PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL STUDIES ON THE AERIAL PARTS EXTRACTS OF *CRINUM LATIFOLIUM* L. (AMARYLLIDACEAE)

Abstract

The present study was undertaken to find the antimicrobial activities, phytochemical presence in various aerial parts extract of *Crinum latifolium* (*C. latifolium*). Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics, flavonoids and alkaloids was determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic, flavonoids and alkaloids was carried out by Folins Ciocalteau reagent, aluminium chloride and bromocresol green method respectively. The *in vitro* antimicrobial activity was done by well diffusion assay method against *S. mutans* and *S. bongori* using standard ofloxacin, ciprofloxacin (10-30µg/ml). The antimicrobial activity was determined by measuring the diameter of the zone of inhibition in term of millimeter (mm). The phytochemical analysis showed the presence of tannins, glycosides, flavonoids and alkaloids ect. The antimicrobial activity of hydroalcoholic extract of aerial parts against all microorganisms was concentration dependent manner but less than standard drug. It is concluded that the antimicrobial activity showed by the plant is due to the presence of these phytochemicals. For future studies, phytochemcials responsible for these activities can be isolated and modified for pharmacological purpose.

Keywords: Infectious diseases, *Crinum latifolium*, Antimicrobial activity, phytochemical analysis.

INTRODUCTION

Due to the widespread idea that green medicine is safe, easy to get, and has fewer side effects, herbal medicines have grown more popular in the treatment of all ailments. Many plants are less expensive and more accessible to the majority of people, especially in poor countries, than traditional treatment, and they have a lower risk of side effects. These factors may explain for their widespread popularity and use [1]. Some researches have documented the therapeutic benefits of some plants [2-4]. The major source of innovative medications and healthcare items is medicinal plants [5]. The extraction and characterisation of various phyto chemicals from these green factories has resulted in the development of a number of medicines with high activity profiles [6]. Indeed, market and public demand have grown to such an extent that many therapeutic plants are now at risk of extinction or genetic diversity loss [7]. The continuing appearance or persistence of drug-resistant organisms, as well as pathogenic organisms' increasing evolutionary adaptations to routinely used antimicrobials,

have lowered the efficiency of presently used antimicrobials. Furthermore, antibiotics are linked to side effects, necessitating the hunt for alternative medications from unconventional sources such as plants. It has been stated that more than 80% of the world's population relies on plants to satisfy their basic health care needs [8]. Plants are still a key source of commercially used medications. Many synthetic medications have been shown to be effective. In recent years, there has been an upsurge in the use of natural goods, and active plant extracts are regularly evaluated for novel medication discoveries [9]. Because there is little information on the antibacterial activity of C. latifolium leaves, the current study aims to explore the antimicrobial activity of hydroalcoholic extracts from A C. latifolium leaves against bacterial and fungal species. These extracts are also subjected to preliminary phytochemical analyses in order to identify bioactive chemicals with antibacterial activity. Spectrophotometric analysis was used to evaluate the total phenol, flavonoid, and alkaloids content.

MATERIALS AND METHODS

Plant material

In the month of November 2021, aerial portions of *C. latifolium* were taken from a local location in Bhopal (M.P.). To eliminate clinging dust particles and other undesired elements, the aerial sections were separated and rinsed with sterile distilled water. The aerial pieces were dried in the open air at room temperature. The powdered plant samples were chopped and ground from dry plant samples. For future usage, the powdered samples were kept in a clean, dry, and sterile container.

Reagents for chemistry

Hi Media Laboratories Pvt. Ltd. (Mumbai, India), SD Fine- Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all of the chemicals utilised in this investigation (Mumbai, India).

The substances employed in this experiment were all analytical grade. Quercetin, atropine and gallic acid was kindly provided by Scan Research Laboratories, Bhopal (India). The test organisms *S. mutans, S. bongori* was obtained from the stocks of Scan Research Laboratories, Bhopal (India).

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' % yield was calculated. After that, the extracts were reduced in a rotary evaporator before being kept in airtight containers at 4°C for later 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 [10, 11].

Total phenol determination

The total phenolic content was calculated using Parkhe et al's technique [12]. 1 ml Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml extracts or standards. The mixture was allowed to sit at room temperature for 15 minutes. A UV/visible spectrophotometer was used to measure the colour generated at 765 nm. The total phenolic content was determined using the gallic acid standard graph, and the findings were represented in milligrammes per 100 milligrammes of gallic acid.

Total flavonoids determination

The total flavonoid content was determined using the method of Parkhe *et al* [12]. 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

Total alkaloids determination

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform [13]. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

Antimicrobial activity of *C. latifolium extract*

Using Bauer et al [13] conventional protocol, the antibacterial activity of the extract produced from *C. latifolium* was determined using the well diffusion method. Ofloxacin and ciprofloxacin of IP grade were employed in the usual formulation. *S. Mutans* and *S. bongori* were cultured for 24 hours to test antibacterial activity. In antibiogram investigations, three

concentrations were used: 25, 50, and 100 mg/ml for each isolated phytochemical. The placement of wells containing antibiotics on the surfaces of agar soon after inoculation with the organism examined is a key component. Inoculums made from undiluted overnight broth cultures should never be utilised. The plates were incubated for 24 hours at 37°C. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug. The diameter of zone of inhibition of each wall was recorded.

RESULTS AND DISCUSSION

To achieve the real yield of extraction, the crude extracts produced after each consecutive maceration extraction step were concentrated on a water bath by fully evaporating the solvents. Table 1 shows the yield of extracts produced from C. latifolium aerial parts employing chloroform, ethyl acetate, hydroalcoholic, and water as solvents. Table 2 shows the findings of a qualitative phytochemical study of the crude powder aerial parts of C. latifolium. Flavonoids, alkaloids, saponins, phenolics, carbohydrate, and tannin were discovered in a hydroalcoholic extract of the plant. Total phenolic compounds (TPC) were calculated as mg/100mg gallic acid equivalent of dry extract sample using the equation 0.019x + 0.016, R2 = 0.999, where X represents gallic acid equivalent (GAE) and Y represents absorbance. Total flavonoids were estimated as quercetin equivalent (mg/100mg) using the equation y = 0.032x + 0.002, R 2 =0.999, where X is the quercetin equivalent (QE) and Y is the absorbance. The total alkaloid content was determined as atropine equivalent mg/100mg using the equation y = 0.007x + 0.006, R 2 =0.999, where X represents the Atropine equivalent (AE) and Y represents the absorbance. Table 3 shows the total phenolic, flavonoids, and alkaloids content of extracts of *C. latifolium* arial sections. Flavonoids are secondary metabolites found in plants that play a critical role in human health and nutrition. Tables 4 and 5 reveal that the antibacterial activity of *C. latifolium* hydroalcoholic arial parts extract showed bioactivity by reducing the growth of the microbiological species tested. The extracts' zone of inhibition was equivalent to that of the conventional medication. It is effective against *S. mutans* and *S. bongori* at different concentrations.

Table 1 Extractive values of *Crinum latifolium*

Sr. No	Extracts	% Yield (W/W)	Colour of extractive
1	Petroleum ether	0.95	Light Yellow
2	Chloroform	2.14	Brown
3	Ethyl acetate	4.51	Brown
4	Hydroalcoholic	10.76	Brown
5	Distilled water	7.84	Brown

Table 2 Result of phytochemical screening of *Crinum latifolium*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Hydroalcoholic extract	Aqueous extract
1.	**Alkaloids** Hager's Test: Wagner's Test:	 -ve -ve	 -ve -ve	 +ve +ve	 -ve +ve
2.	**Glycosides** Legal's Test:	 -ve	 +ve	 +ve	 +ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 -ve +ve	 +ve +ve	 +ve +ve	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 -ve	 -ve	 +ve	 +ve
5.	**Phenol** Ferric Chloride Test:	 -ve	 -ve	 +ve	 +ve
6.	**Proteins** Xanthoproteic Test:	 -ve	 -ve	 +ve	 -ve
7.	**Carbohydrate** Fehling's Test:	 -ve	 +ve	 +ve	 +ve
8.	**Saponins** Froth Test:	 -ve	 -ve	 +ve	 +ve
9.	**Tannins** Gelatin test:	 -ve	 -ve	 +ve	 -ve
10.	***Triterpenoid***	-ve	-ve	+ve	-ve

+Ve = Positive, -Ve= Negative

Table 3 Results of total phenol, flavonoids and alkaloid content

S. No.	Extracts	Total phenol content	Total flavonoids content	Total alkaloid content
		mg/100mg		
1	Chloroform	-	0.263	-
2	Ethyl acetate	-	0.415	-
3	Hydroalcoholic	0.568	0.986	0.091
4.	Aqueous	0.475	0.741	0.053

Table 4 Antimicrobial activity of *standard drug* against selected microbes

S.	Name of drug	Microbes	Zone of Inhibition (mm)

No.			10 µg/ml	20 µg/ml	30 µg/ml
1	Ofloxacin	*Streptococcus mutans*	12±0.15	15±0.13	17±0.19
2.	Ciprofloxacin	*Salmonella bongori*	17±0.15	23±0.86	25±0.5

***Average of three determination, Mean ± SD**

Table 5 Antimicrobial activity of hydroalcoholic extract of *Crinum latifolium* against selected microbes

S. No.	Name of microbes	Zone of inhibition (mm)		
		Hydroalcoholic extract		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Streptococcus mutans*	20±0.47	22±0.47	25±0.94
2.	*Salmonella bongori*	12±0.47	15±0.47	16±0.47

***Average of three determination, Mean ± SD**

CONCLUSION

In this investigation, extracts of *C. latifolium* showed wide antibacterial activity against a variety of microbial species. The plant extract's antibacterial action, which may be related to the discovered phytoconstituents, supports its usage as a folkloric medicine health treatment. As a result, bioactive compounds from this plant can be used to create antimicrobial medicines for the treatment of a variety of bacterial illnesses. Identification of these phytoconstituents, as well as assessment of their antimicrobial potencies and toxicological evaluation with the goal of developing new chemotherapeutic drugs, should be the focus of future research.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Sofowora E. Medicinal plants and medicine in Africa 2nd eds. In.: John Wiley and Sons;. hibueze U, Akubugwo E: Nutritive values and phytochemical contents of some leafy vegetables grown with different fertilizers. Agricultural Biology Journal of North America 2011; 2:1437-1444.

2. Ukpabi C, Akubugwo E. Nutritive values and phytochemical contents of some leafy vegetables grown with different fertilizers. Agriculture and Biology Journal of North America 2011; 2(12):1437-1444.
3. Idu M, Obaruyi G, Erhabor J. Ethnobotanical uses of plants among the binis in the treatment of ophthalmic and ENT (ear, nose and throat) ailments. Ethnobotanical Leaflets 2009; (4): 9-15.
4. Banso A, Adeyemo S. Evaluation of antibacterial properties of tannins isolated from Dichrostachys cinerea. Afr J Biotechnol 2007; 6(15):1785-1787
5. Ivanova D, Gerova D, Chervenkov T, Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J Ethnopharmacology 2005; 96(1-2):145-150
6. Mandal V, Mohan Y, Hemalatha S. Microwave assisted extraction-an innovative and promising extraction tool for medicinal plant research. Pharmacogn Rev 2007; 1(1):7-18
7. Misra A. Studies on biochemical and physiological aspects in relation to phyto-medicinal qualities and efficacy of the active ingredients during the handling, cultivation and harvesting of the medicinal plants. J Med Plants Res 2009; 3(13):1140-1146.
8. WHO Traditional medicine: Growing needs and potential, WHO Policy perspectives on medicines. p. 1, World Healh Organisation, Geneva 2002.
9. Joshi S, Parkhe G, Aqueel N, Dixit N, Jain DK. Estimation of total phenolic, total flavonoids and total protein content of hydroalcoholic extract of Anacyclus pyrethrum. Pharmacologyonline 2019; 1:27-33.
10. Pradhan A, Jain P, Pal M, Chauhan M, Jain DK. Qualitative and quantitative determination of phytochemical contents of hydroalcoholic extract of salmalia malabarica. Pharmacologyonline 2019; 1:21-26.
11. Parkhe G, Jain P, Jain DK. Total phenolic content, total flavonoid & anti-bacterial activity of hydroalcoholic acacia catechu (l.f.) Willd roots extracts for treatment of mouth ulcer. Pharmacologyonline 2018; 2: 227-233.
12. Fazel S, Hamidreza M, Rouhollah G, Mohammadreza V. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci. 2008; 32: 17-20.
13. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 1966; 45:493–496.