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

Abstract

Infectious (or Communicable) diseases are not only the past but also the present problem in developing as well as developed countries. It is caused by various pathogenic microbes like fungi, bacteria, parasites and virus etc. The medicinal plants have been used against the pathogenic microbes. Herbal medicines are generally used for healthcare because they have low price and wealthy source of antimicrobial properties. Since ancient to date most of the countries have been used herbal medicines, but in Asia, some medicinal plants are commonly used in rural and backward areas as a treatment for infectious diseases. 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

Herbal medicines have become more popular in the treatment of any diseases due to the popular belief that green medicine is safe, easily available and with fewer side effects. Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine and there is a lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use [1]. The medicinal properties of

some plants have been documented by some researchers [2-4]. Medicinal plant constitutes the main source of new pharmaceuticals and healthcare products [5]. Extraction and characterization of several phyto compounds of these green factories have given birth to some high activity profile drugs [6]. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today face either extinction or less of genetic diversity [7]. The continued emergence or persistence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials have reduced the efficacy of antimicrobial agents currently in use. In addition to this, antibiotics are associated with adverse effects; therefore, the search for new drugs from novel sources, such as plants, is necessary. It has been pointed out that more than 80% of world is population depends on plants to meet their primary health care needs [8]. Plants continue to be a major source of commercially consumed drugs. Even many synthetic drugs have their origin from natural plant products. The trend of using natural products has increased in recent years and the active plant extracts are frequently screened for new drug discoveries [9]. Limited information is available regarding antimicrobial activity of *C. latifolium* leaves therefore; present study is carried out to investigate antimicrobial activity of hydroalcoholic extracts from leaves of *A C. latifolium* against bacterial and fungal species. Preliminary phytochemical studies of these extracts are also undertaken to find out bioactive compounds having antimicrobial activity. Total phenol, flavonoid and alkaloids content were determined spectrophotometrically.

MATERIALS AND METHODS

Plant material

The aerial parts of *C. latifolium* were collected from local area of Bhopal (M.P.) in the month of Nov, 2021. The aerial parts were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. The aerial parts was air dried under room temperature. The dried plant samples were cut and grinded to make it in powder form.The powdered samples were stored in clean, dry and sterile container for further use.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. Mumbai, India), SD Fine- Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of 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

160 gram of powdered aerial parts of *C. latifolium* was exhaustively extracted with different solvent (petroleum ether, chloroform, ethyl acetate, hydroalcoholic and distilled water) by maceration method. The extract was evaporated above their boiling points. Finally, measured the percentage yield of the dried extracts. The recovered extracts were then reduced in a rotary evaporator and finally stored in airtight containers at 4°C for further use.

Phytochemical screening of the extract

The extract of *C. latifolium* was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids [10, 11].

Total phenol determination

The total phenolic content was determined using the method of Parkhe *et al* [12]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

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*

The well diffusion method was used to determine the antimicrobial activity of the extract prepared from the *C. latifolium* using standard procedure of Bauer *et al* [14](16).The drug used in standard preparation was ofloxacin and ciprofloxacin of IP grade. The antimicrobial activity was performed by using 24hr culture of *S. Mutans and S. bongori.* There were 3 concentration used which are 25, 50 and 100mg/ml for each extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37^{o}C for 24 hr. 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

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the aerial parts of *C. latifolium* using chloroform, ethyl acetate, hydroalcoholic and water as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder aerial parts of *C. latifolium* are shown in Table 2. Hydroalcoholic extract of plant revealed the presence of flavonoids, alkaloids, saponins, phenolics, carbohydrate, and tannin. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $0.019x + 0.016$, $R^2 = 0.999$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $y = 0.032x + 0.002$, $R^2 = 0.999$, where X is the quercetin equivalent (QE) and Y is the absorbance. Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: $y = 0.007x + 0.006$, $R^2 = 0.999$, where X is the Atropine equivalent (AE) and Y is the absorbance. The total phenolic, flavonoids and alkaloids content of extracts of *C. latifolium* arial parts was given in Table 3. Flavonoids compounds are secondary metabolites in plants which play an immensely important role in human health and nutrition. The antimicrobial activity of hydroalcoholic arial parts extract of *C. latifolium* showed bioactivity by inhibiting growth of microbial

species selected for the test as shown in table 4 and 5. The zone of inhibition shown by the extracts was comparable to the standard drug. It is effective against *S. mutans* and *S. bongori* in concentration dependent manner.

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:	-ve	-ve	+ve	-ve
	Wagner's Test:	-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**				+ve
	Froth Test:	-ve	-ve	+ve	
9.	**Tannins**				-ve
	Gelatin test:	-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. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			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

Extracts of *C. latifolium* in this study demonstrated a broad-spectrum of antimicrobial activity against selected microbial species. The antimicrobial activity of the plant extract, possibly due to the identified phytoconstituents, further confirms its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial infections. Identification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view of formulating novel chemotherapeutic agents should be the future direction for investigation.

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 (1993) 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 Chibueze, E. Akubugwo, (2011) Nutritive values and phytochemical contents of some leafy vegetables grown with different fertilizers. Agriculture and Biology Journal of North America 2(12):1437-1444.
3. Idu M, Obaruyi G, Erhabor J (2008) Ethnobotanical uses of plants among the binis in the treatment of ophthalmic and ENT (ear, nose and throat) ailments. Ethnobotanical Leaflets 2009(4):9
4. Banso A, Adeyemo S (2007) Evaluation of antibacterial properties of tannins isolated from Dichrostachys cinerea. Afr J Biotechnol 6(15):1785-1787
5. Ivanova D, Gerova D, Chervenkov T, Yankova T (2005) Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J Ethnopharmacology 96(1-2):145-150
6. Mandal V, Mohan Y, Hemalatha S (2007) Microwave assisted extraction-an innovative and promising extraction tool for medicinal plant research. Pharmacogn Rev 1(1):7-18
7. Misra A (2009) 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 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. Ncube N.S., Afolayan A.J., Okoh A.: Afr. J. Biotechnol. 7, 1797 (2008).
10. 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.
11. 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.

12. 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.
13. Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi, Mohammadreza Verdian-rizi. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm Sci. 2008; 32: 17-20.
14. 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.