

PHYTOCHEMICAL COMPOSITION AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *CALLISTEMON LANCEOLATUS* D.C.**VEERANNA PALURI, SUBRAMANIYAM RAVICHANDRAN, GAURAV KUMAR, LOGANATHAN KARTHIK, KOKATI VENKATA BHASKARA RAO*****Molecular and Microbiology Research Laboratory, Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore, Tamil Nadu 632014, India. Email: kokatibhaskar@yahoo.co.in***Received: 29 Dec 2011, Revised and Accepted: 2 Feb 2012***ABSTRACT**

Callistemon lanceolatus D.C. (Myrtaceae) is an ornamental plant distributed throughout India. In this study, methanolic extract of leaves of *C. lanceolatus* was screened for its phytochemical composition and antimicrobial activity by *in vitro* methods. Extract was screened for the presence of carbohydrates, phenolic compounds, saponins, alkaloids, oil and fats, flavonoids, phytosterols, tannins, glycosides and proteins. *In vitro* antimicrobial activity was performed by agar well diffusion method. The antimicrobial potential of the extract was compared with positive control to calculate relative percentage inhibition. The extract exhibited the presence of carbohydrates, phenolic compounds, saponins, alkaloids, oil and fats, flavonoids, phytosterols and tannins. Among the test microorganisms, extract exhibited maximum zone of inhibition against *Staphylococcus aureus* (20.0±1.73 mm) and minimum zone of inhibition against *Candida albicans* (3.33±1.52 mm). No activity was recorded against *Kelebsiella pneumoniae* and *Escherichia coli*. Extract exhibited maximum relative percentage inhibition against *Staphylococcus aureus* (194.79%) and lowest against *Candida albicans* (4.52%).

Keywords: *Callistemon lanceolatus*, Myrtaceae, Phytochemical composition, Antimicrobial activity

INTRODUCTION

Microbial infections were one of the major causes of death among humans and animals in pre antibiotic era. Discovery and application of penicillin and other antibiotics have significantly reduced the complications and mortality rate of infectious diseases, however failed to control completely. Recently, several studies reported the incidence of microbial drug resistance around the world¹⁻⁴. This may be attributed to the overuse of antibiotic drugs in medicine, food, dairy, poultry and agriculture industries. These drug resistant microorganisms are resistant to one or more commonly used drugs, therefore difficult to control. Thus, there is a continuous need to develop newer drugs from alternate sources.

Since the advent of human civilization, plants and other natural sources have been utilized as a source of medicine. Ancient literatures of Indian, Chinese, Korean, and Tibetan medicines describe the role of plants in traditional health care. Recently, extracts and other byproducts of many plants have been reported to possess a variety of medicinal properties such as antimicrobial activity⁵, anticancer activity⁶, anti-inflammatory activity⁷, anti-helminthic activity⁸, antioxidant activity⁹, antidiabetic activity¹⁰, antimalarial activity¹¹, anti-artheritic activity¹², hepatoprotective activity¹³, neuroprotective activity¹⁴ and nephroprotective activity¹⁵.

Callistemon lanceolatus D.C. (Myrtaceae) is a slow-growing ornamental shrub that grows to a height of around 10 meters. *C. lanceolatus* is a native tree to Australia, but is also widely distributed in Asian countries. It is commonly known as crimson bottle brush tree because of its spiky inflorescence that resembles a bottle brush. The inflorescence is crimson in color and cylindrical, and flowers are borne in spring and summer. The plant has been used by tribal communities of India for the treatment of gastrointestinal disorders, pain, and infectious diseases¹⁶. Over the years, *C. lanceolatus* have been extensively analyzed scientifically and reported to possess anticholinestrase activity¹⁷, wound healing activity¹⁸, hepatoprotective activity¹⁹, inhibit elastase activity²⁰, cardioprotective activity²¹, anti-inflammatory activity²², antidiabetic activity, hypolipidemic activity and antioxidant activities²³. These reports indicate the potential of *C. lanceolatus* to be a good source of bioactive compounds with several medicinal properties.

In this study, we have elucidated the phytochemical composition and antimicrobial activity of methanol extract of the *C. lanceolatus*

leaves against a variety of clinically isolated pathogenic bacteria and fungi.

MATERIALS AND METHODS**Chemicals**

Nutrient agar, Mueller Hinton broth (MHB), Mueller Hinton agar (MHA), Potato Dextrose broth (PDB), Potato Dextrose agar (PDA), Bacitracin discs, Erythromycin discs, Chloramphenicol discs and Fluconazole discs were purchased from Himedia Pvt. Ltd., Mumbai, India. Other chemicals and reagents used for the study were of analytical grade.

Plant material

Fresh and mature leaves of *C. lanceolatus* were collected from the VIT University campus, Vellore (Lat. 12°58' N; Long. 79°09' E) during August, 2011. Sample was collected in sterile plastic bag and brought to the Molecular Biology and Microbiology Research Laboratory, VIT University, Tamil Nadu, India. A voucher specimen was maintained in our laboratory for future reference.

Extract preparation

The leaves of *C. lanceolatus* were washed thoroughly with distilled water and dried at room temperature. Dry leaves were uniformly grounded using a mechanical grinder to yield fine powder. Ten grams of the powder was mixed with 100 ml of methanol in an Erlenmeyer flask and kept in shaking incubator at 100 rpm for 24 hours. The mixture was filtered using Whatman filter paper no 1. Extract was dried and stored in an airtight container at 4°C until further use.

Phytochemical screening

Phytochemical screening of the leaves of *C. lanceolatus* was carried out by using the standard protocols²⁴. Methanolic extract of *C. lanceolatus* was screened for presence of carbohydrates, phenolic compounds, saponins, alkaloids, proteins, oil and fats, flavonoids, glycosides, phytosterols and tannins.

Antimicrobial activity of the plant extract**Test microorganisms**

The following clinical isolates of bacteria and fungi were used for the study: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia*

coli, *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella typhi*, *Candida albicans*, and *C. tropicalis*. Microbial cultures were grown on nutrient agar and potato dextrose agar for bacteria and fungi respectively and maintained at 4°C in a refrigerator for further studies.

Controls used in the study

Bacitracin (10µg/disc) was used as positive control for *S. aureus*; Erythromycin (10µg/disc) was used for *P. aeruginosa*, *E. coli*, *M. luteus*, and *S. typhi*; Chloramphenicol (30µg/disc) was used for *K. pneumoniae*; Fluconazole (10µg/disc) was used for *C. albicans* and *C. tropicalis*. Sterilized distilled water was used as negative control for the study,

Antimicrobial assay

Antimicrobial activity of the crude extracts was determined by the agar well diffusion method²⁵. All test organisms were inoculated on MHB and PDB for 8 hours. Microbial isolates were inoculated on MHA plates and PDA plates by using sterile cotton swabs for bacteria and fungi respectively. Agar surface was bored by using sterilized gel borer to make wells (7 mm diameter). 100 µl of the test extract and 100 µl of sterilized distilled water (negative control) were poured in to separate wells. The standard antibiotic disc was placed on the agar surface as positive control. Bacterial plates were incubated at 37°C for 24 hours and fungal plates were incubated at room temperature for 48 to 72 hours. Experiment was performed in triplicates.

Determination of relative percentage inhibition

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula^{26,27}.

$$\text{Relative percentage inhibition of the test extract} = \frac{100 \times (x-y)}{(z-y)}$$

Where,

x: total area of inhibition of the test extract

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2 ; where, r = radius of zone of inhibition.

Statistical Analysis

The values of antimicrobial activity of the methanolic extract of *C. lanceolatus* leaves are expressed as mean \pm standard deviation of the response of 3 replicates determinations per sample. Results were analyzed by using Microsoft Excel 2007 (Roselle, IL, USA).

RESULTS AND DISCUSSION

Percentage yield

10 gms of dried leaf powder of *C. lanceolatus*, when extracted with methanol resulted in the production of 2.3 gms of extract. Thus, the quantity of extract obtained was 23% of the weight of the initial plant powder.

Phytochemical analysis

The methanolic extract showed the presence of carbohydrates, phenolic compounds, saponins, alkaloids, oil and fats, flavonoids, phytosterols and tannins. Glycosides and proteins were found to be absent in the extract (Table 1). These phytochemical compounds may be the key in unlocking the therapeutic potential of this plant. Earlier, *C. lanceolatus* has been reported to contain several phytochemicals such as volatile oils^{28, 29}, polyphenols^{30, 31} and triterpenoids^{32,33}.

Table 1: Phytochemical analysis of methanolic extract of *C. lanceolatus* leaves

Phytochemical	<i>Callistemon lanceolatus</i>
Tannins	++++
Flavonoids	++++
Phenolic compounds	++++
Carbohydrates	+++
Alkaloids	+++
Saponins	++
Oil and fats	++
Phytosterols	++
Proteins	-
Glycosides	-

Here, +: present, -: not present

Antimicrobial activity

The methanolic extract of *C. lanceolatus* leaves exhibited broad spectrum antimicrobial activity against Gram positive bacteria, Gram negative bacteria and fungal cultures. Antimicrobial activity was measured as zone of inhibition and represented as mean \pm standard deviation (n=3). Extract exhibited maximum antimicrobial activity against *S. aureus* (20.0 \pm 1.73), followed by *S. typhi* (17.33 \pm 0.57), *M. luteus* (12.66 \pm 1.15), *C. tropicalis* (10.66 \pm 2.08), *P. aeruginosa* (6.66 \pm 1.52) and *C. albicans* (3.33 \pm 1.52). Results are summarized in Table 2. *K. pneumoniae* and *E. coli* were found to be resistant towards the methanolic leaf extract of *C. lanceolatus*.

Earlier, hot water extract of *C. lanceolatus* bark was reported to exhibit efficient antimicrobial activity against *Xanthomonas campestris* pv. *campestris*³⁴. Sweet essential oil of *C. lanceolatus* was reported for antifungal activity against a wide variety of fungal cultures included *Absidia ramose*, *Alternaria alternata*, *Aspergillus fumigatus*, *A. niger*, *A. oryzae*, *Chetomium* sp., *Dreschlera* sp., *Fusarium nivale*, *F. oxysporium*, *Mucor* sp., *Penicillium citrinum* and *Trichoderma* sp.³⁵. In past, various plants have been reported to possess a broad spectrum antimicrobial activity. Some examples are *Calotropis gigantea*⁵, *Bauhinia racemosa*²⁵, *Dioscorea villosa*³⁶, *Swertia chirata*³⁷, *Psidium guajava*, *Azadirachta indic*³⁸ etc. All these studies explain the antimicrobial properties of plant extracts and their possible use in pharmaceutical industries for the development of newer and much safer drugs.

Table 2: Antimicrobial activity of methanolic extract of *C. lanceolatus* leaves

Organisms	Zone of inhibition (mm)		
	Methanol extract	PC	NC
<i>Pseudomonas aeruginosa</i>	6.66 \pm 1.52	14.0 \pm 1.0	n.a.
<i>Salmonella typhi</i>	17.33 \pm 0.57	16.0 \pm 4.35	n.a.
<i>Kelebsiella pneumoniae</i>	n.a.	15.66 \pm 3.78	n.a.
<i>Escherichia coli</i>	n.a.	33.66 \pm 1.52	n.a.
<i>Staphylococcus aureus</i>	20.0 \pm 1.73	14.33 \pm 0.57	n.a.
<i>Micrococcus luteus</i>	12.66 \pm 1.15	27.66 \pm 2.08	n.a.
<i>Candida albicans</i>	3.33 \pm 1.52	15.66 \pm 1.52	n.a.
<i>Candida tropicalis</i>	10.66 \pm 2.08	21.66 \pm 1.52	n.a.

PC: positive control, NC: negative control, n.a.: no activity

Values are expressed as mean \pm standard deviation of the three replicates

Zone of inhibition not include the diameter of the well.

Relative percentage inhibition

Antimicrobial activity of *C. lanceolatus* leaf extract was compared with the antimicrobial activity of standard drugs for evaluating relative percentage inhibition (Table 3). The methanolic extract of *C. lanceolatus* leaves exhibited maximum relative percentage inhibition against *S. aureus* (194.79 %) and *C. tropicalis* (24.22%) for bacteria and fungi respectively.

Table 3: Relative percentage inhibition of methanolic extract of *C. lanceolatus* leaves

Test organisms	Relative percentage inhibition (%)
<i>Pseudomonas aeruginosa</i>	22.63
<i>Salmonella typhi</i>	117.31
<i>Kelebsiella pneumoniae</i>	-
<i>Escherichia coli</i>	-
<i>Staphylococcus aureus</i>	194.79
<i>Micrococcus luteus</i>	20.94
<i>Candida albicans</i>	4.52
<i>Candida tropicalis</i>	24.22

CONCLUSION

C. lanceolatus is primarily an ornamental plant, although it has been used in traditional medicine to cure some diseases. Recent reports of various pharmaceutical properties of this plant establish it as a valuable source of medicinal compounds. In this study, methanolic extract of *C. lanceolatus* leaves was found to possess broad spectrum antimicrobial activity against various clinical isolates of bacteria and fungi. We conclude that methanolic extract of *C. lanceolatus* leaves is a good source of antimicrobial compounds and in future, the bioactive molecule can be isolated and characterized using advanced analytical techniques.

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