

Phytochemical analysis and antibacterial activity of *Crossandra infundibuliformis* Ethanolic stem extract

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Abstract

The aim of the present study was to evaluate *Crossandra infundibuliformis* stem ethanolic extract for its antibacterial activity, and phytochemical constituents. The stem extract was tested against five bacterial species such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Streptococcus mutans*. The ethanolic stem extract of *Crossandra infundibuliformis* showed inhibitory effect on only 3 bacterial species such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The ethanolic stem extract showed no effect on *Escherichia coli* and *Streptococcus mutans*. The inhibition zones for *S. aureus* were 8 mm, 9 mm and 10 mm (including well 4 mm) at concentrations of 15 µl, 20 µl and 25 µl respectively. The ethanolic extract displayed 6 mm, 8 mm and 11 mm against *B. subtilis*. At 25 µl concentration of extract inhibition zone 13 mm was noted for *P. aeruginosa*. The phytochemical analysis showed the presence of Carbohydrates, Flavanoids, Alkaloids, Proteins and Saponins.

Keywords: *Crossandra infundibuliformis*, Phytochemicals, Anti-bacterial activity, Stem extract.

1. Introduction

Medicinal plants gaining lot of importance now a days because of efficacy they have been showing in the traditional healing [1]. The best source of drugs without hazardous effects to human systems could be the plant source and this has been proved by the Traditional healing system and the recent studies conducted on the experimental animals [2]. *Crossandra infundibuliformis* belongs to family Acanthaceae. This plant proved to have various medicinal properties. The leaf extract of *C. infundibuliformis* shows aphrodisiac activity on ethanol induced testicular toxicity in male rats [3]. The petroleum ether extract of dried leaves shows significant hepatoprotection when compared to standard drug Silumarin in albino mice [4]. The aqueous and methanol leaf extracts of *C. infundibuliformis* possess antimicrobial activity tested against some common bacterial and fungal pathogens [5]. The successive leaf extracts of *C. infundibuliformis* possess antibacterial, antifungal & anticandidal activities [6]. It is also reported that the successive leaf extracts of *C. infundibuliformis* shown good anti-inflammatory and analgesic activities [7]. It is also found that the crude extracts obtained from petroleum ether, ethyl acetate and methanol of *C. infundibuliformis* shown larvicidal activity against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* [8].

2. Materials and methods

2.1. Plant material

Stem parts of *Crossandra infundibuliformis* were collected from village seshammachruvu, Achanta mandalam, West Godavari district. The plant was authenticated by Dr. Suryanayana raju, Department of Botany, S V K P & Dr. K S Raju Arts and Science College, Penugonda. Stem parts of the plant have been excised from plants and dried in sunlight for a week and then powdered using blender to get coarse powder.

2.2. Microorganisms

The stem extracts of *C. infundibuliformis* were tested against five pathogenic bacteria. The test organisms include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Streptococcus mutans*.

2.3. Extraction process

Preparation of extract was done according to the procedure done by Sharmila *et al.*, (2011) [9]. 25 g of Stem powder was packed in soxhlet extraction unit and exhaustively extracted using 100 ml of ethanol at 60 °C for 12 hours. The extract was completely dried in water bath at 40 °C and subsequent stored at 4 °C.

2.4. Phytochemical analysis

The phytochemicals were qualitatively analysed by using standard procedures [10, 11, 12, 13, 14]. The phytochemicals were observed by using following reagents and chemicals. Carbohydrates were observed with Molisch's test, Foam test was done for saponins, Phytosterols with Liebermann-Burchard's test, Flavonoids were observed with NaOH/HCl, ferric chloride solution was used to find Tannins and Phenols, Ninhydrin test, Biuret test, Millon's test were done for Proteins and amino acids, Gums with Molish reagents and concentrated sulfuric acid. Mayer's reagent, Hager's reagent and Dagerdorff's reagent were used to find Alkaloids.

2.5. Determination of antibacterial activity

Antibacterial activity was measured by well diffusion method [15]. Nutrient agar (Hi media) was prepared and poured in to the petriplates. After solidification of media, overnight bacterial cultures were inoculated on the surface of media. By using a sterile gel puncher 4 mm of wells were made in each petri plates. Then 15 µl, 20 µl, 25 µl of ethanol, water extracts of

Stems of *C. infundibuliformis* were added in to the three wells respectively. The plates were incubated in the incubator at 37°C for optimum bacterial growth. In the next day, diameter of the zone of inhibition was measured.

3. Results

3.1. Anti-bacterial activity

The ethanolic stem extract of *Crossandra infundibuliformis* showed inhibitory effect on only 3 bacterial species such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*

subtilis. The ethanolic stem extract showed no effect on *Escherichia coli* and *Streptococcus mutans*. The inhibition zones for *S. aureus* were 8 mm, 9 mm and 10 mm (including well 4 mm) at concentrations of 15 µl, 20 µl and 25 µl respectively. The ethanolic extract displayed 6 mm, 8 mm and 11 mm against *B. subtilis*. At 25 µl concentration of extract the inhibition zone 13 mm was noted for *P. aeruginosa*. The anti-bacterial results of ethanolic extract were given below (Table 1 and Fig 1-4).

Table 1: shows the inhibition zones of ethanolic extract against test pathogenic bacteria.

Test bacterial species	Inhibition zones in mm (including well 4mm)		
	15 µl (ethanolic extract)	20 µl (ethanolic extract)	25 µl (ethanolic extract)
<i>Staphylococcus aureus</i>	8 mm	9 mm	10 mm
<i>Pseudomonas aeruginosa</i>	-	-	13 mm
<i>Bacillus subtilis</i>	6 mm	8 mm	11 mm
<i>Escherichia coli</i>	-	-	-
<i>Streptococcus mutans</i>	-	-	-

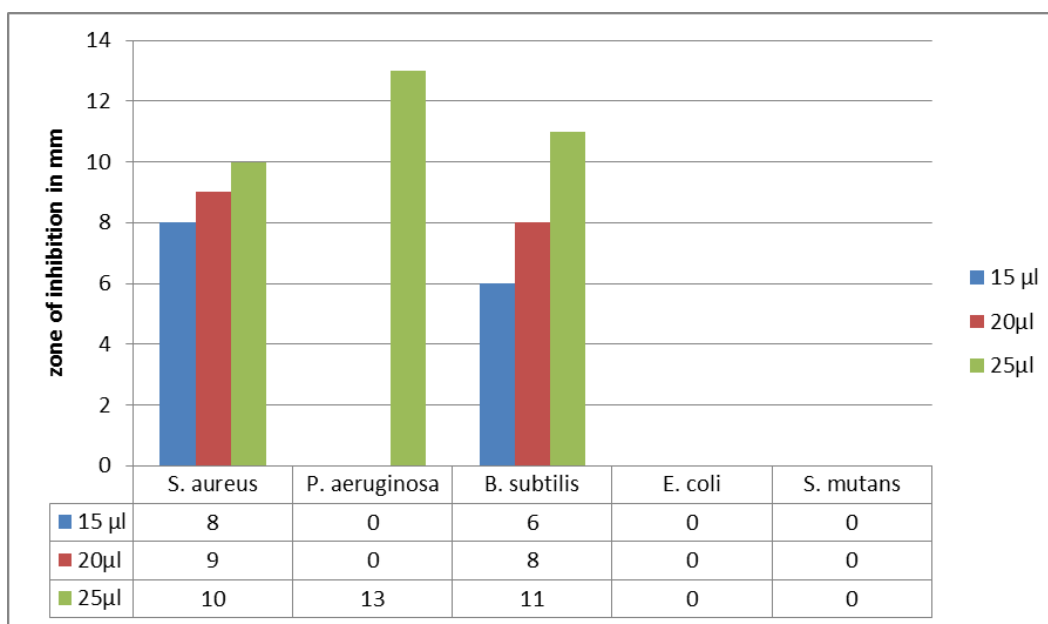


Fig 1: shows the zones of inhibition of ethanolic extract on different bacterial species.

Fig 2-4: shows the sensitivity of ethanol extract of *Crossandra infundibuliformis* stems against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*.

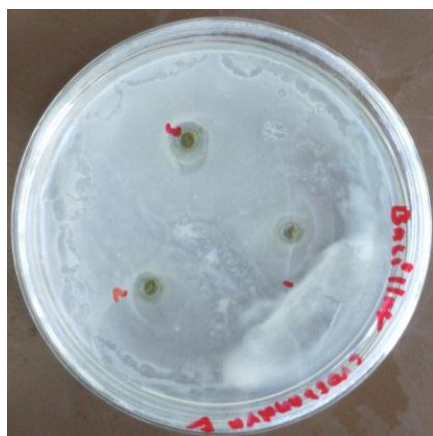


Fig 2: *B. subtilis*

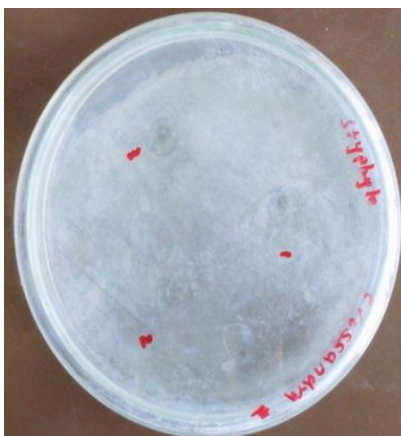


Fig 3: *S. aureus*

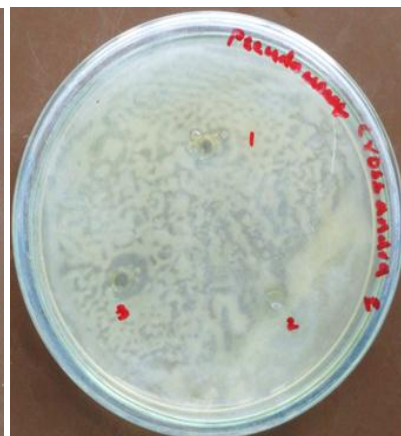


Fig 4: *P. aeruginosa*

3.2. Phytochemical analysis

The phytochemical analysis showed the presence of Carbohydrates, Flavanoids, Alkaloids, Proteins and Saponins (Table 2).

Table 2: shows the presence of phytochemicals in stem ethanolic extract of *Crossandra infundibuliformis*.

S. No	Phyto chemical	Presence of phyto chemical in ethanolic extract
1.	Carbohydrates	+
2.	Flavanoids	+
3.	Alkaloids	+
4.	Tannins and Phenols	-
5.	Proteins	+
6.	Saponins	+
7.	Sterols	-
8.	Gums	-

+ indicates presence; - indicates absence.

4. Discussion

Medicinal plants gaining lot of importance now a days because of efficacy they have been showing in the traditional healing. Researchers focussing on the traditional healers in order to find plant based drugs [1]. As the modern antibiotics have various anarchic toxic effects, these plant extracts could serve as a alternative antibacterial agents. This study showed that the stem extract of *Crossandra infundibuliformis* have moderate antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and no effect on *Escherichia coli* and *Streptococcus mutans*. In single plant many active secondary metabolites are present and medicinal effect can be attributed to either to a single compound or synergistic effect of many compounds and present antibacterial activity can either be due to presence of some specific bioactive molecule or due to the synergistic effect of different phytoconstituents [16]. Previous studies conducted on leaves [5, 9] and flowers [17] of *Crossandra infundibuliformis* showed potent antimicrobial activity. In the present study the stem ethanolic extract has showed moderate antibacterial effect than the previous studies conducted on leaves and flowers of *Crossandra infundibuliformis*.

5. Conclusion

The investigation on ethanolic stem extract of *Crossandra infundibuliformis* showed phytochemicals like Carbohydrates, Flavanoids, Alkaloids, Proteins and Saponins and moderate antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*. Traditional healing system has provided many important herbs have the potential to cure lethal diseases. Based on previous studies this plant *Crossandra infundibuliformis* have various medicinal properties. Hence effective work should be done to isolate of various phyto inhibitors.

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7. References

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