

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *Callistemon linearis* DC LEAF EXTRACT

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Summary

Antibacterial, antifungal & antioxidant activities of methanolic extract obtained from *Callistemon linearis* DC. (Narrow leaf bottle brush) leaf was studied. The antimicrobial assay were carried out against selective microorganism (Bacteria- *Bacillus pumilus*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, , *Enterobacter aerogenes*, *Salmonella typhimurium* and fungus like *Candida albicans*, *Aspergillus niger*) were used for testing by disc diffusion method at different concentration (100,50,25 and 12.5 mgml⁻¹). For testing antioxidant property Hydrogen peroxide scavenging activity and reducing power ability methods were followed. Methanol extract exhibited potential antimicrobial activity against both gram positive as well as gram negative bacteria and moderate activity against fungal species. This extract also shows good antioxidant activity (comparable with standard Ascorbic acid) which is concentration dependent.

Key Words *Callistemon linearis* DC. Leaf, Antibacterial, Antifungal, Antioxidant, Ascorbic acid.

Introduction

Antibacterial therapy was developed in the first half of the last century. The development of antimicrobial resistance in many bacterial and fungal organism however constitutes one of the most serious problems in the control of most infectious diseases. Phytomedicines are defined as the use of plant or their extracts for medicinal purposes, comes handy in this fight against microbial resistance (1). Recently, in different parts of the world, attention has been paid to exploiting higher order plant product as novel chemotherapeutants to protect from various fungal infection. Therefore it has been thought desirable to discover the antifungal potencies of higher order plant products against fungal diseases (2).

Antioxidants are the nutraceuticals whose deficiency states are associated with variety of dreaded diseases conditions, like cardiovascular diseases, diabetes, cataracts, rheumatoid arthritis, Alzheimer diseases and many others. Free radicals are chemical species possessing an unpaired electron that can be considered as fragment of molecules and which are extremely reactive and short lived. They are produced continuously in cells either as accidental by products of metabolism or deliberately during different pathological disorders and phagocytes (3). *Callistemon linearis* DC. (narrow leaved bottle brush tree) is a small, evergreen tree, family myrtaceae, with rough fissured bark and dropping branches. This plant is up to 2.7 m in height, native to Australia. Now a day it is grown in India as an ornamental tree. (4,5). This plant is not well studied from phytochemical & pharmacological point of view. The aim of the present work is to investigate the antimicrobial and antioxidant activities of *Callistemon linearis* DC. Leaf extract.

Materials and Methods

Collection of plant material

The plant *Callistemon linearis* DC. (Bottle brush tree) had been collected from Dibrugarh University campus and was identified by Botanical Survey of India (BSI), Shillong. A voucher specimen (DU/PHC/HRB-4/08) has been kept in the departmental herbarium store.

Methanolic extract:

About 20 gm each of the plant materials (leaves) are placed in clean flasks containing 200 ml of 78% methanol as extraction solvent. The filtrates or resultant of extract are evaporated in a water bath at 40°C.

The samples were then weighed and kept in the refrigerator for further analysis. Microorganisms like *Bacillus pumilus*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhimurium* and fungus like *Candida albicans*, *Aspergillus niger* were used for testing.

Antimicrobial Testing

The crude plant extracts (leaf extract) were test for their anti bacterial & antifungal activity using Disc Diffusion Method (6) was followed. The test plates were prepared with Mueller – Hinton agar and inoculated on the surface with a cell suspension in sterile dissolution on of 0.9% saline. In all cases, the concentration was adjusted to 1.5×10^8 CFU/ml. sterile 5 mm diameter bloating paper disc were impregnated with 10 mg of each extract which were dissolved in solvent from which extract were prepared (0.5-1 ml) and placed on nutrient agar seeded with the micro organisms. The plates were incubated for 24 hrs at 37⁰C for bacteria and 48 hours for fungi (7). Control discs were soaked with the same extraction solvents and treated as the sample discs. The positive results were established by the presence of clear zone of inhibition around active extracts which were measured with a meter rule and diameters recorded in cm standard Disc of Ampicillin was used as positive antibacterial controls.

Antioxidant Testing

Preparation of Extracts

The fresh leaves of *C linearis* DC were shade dried and grounded to a fine powder 4 gm of the powdered sample was then extracted for 5 hrs with methanol (50 ml) under continuous-stirring at room temperature (28⁰C) and the extraction process was repeated until the solvent became colorless. The extracts were then concentrated in water both 40⁰C. The solid mass obtained was re-suspended in methanol & stored at 4⁰C.

Method -1

Hydrogen peroxide Scavenging Activity

The antioxidant activity of methanol extract of leaves of *Callistemon linearis* and the standard compound Ascorbic acid AR grade (S D Fine chemicals Ltd, Mumbai) was measured in terms of hydrogen peroxide Scavenging ability using the hydrogen peroxide (Rankem, New Delhi).

For this the methanolic extract at various concentration were prepared and hydrogen peroxide solution were prepared by mixing with phosphate buffer saline (pH-7.4) the methanolic extract solution & hydrogen peroxide for phosphate buffer solution were mix at same concentration. A control solution was prepared with methanolic extract solution in phosphate buffer saline without hydrogen peroxide solution. The absorbance at 230 nm was recorded using a UV- visible spectrophotometer against blank samples(8). The mean values were obtained from triplicate experiments. The percentage inhibition of Hydrogen peroxide scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \frac{\text{Abs. control} - \text{Abs. of test}}{\text{Abs. control}} \times 100$$

Method II

Reducing power ability

The reductive potential of the extract was determined according to the method of Oyaizu et al 1986. The different concentration of methanolic extract & standard (20, 40, 60, 80, 100 μgml^{-1}) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 molar, pH-6.6) and potassium ferricyanide (Merck, Mumbai) [$\text{K}_3\text{Fe}(\text{CN})_6$]. The mixture was incubated at 50°C for 20 min. A portion (2.5ml) of Trichloroacetic acid (10% w/v) was added to the above mixture, which was then centrifuged for 10 min at 1000-3000 rpm. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) & FeCl_3 (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential (9,10).

Results and Discussion

The *in vitro* antimicrobial activities of the methanolic extracts were resulted in a range of growth inhibition pattern against the used microorganism. The results of the antimicrobial activity of methanolic extract of *Callistemon linearis* leaf are given in the Table – 1.

These data revealed that the methanolic extract showed potential antimicrobial activity against gram positive & gram negative bacteria & *Candida albicans*. It is noteworthy in particular its effect against *Escherichia coli*, *Bacillus pumilus* which was comparable with ampicillin.

Table-1– Growth inhibition zones (mm) at different concentration

Microorganisms	<u>Concentration (mgml⁻¹)</u>				
	100	50	25	12.5	12.5**
<i>B.Pumilus</i>	13.8	13.8	13.1	11.2	14.2
<i>B.Cereus</i>	11.2	10.8	9.3	8.7	13.1
<i>St.aureaus</i>	12.8	10.2	8.8	8.0	10.6
<i>E.coli</i>	13.3	11.5	9.5	8.1	9.2
<i>E.aerogenes</i>	10.6	9.8	8.5	8.0	10.8
<i>S.typhimurium</i>	6.1	4.3	2.9	+/-	5.6
<i>C.albicans</i>	5.5	4.2	3.3	2.5	3.0
<i>A.aegyptiacus</i>	2.4	2.0	+/-	+/-	-

+/- = zone of inhibition <2 mm , (-) = no inhibition, **Concentration of Ampicillin

=Standard drug.

The Hydrogen peroxide scavenging activity of Methanolic extract of leaves of *Callistemon linearis* exhibited effective antioxidant activity at all the concentration tested. The effect of various concentration of methanol Extract (100-500 µg/ml) with their % inhibition revealed that the antioxidant activity of the extract increased with increasing concentration Fig-1. The % inhibition of methanol Extract in Hydrogen peroxide scavenging system in various concentrations is 46.91%, 58.56%, 63.30%, 72.05%, and 79.33%. The % inhibition of 500 µg/ml concentration of ascorbic acid was found to be 80.42%. The IC₅₀ value of methanol extract and ascorbic acid is: 225.4 & 241.0 respectively. The reducing power of the methanolic extract and the reference compound ascorbic acid increased steadily with increase in concentration the reducing power (absorbance at 700 nm) of methanolic extract and ascorbic acid were 1.137 & 0.852 respectively Fig-2.

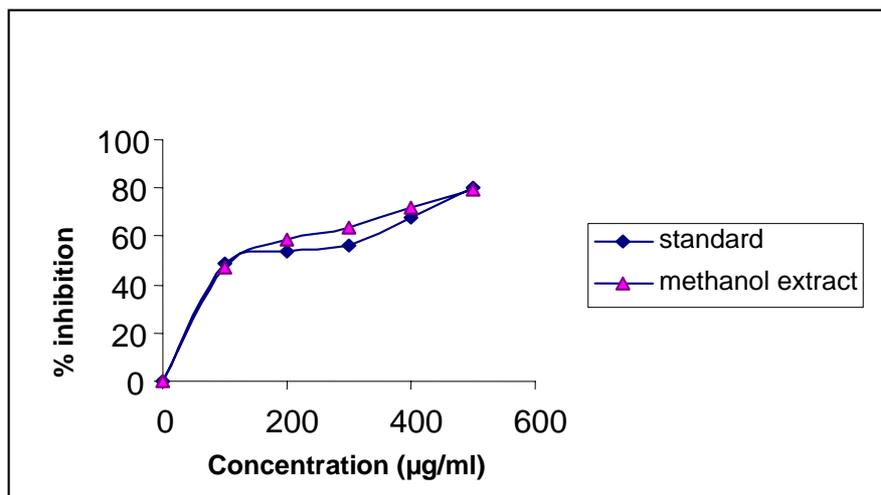


Fig-1: Comparative scavenging activity of *C. linearis* leaf extract

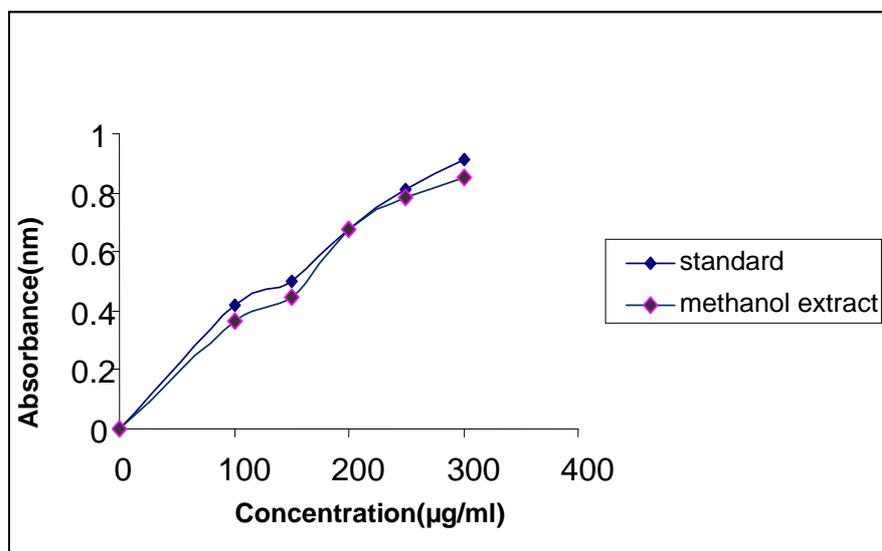


Fig-2: Comparative reducing power activity of *C. linearis* leaf extract

Methonolic extracts of *Callistemon linearis* leaf showed good to moderate antimicrobial activity and among the different leaf extract alcoholic extract showed better antimicrobial activity.

The result obtained in the study of Antioxidant activity of Methanol Extract of leaves of *Callistemon linearis* DC, had revealed the reducing power and hydrogen peroxide scavenging activity which is comparable to that of standard antioxidant such as ascorbic acid. The methanol extract of *Callistemon linearis* leaf possess antioxidant properties which is concentration dependant.

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