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Preliminary phytochemical analysis, antibacterial, antifungal and anticandidal activities of successive extracts of *Crossandra infundibuliformis*

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ABSTRACT

Objective: To investigate the phytochemical, antibacterial, antifungal and anticandidal activity of successive extracts of *Crossandra infundibuliformis* (Acanthaceae) leaves. **Methods:** Preliminary screening on the presence of alkaloids, saponins, phytosterols, phenolic compounds, flavanoids, tannins, carbohydrates, terpenoids, oils and fats were carried out by phytochemical analysis. The antibacterial, antifungal and anticandidal activities were done by agar well diffusion technique. **Results:** The successive extracts have an array of chemical constituents and the MIC values of antibacterial activity ranges from 0.007 8 to 0.015 0 μg/mL. In case of antifungal and anticandidal activities the MIC values were between 0.125 and 0.250 μg/mL. **Conclusions:** These findings demonstrate that the leaf extracts of *C. infundibuliformis* presents excellent antimicrobial activities and thus have great potential as a source for natural health care products.

1. Introduction

Over the last few decades, the incidence of opportunistic infections in patients treated with immunosuppressive drugs or intensive chemotherapy is increasing at an alarming rate[1]. Resistance to antibiotics is a major problem in the management of infections caused by microbes. Several new strategies to control such infections have been considered in recent years. These include the use of antibiotic combinations, the development of new members of existing antibiotic classes and the introduction of novel agents[2,3].

The increase in microbial resistance to classical drugs, their toxicity, and the treatment cost justify the search for new strategies. As a result, antibiotics are playing a major role in health care and thus screening of traditional plants for discovery of novel drugs is now important.

Higher plants, which are able to do photosynthesis, produce hundreds to thousands of diverse chemical compounds with different biological activities[4]. It is believed that these compounds have an important ecological role. They can work as pollinator attractants and as chemical defenses against insects, herbivores and microorganisms[5].

These antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms[6]. There are several reports in the literature regarding the antimicrobial activity of plant crude extracts and the bioassay-guided fractionation of those extracts that yielded active principles[7-11]. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries[12]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases[13]. Therefore, researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against micro-organism infections[6,14].

Crossandra infundibuliformis (C. infundibuliformis), an ornamental plant, is marketed as such. The species is often grown to beautify kitchen gardens as it is small in size and sports attractive flowers[15]. Thus, we are excited to learn from literature and also from folk[16-22], the medicinal uses of *C. infundibuliformis*. In continuation of our work in medicinal plants[23-25], the present study was initiated

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with preliminary work relative to its pharmacological, phytochemical studies which are presented in this paper. Hence forth, antibacterial, antifungal and anticandidal activities of successive extracts of *C. infundibuliformis* have been reported here. The plant extracts were assessed for antibacterial activity against 23 bacterial strains, antifungal activity against 4 fungal organisms and anticandidal activity against 2 candidal strains, thus providing a preliminary screening assessment.

2. Materials and methods

Chemicals and solvents were procured from SD Fine of analytical grade. All other reagents were purchased from Hi-Media. Ethnobotanical reviews of plant species were carried out and the plant species were selected for analysis. The leaves of *C. infundibuliformis* were obtained from the Vellore District, Tamil Nadu. The plant specimens were prepared and authenticated in Botanical Survey of India (No: BSI/SC/5/23/09-10/Tech.-1718) and a herbarium is stored in the Pharmaceutical Chemistry division, VIT University for future references. Once the leaves are harvested, they were cleaned, air-dried and stored at 0-4 ℃.

The microorganisms used in this study were obtained from Christian Medical College, Vellore District, Tamil Nadu. The bacterial strains used for the study are *Bacillus brevis* (Ia), *Bacillus circulans* (Ib), *Bacillus firmus* (Ic), Bacillus *coagulans* (Id), *Blicheis formis* (Ie), *Blicheis megaterium* (If), *Blicheis sterothermophilus* (Ig), *Clostridium autobotylicum* (Ih), *Klebsilla pneumonia* (Ii), *Bacillus cerus* (Ij), *Bacillus pumilus* (Ik), *Bacillus substilis* (Il), *Enterobacter aerogenes* (Im), *Escherichia coli* 2810 (In), *Escherichia coli* 2809 (Io) *Proteus mirabilis* (Ip), *Staphylococcus aureus* (Iq), *Staphylococcus epidermis* (Ir), *Strepococci facealis* (Is), *Streptococcus pyogenes* (It), *Citrobacter frendil* (Iu), *Proteus vulgaris* (Iv) and *Pseudomonas aeruginosa*(Iw). The fungus and Candida used were *Aspergillus niger* (IIa), *Aspergillus flavus* (IIb), *Aspergillus fumigatus* (IIc), *Pencillium chrysogenum* (IId), *Candida kruseii* (IIIa) and *Candida gullirmondi* (IIIb). The microbial isolates were subcultured and prepared for assessment of plant extract activity. Rifampicin and amphotericin B were used as positive control for antibacterial, antifungal and anticandidal activities. The negative control used is dimethyl sulfoxide (DMSO).

The air-dried leaves of *C. infundibuliformis* (100 g) were extracted with petroleum ether (A), ethyl acetate (B) and methanol (C) in Soxhlet apparatus for 15 to 20 h using 500-800 mL of solvent. The extracts were concentrated by rotator evaporator and stored in refrigetor below 20 ℃.

2.1. Preliminary phytochemical screening

Preliminary screening for the presence of alkaloids, saponins, phytosterols, phenolic compounds, flavanoids, tannins, carbohydrates, terpenoids, oils and fats was carried out by the reported protocol[26].

2.2. Minimum inhibitory concentration (MIC)

2.2.1. In vitro antibacterial activity

The extracts of petroleum ether, ethyl acetate and methanol were evaluated for their antibacterial activity against pathogenic bacteria. The agar diffusion method was performed using Muller-Hinton agar (Hi-Media) medium. Suspension of each microorganism was prepared and applied to plates with serially diluted compounds (DMSO, solvent control) to be tested and incubated for 20 h at 37 ℃. The compounds were tested at concentrations ranging from 0.015 0 to 0.007 8 μg/mL. Rifampicin was used as a reference standard. The bacterial strains that were used are *B. brevis* (Ia), *B. circulans* (Ib), *B. firmus* (Ic), *B. coagulans* (Id), *B. formis* (Ie), *B. megaterium* (If), *B. sterothermophilus* (Ig), *C. autobotylicum* (Ih), *K. pneumonia* (Ii), B. cerus (Ij), *B. pumilus* (Ik), *B. substilis* (Il), *E. aerogenes* (Im), *E. coli* 2810 (In), *E. coli* 2809 (Io) *P. mirabilis* (Ip), S. aureus (Iq), *S. epidermis* (Ir), *S. facealis* (Is), *S. pyogenes* (It), *C. frendil* (Iu), *P. vulgaris* (Iv) and *P. aeruginosa*(Iw).

2.2.2. In vitro antifungal and anticandidal activity

The extracts were evaluated for their *in vitro* antifungal and anticandidal activity against pathogenic fungi using agar diffusion method with Saburoud's dextrose agar (Hi-Media). Suspension of each fungus were prepared and applied to agar plates with serially diluted compounds to be tested. The compounds were tested at concentrations ranging from 0.015 0 to 0.007 8 μg/mL. Amphotericin B was used as a reference standard. The plates were incubated at 26 ℃ for 72 h and MIC was determined. The fungus strains *A. niger* (IIa), *A. flavus* (IIb), *A. fumigatus* (IIc), *P. chrysogenum* (IId), *C. kruseii* (IIIa) and *C. gullirmondi* (IIIb) were used for the study.

3. Results

In the present investigation, preliminary phytochemical screening has been done for petroleum ether (A), ethyl acetate (B) and methanol (C) extracts for the presence of phytochemical constituents namely alkaloids, carbohydrates, saponins, proteins, tannins, flavanoids, oils, fats, phytosterols, and terpenoids which are tabulated in Table 1. The results of antibacterial activity evaluation of the successive extracts of *C. infundibuliformis* are presented in Table 2. The results of antifungal and anticandidal assay for the crude extracts were tabulated in Table 3.

Table 1
Summary of preliminary phytochemical analysis of crude extracts, A–C.

Tests	A	B	C
Alkaloids	–	+	+
Carbohydrates	–	–	–
Saponins	–	+	–
Phenolics & Tannins	+	+	+
Flavanoids	+	+	+
Oils & Fats	–	–	–
Phytosterols	+	+	–
Terpenoids	+	–	–

A = Petroleum ether extract, B = Ethylacetate extract, C = Methanol extract. (+) = presence of compound, (–) = absence of compound.

Table 2
Antibacterial activity: MIC of crude extracts, A–C (g/mL).

Organisms	Successive extracts			S1
	A	B	C	
Ia	0.125 0	0.015 0	0.062 0	0.031
Ib	0.062 0	0.062 0	0.031 0	0.031
Ic	0.125 0	0.500 0	0.500 0	0.125
Id	0.062 0	0.250 0	0.031 0	0.031
Ie	0.007 8	0.250 0	0.007 8	0.031
If	0.007 8	0.015 0	0.031 0	0.031
Ig	0.015 0	0.031 0	0.031 0	0.031
Ih	0.062 0	0.007 8	0.031 0	0.031
Ii	0.125 0	2.000 0	0.500 0	0.031
Ij	0.015 0	0.500 0	0.250 0	0.125
Ik	0.500 0	0.125 0	0.007 8	0.031
Il	0.062 0	0.062 0	0.007 8	0.031
Im	0.500 0	1.000 0	0.500 0	0.031
In	0.125 0	1.000 0	0.125 0	0.031
Io	0.031 0	0.500 0	0.500 0	0.031
Ip	0.031 0	0.500 0	0.500 0	0.031
Iq	0.250 0	0.015 0	0.500 0	0.125
Ir	0.125 0	2.000 0	0.500 0	0.031
Is	0.500 0	2.000 0	0.125 0	0.125
It	0.007 8	1.000 0	0.125 0	0.125
Iu	0.250 0	0.250 0	0.125 0	0.125
Iv	0.500 0	0.062 0	0.250 0	0.031
Iw	0.250 0	0.125 0	0.250 0	0.125

S1= Rifampicin (Standard drug).

Table 3
Antifungal and anticandidal activity: MIC of crude extracts, A–C (g/mL).

Organisms	Successive extracts			S2
	A	B	C	
IIa	0.250	0.125	0.125	0.125
IIa	0.125	0.125	0.125	0.125
IIc	0.250	0.500	0.125	0.125
IId	0.250	0.125	0.062	0.125
IIIa	0.125	0.125	0.125	0.125
IIIb	–	–	–	0.125

A = Petroleum ether extract, B = Ethylacetate extract, C = Methanol extract. S2 = Amphotericin B (Standard drug).

4. Discussion

The presence of phenolics, tannins and flavanoids were observed in all extracts in Table 1. Alkaloids are present in the ethyl acetate (B) and methanol extracts (C). Phytosterols are present only in petroleum ether (A) and ethyl acetate extracts (B). The over all results of phytochemical analysis is presented. The crude extracts from plants are always a mixture of active and non-active compounds. MIC of less than 100 mg/L has suggested as a good antimicrobial activity. The extracts showed a strong antibacterial activity which was remarkably shown in inhibition of growth of bacteria. The petroleum ether extract (A) have a high degree of activity against *Blicheis formis* (Ie), *Blicheis megaterium* (If), *Blicheis sterothermophilus* (Ig), *Bacillus cereus* (Ij), and *Strepococcus pyrogenes* (It) with minimum inhibitory concentration range between 0.015 0 to 0.007 8 μg/mL and when compared to standard drug MIC value of petroleum ether extract have been effectively very less. The usage of ethyl acetate extract (B) has demonstrated significant levels of inhibition against only two organism such as *Clostridium autobotylicum* (Ih), and *Staphylococcus aureus* (Iq) at 0.0078 and 0.015 μg/mL when compared with standard which showed inhibition at 3.1 μg/mL. The methanol extract (C) showed relatively high level of inhibition against *Bacillus pumilus* (Ik), and *Bacillus substilis* (Il) with MIC value of 0.007 8 μg/mL.

Petroleum ether extract (A) has a good degree of inhibition against *Bacillus firmus* (Ic), *Escherichia coli* 2809 (Io) *Proteus mirabilis* (Ip) when compared to the standard drug. Ethyl acetate extract (B) showed remarkable potential against *Pseudomonas aeruginosa* (Iw) with that of standard. Methanol extract (C) has a promising potential against *Bacillus circulans* (Ib), *Bacillus coagulans* (Id), *Citrobacter frendil* (Iu).

Bacteria's, *Klebsilla pneumonia* (Ii), *Enterobacter aerogenes* (Im), *Escherichia coli* 2810 (In), *Staphylococcus epidermis* (Ir), *Strepococci facealis* (Is), *Citrobacter frendil* (Iu), *Proteus vulgaris* (Iv) have very feeble inhibition against extracts A, B and C when compared to standard drug.

With reference to antifungal activity, Petroleum ether extract (A) has a very high degree of inhibition against panel of fungal organisms. Whereas, ethyl acetate extract (B) showed a remarkable activity against *A. niger* (IIa), and *P. chrysogenum* (IId), and the extract has moderate activity against the other two organisms such as *A. flavus* (IIb), and *A. fumigatus* (IIc). The methanol extract (C) was noted to have very feeble inhibition against all organisms expect *P. chrysogenum* (IId). When compared to standard all extracts A, B and C exhibits moderate inhibition.

In case of anticandidal activity, ethyl acetate extract (B) has recorded a good inhibition than petroleum ether extract (A) and methanol extract (C) against *C. kruseii* (IIIa) but in case of *C. gullirmondi* (IIIb) all the extracts were inactive.

In conclusion, based on the present study it can be summarized that crude extracts from dried leaves of *C. infundibuliformis*, were having good antibacterial activity

against all the bacterial strains tested. The MIC values of extracts are found to vary for the bacterial strains and it is observed that MIC values are much lower than the standard drug. The extracts were showing promising results in case of antifungal activity. The extracts showed a moderate inhibition against the candidal strains.

The present study indicated that *C. infundibuliformis* possess a good antibacterial, antifungal and anticandidal activities. Thus, the results prompted us for further fractionation and identification of bioactive compound would promote an antibiotic drug. The future studies will be focused on antioxidant property and characterization of active components in all three extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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