INT J CURR SCI 2011, 1: 72-77
RESEARCH ARTICLE

Phytochemical screening and antimicrobial activity of leaf extracts of *Crossandra infundibuliformis* (L.) nees on common bacterial and fungal pathogens

Elamathi R, T. Deepa, R. Kavitha, P. Kamalakannan, S. Sridhar* and J. Suresh Kumar

Department of Botany, Government Arts College, Thiruvannamalai-606 603

Thiruvalluvar University, Tamil Nadu, India

**Correspondence to: E-mail: sekarsridhar@rediffmail.com; Phone: +91-9443105935*

Abstract

The medicinally active substances were isolated from leaves of *Crossandra infundibuliformis* by Soxhlet extractor and identified by phytochemical tests. The soxhlet extraction in powdered form was performed using aqueous and methanol. The results of each extracts confirm the active substances such as carbohydrates, alkaloids, steroids, tannins, phenols, saponins, fixed oils and fats, gums and mucilage, proteins, flavonoids and volatile oils. The evaluation of the leaf powder was supported by the physico-chemical analysis. The *C. infundibuliformis* showed potential antimicrobial activities against some selected strains and a maximum inhibition zone 37 mm was recorded from 200 mg of methanol extract of *C. infundibuliformis* against *Staphylococcus aureus* and minimum (10 mm) by *Salmonella typhi* at 50 mg of the above extract. The methanolic extract showed a maximum antifungal activity 32 mm inhibition zone was recorded from 200 mg of extract against *Aspergillus flavus* and minimum 9 mm by 50 mg of aqueous extract against *Rhizopus indicus.*

Key words: antimicrobial, alkaloids, *Crossandra*, flavonoids, phytochemical

Received: 2nd September; Revised: 25th September; Accepted: 29th September;

Introduction

The use of medicinal plants as a source of relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyback, these plants are still widely used in ethno medicine around the world (Stockwell, 1988). The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 2,50,000-5,00,000 plant species only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Kroschwitz and Howe-Grant, 1992). Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine. On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons (Newman et al., 2000).

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). A wide range of medicinal plant parts is used for extract as

raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of the raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal et al., 2006). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them not been adequately evaluated (Balandrin et al., 1985). *Crossandra infundibuliformis* is one of the perennial crops which occupies in the same rhizosphere soil for more than years and remove most of the available nutrients from the rhizosphere soil and leads to pour content of nutrients particularly phosphorus. In order to overcome phosphorus deficiency the plants like *Crossandra* always requires interaction of Arbuscular Mycorrhizal (AM) fungi if the plants having interaction of AM fungi in such type of soil never express deficiency symptoms to phosphorus because AM fungal mycelium acts something like feeder roots and scavenge required phosphorus from the surrounding soil (Bharathiraja and Tholkappian, 2011).

Materials and methods

Preparation of plant extracts

Leaves of fresh plants of *Crossandra infundibuliformis* was collected from Manmalai village near Chengam, Thiruvannamalai district, Tamil Nadu, India and the plant were identified with the help of Gamble's flora.

Preparation of powder

The leaves of plants were collected and dried under shade. These dried materials were mechanically powdered sheaved using 80 meshes and stored in an airtight container (Harborne, 1973). These powdered materials were used for further physiochemical, phytochemical and fluorescent analysis.

Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1966). The leaves were shade dried and they were subjected to pulverization to get coarse powder. The coarse powder was subjected to Soxhlet extraction separately and successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analyzed for phytochemical screening of compounds, antimicrobial and pharmacological activities.

Qualitative phytochemical studies

Qualitative phytochemical analyses were done by using the procedures of Kokate et al. (1995). Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, phytosterol, proteins and amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analyzed.

Test organisms

The stored culture of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Leuconostoc lactis* and *Salmonella typhi* were collected from the Microbial Type Culture Collection (MTCC), The Institute of Microbial Technology, Sector 39-4, Chandigarh, India. The pathogenic fungal strains *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus indicus* and *Mucor indicus* were collected from the Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India.

Antibacterial Studies

Bacterial Media

Thirty Six grams of Muller-Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The

solidified plates were bored with 6 mm dia cork porer. The plates with wells were used for the antibacterial studies.

Antifungal studies: Fungal media

Two hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20 g of dextrose was mixed with potato infusion. 20 g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6 mm dia cork porer.

Well diffusion method

Antibacterial and antifungal activity of the plant extract was tested using well diffusion method (Bauer et al., 1996). The prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 6 mm cork borer. The extracts were poured into a well using sterile syringe. The plates were incubated at 37±2^{o}C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

Results

The physico-chemical characters such as total ash, acid insoluble ash, acid soluble ash, solubility in alcohol and solubility in water of the dried leaf powder were calculated in terms of air dried sample as shown in Table 1. Ash values are indicating the purity of drug. The results of qualitative phytochemicals are presented in Table 2. Different chemical compounds such as carbohydrates, flavonoids, saponins, gums and mucilage, proteins, tannins and phenolic compounds in the extracts obtained from the leaves of *Crossandra infundibuliformis* which could make the plant useful in treating different ailments and having potential for providing useful drug for human use. This is because the pharmacological activity of any plant is usually traced to particular compound.

Table 1. Analysis of fluorescent properties of *Crossandra infundibuliformis* leaf powder in different chemical reagents

Chemical reagent	Appearance
Powder colur	Green
5% NaOH	Brown
10% NaOH	Brown
Con. H_2SO_4	Brown
Acetic Acid	Brown
1N NaOH in H_2O	Dark brown
5% KOH	Dark brown
50% HNO_3	Dark brown
5% $FeCl_2$	Brownish green
1N HCl	Light brown
Con.HNO_3	Light brown
1N NaOH in ethanol	Dark green
50% H_2SO_4	Dark green
50% HCl	Light brown
Con. HCl	Dark brown

The leaf extracts of *C. infundibuliformis* were tested for their antibacterial activity against *E. coli*, *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *L. lactis*, *S. typhi* and antifungal activity against *A. niger*, *A. flavus*, *R. indicus* and *M. indicus* the results are presented in Table 3 and 4. *Streptococcus aureus* was found to be more susceptible towards the methanol extract of leaf with a maximum inhibitory zone (37 mm). *Salmonella typhi* was found to be more sensitive to the methanol extract of leaf with a maximum inhibitory zone (32 mm) followed by aqueous extract (24 mm). *Pseudomonas aeruginosa* was found to be more susceptible towards the methanol extract of leaf with a maximum inhibitory zone (30 mm) and both methanol and aqueous extracts did not show any inhibition against *Escherichia coli*, *Leuconostoc lactis* and *Streptococcus pyogenes*. Antifungal activity of methanol extract (50, 100 and 200 mg) and aqueous extract (50, 100 and 200 mg) tested against various fungus but a maximum activity was shown by methanol extract against *Aspergillus flavus* and

Aspergillus niger showed moderate activity. But in the case of aqueous extract did not show any activity against the test fungus except *Rhizopus indicus*.

Table 2. Results of phytochemical screening of aqueous leaf extracts of *Crossandra infundibuliformis*

Name of the test	Status of the substances	
	AE	ME
Carbohydrates:		
Fehling's	+	+
Benedict's	+	++
Alkaloids:		
Mayer's	-	-
Hager's	-	-
Wagner's	-	-
Dragen Dorfff's	-	-
Steriods:		
Chloroform + Acetic acid + H_2SO_4	-	-
Tannins and Phenols:		
10% Lead acetate	-	+
5% Ferric chloride	+	+
1% gelatin	-	-
Saponins:		
Foam test	+++	+++
Fixed oils and fats:		
Spot test	-	-
Gums and mucilage:		
Alcoholic precipitation	+	++
Proteins:		
Biuret test	+	+
Flavonoids:		
NaOH / HCl	+	+
Volatile oils:		
Hydro distillation method	-	-

++++ High rich amount; +++ Rich amount; ++ Moderate amount; + Minimum amount; - Absent; AE – aqueous extract; ME – Methanol extract

Discussion

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics becomes an ever increasing therapeutic problem (Austin et al., 1999; Venkatesan and Karrunakaran, 2010). The presence of antifungal and antimicrobial substances in the higher plants are well established as they have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine have been used for the treatment of diseases as in Unani and ayurvedic system of medicines, a natural blueprint for the development of new drugs. Much of the exploration and utilization of natural product as antimicrobial arise from microbial sources. The present study was conducted to analysis the phytochemical, fluorescence characteristics, antibacterial and antifungal potential of leaf extracts of *C. infundibuliformis*.

The results of antibacterial activity of methanolic extracts of *C. infundibuliformis* were consistent with previous reports. Methanolic extract was showed higher activity compared to the aqueous extract. Antimicrobial activity of tannins (Doss et al., 2009), flavonoids (Mandalari et al., 2007), saponins (Avato et al., 2006), terpenoids (Funatogawa et al., 2004) and alkaloids (Navarro and Delgado, 1999) have been well documented. In the present investigation, phytoconstituents namely flavonoids, alkaloids, steroids and saponins were detected in the extracts which may account for the activities. Geraniol extracted from the essential oil of fruits of *Zanthoxylum alatum* was shown to have strong antifungal activity against *Colletotrichum falcatum* and *Ceratocystis paradoxa* fungal pathogens of sugar cane and was more potential than commercial synthetic fungicides (Rao and Singh, 1994). Essential oils from *Cinnamomum camphora* have been reported to have antifungal activity against *Fusarium graminearum* (Liu et al., 2001). Mishra et al. (1992) reported antifungal activity of aqueous leaf extract of *Thuja orientalis* against *Curvularia lunata*. Aqueous and ethanolic extracts of *Vitex negundo* leaves has been shown to be inhibitory against *Pyricularia oryzae* (Rajeswari and Mariappan, 1992). Similarly, chloroform leaf extract of *Ipomea carnea* has been reported to have strong antifungal activity against *Rhizoctonia solani* (Kagale et al., 2004). Among the solvents the highest antifungal activity was noticed in the methanolic extract of *C. infundibuliformis* followed by the aqueous extract.

Table 3. Inhibition zone of aqueous and methanol extracts of *Crossandra infundibuliformis* against bacterial pathogens

Name of the organism	Zone of inhibition					
	Aqueous extract (in mg)			Methanol extract (in mg)		
	50	100	200	50	100	200
S. aureus	12±1.4	17±2.8	24±2.4	29±2.8	33±3.7	37±2.8
E. coli	-	-	-	-	-	-
L. lactis	-	-	-	-	-	-
S. typhi	16±2.8	21±2.4	24±4.9	10±2.4	18±3.7	32±2.4
P.aeruginosa	-	-	-	18±2.4	21±1.4	30±2.4
S. pyogenes	-	-	-	-	-	-

Table 4. Inhibition zone of aqueous and methanol extracts of *Crossandra infundibuliformis* against fungal pathogens

Name of the organism	Zone of inhibition					
	Aqueous extract (in mg)			Methanol extract (in mg)		
	50	100	200	50	100	200
A. flavus	-	-	-	14±2.8	23±3.7	32±2.4
M. indicus	-	-	-	-	-	-
A. niger	-	-	-	13±2.8	18±3.7	30±6.2
R. indicus	09±2.4	12±2.8	16±2.4	-	-	12±2.8

References

Anonymous (1966). Pharmacopoeia of India (The Indian Pharmacopoeia) 2nd Edition, Manager of Publications, New Delhi, India, pp. 947-48.

Austin DJ, Kristinsson KG, Anderson RM (1999). The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc Natl Acad Sci USA 96:1152-1156.

Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta, M (2006). Antimicrobial activity of saponins from *Medicago* sp. structure-activity relationship. Phytotherapy Res 20 (6): 454-457.

Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH (1985). Natural plant chemicals: Sources of Industrial and Medicinal materials. Science 228: 1154-1160.

Bauer AW, Kirby WM, Sherris JC, Jurck M (1996). Antibiotic susceptibility testing by a standard single disc method. Am J Clin Pathol 451: 493-496.

Bharathiraja S, Tholkappian P (2011). AM fungal interaction and its beneficial effects on *Crossandra infundibuliformis*. International Journal of Current Research 3(6): 100-102.

Funatogawa K, Hayashi S, Shimomura H, Yoshida T, Hatano T, Ito H, Hirai Y (2004). Antibacterial activity of hydrolyzable tannins derived from medicinal plants against *Helicobacter pylori.* Micro and Immunol 48(4): 251-261

Harborne JB (1973). Phytochemical methods. In: A guide to modern techniques of plant analysis. Harborne JB (ed.), Chapman and Hall, London. pp.279.

Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R (2004). Antimicrobial activity and induction of systemic acquired resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia*

solani and *Xanthomonas oryzae* pv. *oryzae*. Physiol Mol Plant Pathol 65: 91-1000.

Kokate CK, Purohit AP, Gokhale SB (1995). Pharmacognosy, 3rd edition, Nirali Prakashan, Pune, India.

Kroschwitz JI, Howe-Grant M (1992). Kirk-Othmer encyclopedia of chemical Technology 2: 893.

Liu CH, Mishra AK, He B, Tan RX (2001). Composition and antifungal activity of essential oils from *Artemisiaprinceps* and *Cinnamomum camphora*. Inter Pest Cont 43: 72-74.

Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ, Narbad A (2007). Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. J App Microb 103(6): 2056-2064.

Mishra M, Malik SS, Tiwari SN (1992). Allelopathic effect of certain botanicals against six fungal pathogens of rice. In: Proceedings of First National Symposium on Allelopathy in Agroecosystems (Agriculture and Forestry). Tauro P, Narwal SS (Eds.). CCS HAU, Hisar, India, 191-193.

Navarro V, Delgado G (1999). Two antimicrobial alkaloids from *Bocconia arborea*. J Ethnopharmacol 66(2): 223-226.

Newman DJ, Cragg GM, Snader KM (2000). The influence of natural products upon drug discovery. Nat Prod Res 17: 215-234.

Rajeswari E, Mariappan V (1992). Effect of plant extracts on in vitro growth of rice blast (Bl) pathogen *Pyricularia oryzae*. Inter Rice Res News Let 17: 6.

Rao GP, Singh SB (1994). Efficacy of geraniol extracted from the essential oil of *Zanthoxylum alatum* as a fungitoxicant and insect repellent. Sugar Cane 4: 16-20.

Srivastava J, Lambert J, Vietmeyer N (1996). Medicinal plants: An expanding role in development. World Bank Technical Paper No. 320.

Uniyal SK, Singh KN, Jamwal P, Lal B (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. J Ethnobiol Ethnomed 2: 1-14.

Venkatesan D, Karrunakaran CM (2010). Antimicrobial activity of selected Indian medicinal plants. Journal of Phytology 2(2): 44-48.