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Antibacterial, Antioxidant activity and Phytochemical studies of *Crossandra infundibuliformis* leaf extracts

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Abstract

The aim of the present study was to evaluate *Crossandra infundibuliformis* (Acanthaceae family) for its antibacterial, antioxidant activity and phytochemical constituents. The leaves of *C. infundibuliformis* were screened for antibacterial activity against six pathogenic bacteria isolated from clinical samples such as Shigella dysenteria, Pseudomonas aeruginosa, Serratia marcescens, Salmonella typhimurium, Proteus mirabilis & Staphylococcus aureus by extracting them in ethanol, petroleum ether and water. Ethanol extract exhibited inhibition zone against all the pathogens comparable to the standard (Amikacin), whereas no zone of inhibition was observed in petroleum ether and water extracts. Phytochemical analysis showed the presence of flavonoids, saponins, terpenoids, cardiac glycoside, reducing sugars and tannins. The ethanol extract of *C. infundibuliformis* leaves appeared to be good antioxidant agent with maximum inhibition percentage of 89.27 ±0.284 % at 16µg/ml whereas standard Quercetin showed maximum inhibition percentage of 94.30 ±0.272 % at 22µg/ml.

Keywords: *Crossandra infundibuliformis*, leaf extracts, antibacterial activity, phytochemicals, antioxidant activity.

Introduction

Crossandra infundibuliformis (Acanthaceae family) is a plant which is important in horticulture. This plant is found abundantly in tropical areas such as South India and Srilanka. It reaches 2m in height and can withstand high temperature which makes it to survive in very high humidity. Due to its medicinal value, the various parts of this plant are used for many treatments. Paliyar tribes of Shenbangathope in Virudhanagar district of Tamil Nadu use flowers of *C. infundibuliformis* in combination with pepper in wound healing [1]. The leaf extract of *C. infundibuliformis* shows aphrodisiac activity on ethanol induced testicular toxicity in male rats [2]. It is also shown that the increase in aphrodisiac activity is due to the increase in testosterone level. The petroleum ether extract of dried leaves shows significant hepatoprotection when compared to standard drug Silumarin in albino mice [3]. It is also found that *C. infundibuliformis* shows very good anticorrosive property when coated on steel against 1M HCl [4].

A very less of published journals have revealed that *C. infundibuliformis* have medicinal values. Despite of studies that have revealed the efficiency of this plant as potential source of aphrodisiac activity, wound healing, anticorrosive activity, etc, there is a lack of information about the level of antibacterial, antioxidant and phytochemical constituents.

Therefore this study was proposed to investigate antibacterial activity, antioxidant activity and screening phytochemical constituents.

Materials and Methods

Plant material

Crossandra infundibuliformis plants were collected from a village Sennanur, Coimbatore, Tamil Nadu, India. The plant leaves were washed thoroughly using tap water before drying it completely under shade for 11 days. The dried leaves were grinded using grinder machine to increase its surface area. About each 25g of leaves powder was packed in soxhlet extraction unit and exhaustively extracted using 100ml of solvents such as ethanol, petroleum ether and water respectively at 60ºC for 12 hours.

The extract was completely dried in water bath at 40ºC and subsequent stored at 4ºC.

Test organisms

The leaf extract of *C. infundibuliformis* were screened against six pathogenic bacteria isolated from clinical samples collected from Bioline Laboratory, Coimbatore. The test organisms include Shigella dysenteria, Pseudomonas aeruginosa, Serratia marcescens, Salmonella typhimurium,, Proteus mirabilis & Staphylococcus aureus. The bacterial cultures were revived in nutrient broth medium and incubated at 37ºC for 48 hours. Each bacterial culture was further maintained at 37ºC on nutrient agar slants and nutrient broth after every 48 hours of transferring.

Antimicrobial activity

The well diffusion method was followed for the estimation of antibacterial activity of *C. infundibuliformis* extracts. About 250ml of nutrient agar medium was prepared and poured in to 10 petriplates each containing 20ml of medium. After solidification of the medium, 100µl (10^6 cell/ml) of test bacterial cultures were seeded into respective medium by spread plate method. In each petriplates, four 10mm wells were made using gel puncher. Then, 100µl of each (250µg/ml) ethanol, petroleum ether and water extracts were added into three of the wells respectively. In the fourth well, 100µl of Amikacin (5µg/ml) was added as reference antibacterial agent. The plates were incubated overnight at 37ºC for allowing bacterial growth. After incubation, the diameter of the zone of inhibition was measured. An inhibition zone of 10mm or greater (including diameter of well) was considered antibacterial activity.

Phytochemical analysis

The freshly prepared leaf extracts were subjected to preliminary analysis for the presence of phytoconsituents as described by Ayoola [5]. The tests done are as follows, Fehling's test for reducing sugar, Salkowski test for terpenoids, Ammonia test for flavanoids, Foam test for saponnins, Ferrric chloride test for tannins and Keller-killiani tests for cardiac glycosides.

DPPH Radical Scavenging Activity

The free radical scavenging activity of the ethanol extract was measured in terms of radical scavenging ability using the stable 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical method [6]. A solution of 0.1mM DPPH in ethanol was prepared and 1.0 ml of this solution was mixed with 3.0 ml of extract in ethanol containing 10, 20, 30, 40 and 50µg/ml of the extract. The same procedure was followed by using Quercetin as standard. The reaction mixture was vortexed thoroughly. After thirty minutes incubation, the absorbance of the mixture was measured spectrophotometrically at 517nm.

It was compared to control containing same amount of ethanol and DPPH without an extract or standard. The capability to scavenging the DPPH radical was calculated using the following formula [7]:

$$\% \text{ Inhibition} = \{[Ac - A_a]/A_c\} \times 100$$

Where A_c is the absorption of the control and A_a is the absorption of the extract.

Results & Discussion

Antibacterial activity

The results from the present study showed that only ethanol extract of *C. infundibuliformis* displayed antibacterial activities against all the six pathogenic bacteria, whereas water and petroleum ether extracts shows no activity (Table 1). The ethanol extract exhibits broad spectrum of activity. Ethanol extract antibacterial results showed the diameter of inhibition zones ranging from 15- 29nm, with the highest zone observed against Pseudomonas aeruginosa (29mm), followed by Proteus mirabilis (26mm), Shigella dysenteria (22mm), Serratia marcescens (19mm), Salmonella typhimurium (18mm). Least inhibition zone was observed against Staphylococcus aureus (15mm). It is noteworthy that when comparing, the ethanol extract was seen to be as potent as standard antibiotic Amikacin used. The ethanol extract antibacterial activity seen against all the tested opportunistic and pathogenic bacteria is very encouraging and important considering the role of bacteria in noscomial infection leading to increased morbidity and mortality rates. No zone of inhibition indicates that the water and petroleum ether extraction of *C. infundibuliformis* did not have any role in extracting antibacterial phytochemicals.

Phytochemical analysis

The major phytochemical constituents of interests such as flavonoids, saponins, terpenoids, cardiac glycosides, reducing sugars and tannins are found to be present in the leaf extract of *C. infundibuliformis* (Table 2).

Table1: Antibacterial activity of leaf extract of *C. infundibuliformis* against pathogenic bacteria.

Pathogenic Bacteria	The diameter of Inhibition Zone (including 8mm diameter of well)*			
	Amikacin (5µg/ml)	Ethanol Extract (250µg/ml)	Water Extract (250µg/ml)	Petroleum Ether extract (250µg/ml)
Shigella dysenteria	24	22	-	-
Pseudomonas aeruginosa	17	29	-	-
Serratia marcescens	20	19	-	-
Salmonella typhimurium	20	18	-	-
Proteus mirabilis	28	26	-	-
Staphylococcus aureus	17	15	-	-

*** Each value is the mean of two replicates**
- No Zone of inhibition

Table2: Phytochemical components of leaf extracts of *C. infundibuliformis*.

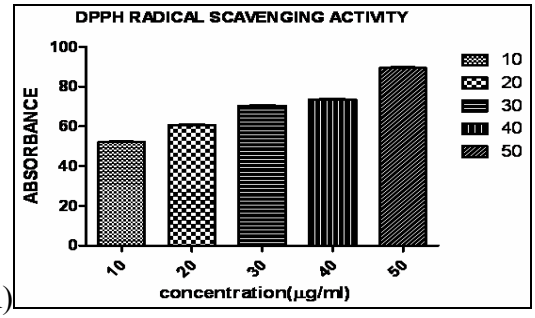
S. No.	Phytochemical constituents	Tests	Presence/Absence
1	Flavanoids	Ammonia test	+
2	Saponins	Foam test	+
3	Terpenoids	Salkowski test	+
4	Cardiac glycosides	Keller-killiani tests	+
5	Reducing sugars	Fehling's test	+
6	Tannins	Ferrric chloride	+

+ indicates the presence of constituents.

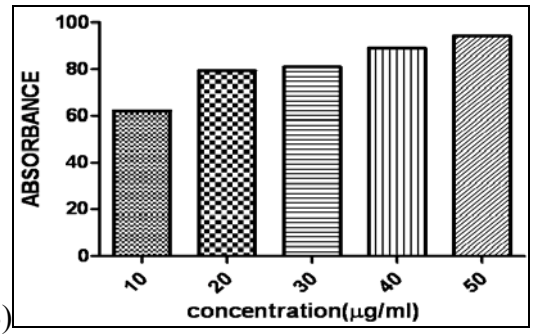
The presence of flavonoids and tannins is likely to be responsible for the free radical scavenging activity. These findings give credence to the traditional medicine application of *C. infundibuliformis* as remedies for sores, rash, internal and external wounds and infections. Several phytoconstituents like flavonoids [8], tannins [9]and saponins [10] are effective antimicrobial substances against a wide range of micro-organisms. Flavonoids show anti-allergic, anti-inflammmatory and anticancer activity. The presence of cardiac glycosides shows that the plant leaves can be used in the treatment of congestive heart failure and cardiac arrhythmia. They are also used to strengthen a weaken heart and allow it to function more efficiently [11]. Saponins posssess hypocholesterolemic and antidiabetic properties [12]. Terpenoids also shows analgesics properties [13]. Saponins are also responsible for Central nervous system activities [14].

DPPH Radical Scavenging Activity

Scavenging activity of *C. infundibuliformis* leaf extract and Quercetin as standard on DPPH radical has been shown in Figure 1.



A)



B)

Figure1: A) DPPH radical scavenging activity of ethanol leaf extract of *C. infundibuliformis,* B) and of Quercetin.

EC_{50} value of 16µg/ml and 22µg/ml were recorded for ethanol leaf extract of *C. infundibuliformis* and Quercetin respectively (Table 3).

Table 3: DPPH radical scavenging activity of ethanol leaf extract of *C. infundibuliformis* (Test) and Quercetin (Standard).

Test (Sample) Concentration (µg/ml)	Percentage Inhibition	Standard (Quercetin) Concentration (µg/ml)	Percentage Inhibition
10	52.07 ± 0.052	10	55.13 ± 0.146
20	60.54 ± 0.417	20	79.48 ± 0.072
30	70.35 ± 0.226	30	81.02 ± 0.083
40	73.56 ± 0.162	40	88.95 ± 0.066
50	89.27 ± 0.284	50	94.30 ± 0.272
EC-50	16µg/ml		22µg/ml

Values are expresses mean ± SEM (n=3)

The DPPH test provides information on the reactivity of the test compounds with a stable free radical. The degree of reduction in absorbance measurements is indicative of antioxidant activity of extract. The ethanol extract of *C. infundibuliformis* leafs shows maximum inhibition percentage of 89.27 ±0.284 % at 16µg/ml whereas maximum inhibition percentage of Quercetin was 94.30 ±0.272 % at 22µg/ml. This very slight difference indicates that this plant could be used as a good antioxidant agent. This result also contributes to the presence of important phytochemical constituent in it.

Conclusion

Results reveal that leaves of *C. infundibuliformis* have many phytochemical constituent which may be responsible for many pharmacological activities. The antibacterial activity results obtained were comparable with those of standard drug Amikacin. It appeared that this plant could be a potential natural source of new antimicrobial agent. The antioxidant activity of *C. infundibuliformis* was found to be comparable to standard Quercetin. For further work on the profile and nature of chemical constituents of *C. infundibuliformis* leafs will provide more

information on the active principles responsible for their pharmacological properties. This may also lead to the development of a new generation of drugs that possess both chemotherapeutic and chemopreventative properties which can results in ways of combating the serious problems of diseases.

Author's contributions

NS collected the plant, prepared the plant extract, carried out phytochemical constituents screening and drafted the manuscript. NS and NG have equal contribution in carrying out antioxidant activity and antibacterial activity.

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