum latifolium*, quantitative phytochemical, antioxidant activity.

Introduction

Free radicals, or highly reactive oxygen species, are produced in the human body by exogenous chemicals and endogenous metabolic activity. These can cause neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis, and other degenerative illnesses by oxidising biomolecules such as nucleic acids, proteins, lipids, and DNA [1, 2]. Antioxidants are molecules that protect cells from free radical damage, lowering the risk of disease [3]. Antioxidant molecules like ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione, as well as enzymes like superoxide dismutase and catalase, protect almost all organisms from free radical damage to some extent. Antioxidant supplements or dietary antioxidants, according to Prior and Cao [4, protect against free radical damage. Natural antioxidants are gaining popularity as a means of preventing oxidative damage caused by free radicals in the human body, notably in the brain. In the last two decades, several medicinal plants have demonstrated efficacy utilising traditional psychoneuropharmacology methodologies [5]. Crinum is a tonic that can be used to treat allergies, inflammation, and tumours. In Hindi, it's called "Sudarshan," while in Sanskrit, it's called "Madhuparnika." The leaf juice is applied externally to skin problems and piles to reduce pain and discomfort [6]. It's also utilised as an analgesic, immunological stimulant, anticancer, antiviral, and antibacterial, as well as a treatment for high blood pressure, rheumatism, and weakness [7]. *C. latifolium is utilised in a variety of Ayurvedic formulations, notably Mahasudarshancurna, which has been used as an antiviral, antimalarial, and antipyretic for centuries [8], and hence has a considerable industrial potential. With this in mind, the current study aimed to compare the antioxidant activity of C. latifolium with that of other plants that have been used for a variety of purposes in the past.*

Material and Method

Material

HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all of the chemicals utilised in this work (Mumbai, India). The chemicals and solvents utilised in this experiment were all of analytical grade.

Methods

Extraction by maceration process

The maceration method was used to extract 160 gramme of powdered aerial parts of *C. latifolium* using several solvents (petroleum ether, chloroform, ethyl acetate, hydroalcoholic, and distilled water). The extract was evaporated at temperatures higher than their boiling points. Finally, the dried extracts' percentage yield was calculated. After that, the extracts were reduced in a rotary evaporator before being kept in airtight containers at 4°C for further use.

Phytochemical screening of the extract

The extract of *C. latifolium* was analysed qualitatively for alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids, among other phytoconstituents [9, 10].

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DPPH method

The spectrophotometer was used to measure the DPPH scavenging activity [11]. The stock solution (6 mg in 100 mL methanol) was produced to give an initial absorbance of 1.5 mL in 1.5 mL methanol. After 15 minutes, there was a decrease in absorbance in the presence of sample extract at various concentrations (10-100g/ml). 1.5 ml of DPPH and 1.5 ml of varying concentrations of the test sample were placed in a succession of volumetric flasks, and the final volume was adjusted to 3 ml using methanol. Three test samples were collected and processed in the same way. Finally, the average was calculated. After 15 minutes at 517 nm, the absorbance of DPPH with varied concentrations showed a final reduction.

$$Calculationof\ \%\ Reduction \frac{\text{Control Absorbance } - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Superoxide radical scavenging assay

The scavenging ability of extracts against superoxide radicals (O_2) was determined using the NBT reagent technique [12-13]. This approach relies on the autooxidation of hydroxylamine hydrochloride in the presence of NBT, which is then reduced to nitrite, to generate O_2. In the presence of EDTA, the nitrite ion produced a colour with a maximum wavelength of 560 nm. Sodium carbonate (1 mL, 50 mM), NBT (0.4 mL, 24 mM), and EDTA (0.2 mL, 0.1 mM) solutions were added to extracts or vitamin C (1 mL, 10-100 µg/mL) test samples, and absorbance was measured at max 560 nm immediately.

Calculation of % Reduction

$$\frac{\text{Control Absorbance -Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Reducing power determination

The potassium ferricyanide-ferric chloride technique was used to investigate the ferric reducing capability of extracts. To decrease ferricyanide to ferrocyanide, 0.2 mL of each of the extracts at varied concentrations, 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide K3Fe(CN)6 (1 percent) were combined and incubated at 50°C for 20 min [14]. The reaction was halted by adding 2.5 mL of 10% (w/v) trichloroacetic acid and centrifuging for 10 minutes at 1000 rpm. Finally, the absorbance was measured at 700 nm using 2.5 mL of

the upper layer combined with 2.5 mL of distilled water and 0.5 mL of FeCl3 (0.1 percent).

Results and Discussion

The yield of extracts produced from *C. latifolium* aerial parts employing hydroalcoholic solvent was determined to be 10.76 percent. Flavonoids, alkaloids, saponins, phenolics, carbohydrate, and tannin were discovered in a hydroalcoholic extract of the plant. The hydrogen donating nature of extracts was evaluated using the DPPH radical scavenging test. Various methods were used to determine the inhibitory concentration 50 percent (IC50) value of aerial portions of C. latifoliumhydroalcoholic extract for DPPH radical scavenging activity. In the investigated models, the extract had dose-dependent free radical scavenging properties. For the DPPH method, aerial portions of *Crinum latifolium* extract had an IC50 value of 46.35µg/ml, which was equivalent to ascorbic acid (IC50=20.03µg/ml) table and figure 1. The IC50 result for the Superoxide radical scavenging experiment was 60.94g/ml, which compares favourably to ascorbic acid (IC50= 20.91g/ml) table and figure 2. The IC50 value was determined to be 50.30g/ml in reducing power determination, which was equivalent to that of ascorbic acid (IC50=20.84g/ml)table and figure 3. The phytochemical profile and antioxidant activity of *Crinum latifolium*, which will be exploited for medicinal purposes, are described in this paper.

Table 1
% Inhibition of ascorbic acid and *Crinum latifolium* extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	37.39	24.32
2	20	52.13	35.41
3	40	67.78	44.53
4	60	69.91	62.77
5	80	83.74	71.88
6	100	85.56	81.46
IC_{50}		20.03	46.35

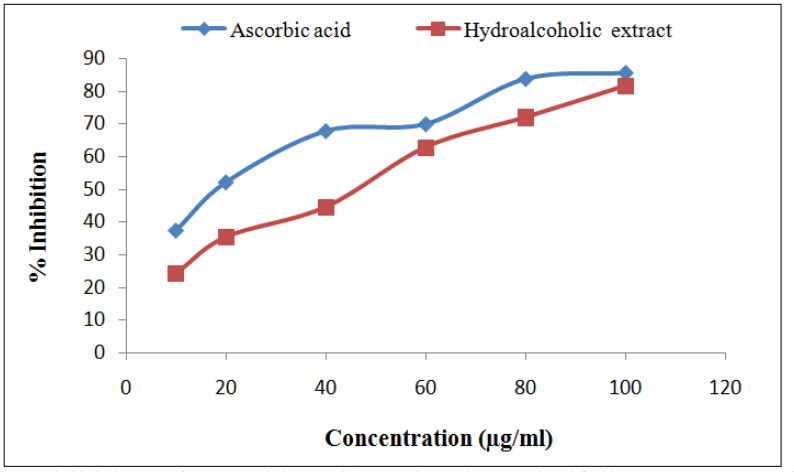

Figure 1. % Inhibition of ascorbic acid and *Crinum latifolium* extract using DPPH method

Table 2
Superoxide radical scavenging activity of hydroalcoholic extract with reference to ascorbic acid

S. No.	Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Hydroalcoholic extract (% inhibition)
1	10	41.49	20.21
2	20	51.06	26.29
3	40	60.79	41.95
4	60	67.48	52.13
5	80	71.88	56.69
6	100	79.33	72.04
IC_{50} Value		20.91	60.94

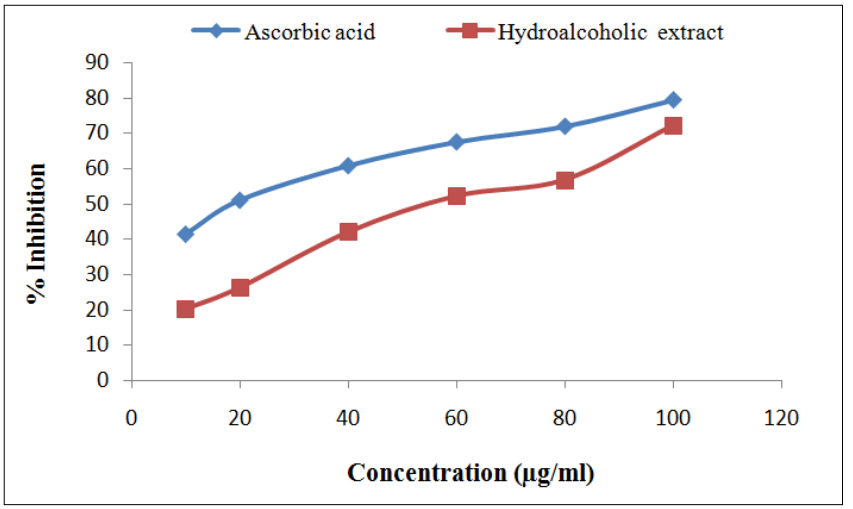

Figure 2. Superoxide radical scavenging activity of hydroalcoholic extract with reference to ascorbic acid

Table 3
% Reducing power determination ofAscorbic acid and hydroalcoholic extract of *Crinum latifolium*

Control	% Inhibition	% Inhibition
	Ascorbic acid	*Crinum latifolium* extract
10	41.49	16.11
20	52.58	26.29
40	56.69	44.53
60	67.78	57.29
80	71.88	73.56
100	73.56	79.48
IC_{50}	20.84	50.30

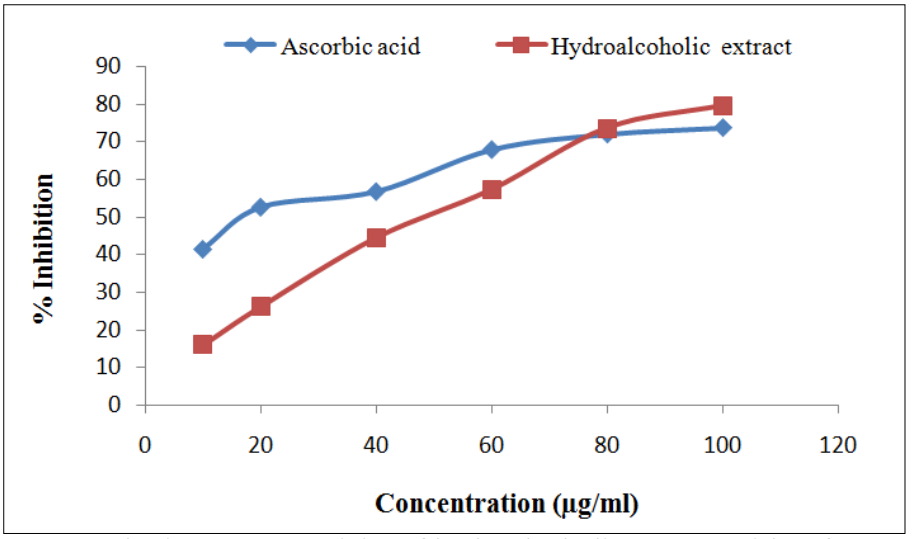

Figure 3. Reducing power activity of hydroalcoholic extract with reference to ascorbic acid

Conclusion

The findings of this study show that the extract may effectively scavenge several reactive oxygen species/free radicals in vitro. This could be because it can produce a large number of stable oxidised products after oxidation or radical scavenging. The extracts' wide spectrum of action suggests that antioxidant activity is mediated by many pathways. The extract's various antioxidant properties exhibited in this study clearly reveals the plants' potential application value. However, before either plant can be used as an antioxidant ingredient in animal feeds or human health foods, its in vivo safety must be thoroughly examined in experimental rodent models. According to the findings, hydroalcoholic extract of *Crinum latifolium* may have antioxidant capabilities. Further research into the antioxidant role of the plants mentioned above in diverse systems could lead to the discovery of natural antioxidants.

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