Antimicrobial Potential of Ethanolic Extract of *Callistemon linearis* DC Leaf

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Summary

The antibacterial and antifungal properties of ethanolic extract of *Callistemon linearis* leaf was screened against different pathogenic bacteria and fungi namely, *Bacillus cereus, Bacillus pyogenes,Bacillus pumilus, Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes,Proteus vulgaris, Salmonella typhimurium, Candida albicans, Aspergillus aegyptiacus,* by using disc diffusion method employing Ampicillin and Amphotericin-B as standard disc. The extract showed significant activity against maximum bacteria which is comparable with standard Ampicillin effect and moderate activity against fungal strain which is also comparable with standard Amphotericin-B.

Key words: *Callistemon linearis*, antibacterial, antifungal, Ampicillin, Amphotericin-B.

Introduction

The use of herbs and medicinal plant as the first medicines is a universal phenomenon. Every culture on the earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in plants for their therapeutic properties. All drugs of the plant were substances with a particular therapeutic action extracted from plants (1).Histologically plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contribution to human health and well being. Their role in the development of new drugs i.e.natural blue prints for herbal medicines and anti-infective properties which offer a therapeutic benefit and more affordable treatment.

Various infections involving microorganism's ie.bacteria, fungi, viruses, nematodes, they cause severe infections in tropical and subtropical countries of the world and search for herbs or plant extracts having antimicrobial properties have been found a prime important to eradicate these infections. In the recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases.

Over the last three centuries, intensive efforts have been made to discover clinically useful antimicrobial drug, (2,3,4).Different medicinal plants are finding their way into pharmaceuticals, cosmetics, food supplements. The World Health Organization (5) estimated that 80% of population of developing countries still relies on plant drugs medicines for their health care needs. Due to unsafe and fatal side effects of unscreened plant drugs, there is an urgent need to study the screening of antimicrobial properties of herbs (5,6).

Callistemon linearis DC. is an evergreen tree, under the family Myrtaceae.It is also known as narrow leaved bottle brush indigenous to Australia. It is now available in Indian garden as an ornamental tree. The Methanolic extract of the fruit showed antibacterial activity against gram positive and gram negative bacteria and also against fungal strains (7).The seeds of a sample from Japan yielded an oil containing β-sitosterol (8,9). In this study we concentrate on the antibacterial and antifungal activity of ethanolic extract of *Callistemon linearis* leaf.

Material 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.

Cold maceration

For cold maceration about 20 gm of plant materials (leaves) were placed in clean flasks containing 200 ml of 70% ethanol, corked and stored for five days at room temperature and there after filtered. The filtrates or resultant extract was evaporated in a water bath at 40^0c.The sample were then weighed and kept in the refrigerator for further analysis. (10,11,12).

Test microorganism

Bacillus cereus, Bacillus pumilus, Bacillus pyogenes Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Salmonella typhimurium, Candida albicans, Aspergillus aegyptiacus.

Antimicrobial assay

The antimicrobial activity of the ethanolic extract was determined by using the zone of inhibition tests. For that purpose, disc diffusion method of Bauer et al.(13) 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 nutrient agar plates containing an inoculums size of 10^6 CFU/ml for bacteria and saborand glucose agar plates containing $2x10^5$ spores for fungi were used. Previously prepared ethanolic extract impregnated disc(5 mm in diameter bloating paper disc) at concentration of 100 μg/ml for bacteria and 1000μg/ml for fungal strains were placed aseptically on sensitivity plates with appropriate control Ampicillin(100μg/ml) and Amphotericin-B (1000μg/ml) were used as standard antibacterial and antifungal antibiotics respectively.. The plates were incubated for 24 hrs at 37^0C for bacteria and 48 hours for fungi (14). Control discs were soaked with the same extraction solvents and treated as the sample discs.

The positive results (sensitivity) were established by the presence of clear zone of inhibition around active extracts which were measured with a meter rule and diameters were recorded in mm.

MIC was determined by tube dilution method for each of the test organism in duplicates. For that purpose,ehtanolic extract are prepared at different concentration(0-100 μg/ml for bacterial strains and 0-1000 μg/ml for fungal strains),2 ml of nutrient broth was added and then a loopful of test organism previously diluted to 0.5 McFarland turbidity standard for (bacteria) and 10^5 cfu/ml(for fungal strains) was introduced to the tubes. The procedure was repeated on the test organisms using standard antibiotics Ampicillin for bacteria and Amphotericin-B for fungi. A tube containing nutrient broth only seeded with the test organism was served as control. Tubes containing bacterial cultures were then incubated for 24 hrs at 37^0C for bacteria and 48 hours for fungi. After inoculation the tubes were examined for microbial growth by observing the turbidity (15).

Results and Discussion

The preliminary antimicrobial activity of Ethanol extract was resulted in a growth inhibition pattern against the used microorganism. The results of the antimicrobial

activity were given in the Table-1.These data revealed that the ethanol extract showed good antimicrobial activity against bacteria and fungi antimicrobial activity It is noteworthy in particular effect against E.coli, St.aureus, B. pumilus and C.albicans which is comparable with Ampicillin for bacteria and AmphotericinB for fungi and has no activity against B.pyogenes. The minimum inhibitory concentration (MIC) of ethanol extract was resulted in a growth inhibition pattern against the used microorganism.

Table -1: Results of preliminary antimicrobial activity of ethanolic extract of *Callistemon linearis* leaf.

Organism	**Zone diameters (mm)**	
Gram positive bacteria	**Ethanol extract (100µg/ml)**	**Ampicillin (100µg/ml)**
Bacillus cereus	11.2	NT
Bacillus pyogenes	-	NT
Bacillus pumilus	13.8	NT
Staphylococcus aureus	12.8	NT
Gram negative bacteria		
Escherichia coli,	13.9	14.2
Enterobacter aerogenes	10.6	12.7
Proteus vulgaris	3.5	6.8
Salmonella typhimurium	7.1	8.8
Fungal strains	**Ethanol extract (1000µg/ml)**	**AmphotericinB(1000µg/ml)**
Candida albicans	7.8	12.5
Aspergillus aegyptiacus	3.4	7.8

'(-)' = no measurable zone of inhibition, 'NT' =Not tested.

The results of the antimicrobial activity were given in the Table-2 .The extract showed its particular effect against E.coli, St.aureus, B pumilus in terms of zone of inhibition at lower concentration (12.5 µg/ml) which is comparable with Ampicillin and showed no activity against B.pyogenes (>100 µg/ml).This extract also showed moderate activity against B.cereus, E.aerogenes and S.typhimurium and weak inhibition against P.vulgaris. (100 µg/ml) in terms of MIC. In preliminary antifungal assay of ethanol extract showed moderate zone of inhibition against Candida albicans at concentration(250-1000 µg/ml) and weak activity against A.aegyptiacus(1000 µg/ml).

Table-2: Result of Minimum Inhibitory Concentration (MIC) of ethanol extract.

	Concentration (µgml$^{-1)}$					
Microorganisms	**100**	**50**	**25**	**12.5**	**12.5***	**125****
B.Pumilus	13.8	13.8	13.1	11.2	+/-	NT
B.Cereus	11.2	10.8	9.3	8.7	-	NT
St.aureaus	12.8	10.2	8.8	8.0	-	NT
B.pyogenes	-	-	-	-	NT	NT
E.coli	13.3	11.5	9.5	8.1	13.2	NT
E.aerogenes	10.6	9.8	8.5	7.7	12.7	NT
S.typhimurium	7.1	6.3	6.1	5.9	8.8	NT
P.vulgaris	3.5	3.3	+/-	+/-	6.8	NT
Fungi	**Concentration(µgml^{-1})**					
	1000	**500**	**250**	**125**		**125****
A.aegyptiacus	3.4	2.0	+/-	+/-	NT	4.2
C.albicans	7.5	7.2	4.3	+/-	NT	12.5

'+/-'= zone of inhibition <2 mm, '(-)' = no measurable zone of inhibition, '*' = Concentration of Ampicillin, '' = Concentration of Amphotericin-B, NT = not tested**

Conclusion

The antimicrobial activities of various plants have been reported by many researchers (16,17).As the plant produce secondary metabolites in order to protect themselves from microorganism ,herbivores and insects, thus antimicrobial effect is some how expected from plants namely flavonoids,alkaloids,and triterpenoid are producing a better opportunity for testing wide range of microorganism. In the present study a wide variety of gram positive, gram negative and fungal strains were selected for screening of antimicrobial effects of ethanol extract of *Callistemon linearis* leaf. The result of this study showed that the ethanol extract exhibited varied range of antimicrobial activity against the tested organism including gram positive, gram positive bacteria and fungal strains, which is comparable to standard ampicillin effect .The antimicrobial activity of ethanol extract may be due to various types of phyto molecules present in the extract. Thus our motto is to develop standardized extract to combat against the wide range of microorganism.

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