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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY OF CALLISTEMON SALIGNUS LEAVES

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ABSTRACT

Purpose: The aim of the present study was to investigate antimicrobial activity of the various extracts of Callistemon Salignus leaves.Method: Callistemon Salignus leaves were extracted in hexane, ethyl acetate, methanol and water and their antimicrobial activities were examined against few selected microorganisms including B.subtilis, S. aureus, M. luteus, S. marcenscens, P. aeruginosa, B. megaterium, E. coli, P. vulgaris, Yeast,A. niger and R. oligoporus using cup plate method.Results: Water extract of Callistemon Salignus leaf showed activity against B. subtilis and S. aureus only. Methanol extract gave the highest zone of inhibition against P. aeruginosa where as minimum zone of inhibition was found against S. aureus and yeast. B. megaterium and yeast were found to be highly susceptible towards ethyl acetate and hexane extracts, respectively whereas A. niger and B. subtilis were found to be least susceptible against ethyl acetate and hexane extracts, respectively. Hexane extract showed the highest activity against yeast among the tested microorganisms.Conclusion: The study confirms the possible antimicrobial potentiality of the leaf extract of Callistemon Salignus.

Keywords: Callistemon Salignus, antimicrobial activity, leaf extracts

INTRODUCTION

The use of natural products with therapeutic properties has a long history, plant, animal, and mineral products were the main source of medicines[1]. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds[2]. Plants can possess antimicrobial natural products to protect themselves from microbial infection and deterioration[3]. In the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects[4]. In recent years, concern over pathogenic and spoilage microorganisms in foods has increased due to the increase in outbreaks of food borne disease[5]. There are growing interests in using

natural antimicrobial compounds, especially extracted from plants, for the preservation of

foods. In addition, there are more consumers who tend to question the safety of synthetic

additives and would prefer natural foodstuffs[6-7]. There is therefore the need to search for plants of medicinal value. *Callistemon Salignus* (Family – Myrtaceae) is widely grown in India and Australia. It is a conspicuous tree, 3-10m tall. The tribal for analgesic and anti-inflammatory used other species of callistemon. Several studies on the various parts of the plant have been reported for their antithrombin[1], anti mycobacterium tuberculosis properties[8], and insecticidal [9] activities. The phytochemical studies revealed the presence of C – methyl flavonoids, lipid and butalinic acid. Therefore, the presents study was planned to study the antimicrobial activity of hexane, ethyl acetate, methanol and water extracts of *Callistemon Salignus* leaves against the selected microorganisms.

MATERIALS AND METHODS

Plant Material and Microorganisms

The fresh leaves of *Callistemon Salignus* were collected in the month of September from Simlipal forest (Odisha) and authenticated by botanical survey of India, Howrah (Voucher No. CNH/1-1(87)/2005-TechII/1326). The bacterial strains used were obtained from the stock culture of the Department of Microbiology, Jadavpur University,Kolkata, India. The organisms included in the present study were *B.subtilis*, *S. aureus*, *M. luteus*, *S. marcenscens*, *P. aeruginosa*, *B. megaterium*, *E. coli*, *P.vulgaris*, Yeast, *A. niger* and *R. oligoporus*. All the bacterial strains used for the experimental purpose were grown and maintained on nutrient agar medium. Yeast was isolated from curd sample on Sabouraud agar medium in the laboratory and maintained on the same medium. *R. oligosporus* and *A. niger* were grown and maintained on potato dextrose agar medium.

Preparation of extracts

Collected fresh leaves were shade dried and ground thoroughly in a grind mill to obtain a coarse powder. The powdered leaf material was extracted successively in increasing polarity by using hexane, ethyl acetate, methanol and water in soxhlet apparatus. The individual extracts were collected and concentrated under reduced pressure. Residues were stored in labeled sterile screw capped bottles at 20°C.

Antimicrobial Assay

The yield of hexane, ethyl acetate, methanol and water extracts of the leaf were found to be 3.12, 10.31, 14.20 and 12.44 %(w/w) respectively. Respective solvents were used to prepare a final concentration of 50mg/ml and sterilized by filtration through a 0.45-µm nylon membrane filter. Various extracts of *Callistemon Salignus* leaves were subjected to antimicrobial assay using the cup plate method[10]. Nutrient agar plates were prepared by pouring 20 ml of nutrient agar in sterile Petri dishes for antibacterial assay. Similarly, potato dextrose agar plates were prepared for anti fungal assay. These were allowed to solidify. The bacterial cultures used for assay were 24 hours old whereas fungus cultures were 4 to 5 days old. Concentration of these organisms was prepared to contain approximately 1×10^6 cfu/ml. Sugar tubes containing molten agar (10 ml) were sterilized and cooled to about $40\text{-}42^0$ C. The tubes were then inoculated with 0.1 ml of the appropriate culture suspension of bacterium or fungus, mixed gently and poured onto previously solidified nutrient agar or potato dextrose agar plates, respectively. After setting, a cup borer (6 mm diameter) was properly sterilized by flaming and used to make four uniform cups in each Petri dish. The cups were then filled with different *Callistemon Salignus* leaf extracts and allowed to diffuse for 45 minutes. The solvents used for extraction were analyzed similarly as control. Ciprofloxacin (10µl/disc) and amphotericin-B (100units/disc) were used as standards for bacteria and fungi, respectively. The plates were incubated at 37^0 C for 24 hours. At the end of the period, inhibition

zones formed on the medium were evaluated in mm using a scale. The experiment was carried out in triplicates.

Statistical analysis

Analysis of variance (ANOVA) and Duncan's test were carried out using SPSS package for statistical analysis.

RESULTS

Table 1: Antimicrobial activity of water, methanol, ethyl acetate and hexane extracts of *Callistemon Salignus* leaves (zone of inhibition in mm)

Microorganisms	Standard	Water extract	Methanol extract	Ethyl acetate extract	Hexane extract	F values
B. subtilis	33.67^{b} ± 0.88	8.33^{a} ±0.33	9.00^{a}± 0.58	9.00^{a}± 0.58	8.67^{a}± 0.33	372.73*
S. aureus	19.33^{d}± 0.67	9.33^{a}±0.33	8.33^{a}± 0.33	12.33^{b}± 0.33	15.33^{c}± 0.33	114.19*
M. luteus	12.33b± 0.33	- -	8.67^{a}± 0.33	8.67^{a}± 0.33	13.67^{c}± 0.33	319.38*
S. marcenscens	33.67^{c}± 0.88	- -	10.00^{a}± 0.58	11.67^{a}± 0.33	20.67^{b}± 0.88	400.92*
P. aeruginosa	27.00^{c}± 0.58	- -	11.00^{a}± 0.58	12.00^{a}± 0.58	19.67^{b}± 0.88	288.34*
B. megaterium	35.00^{d}± 0.58	- -	10.67^{a}± 0.33	13.00^{b}± 0.58	16.33^{c}± 0.33	913.44*
E. coli	23.33^{d}± 0.33	- -	8.67^{a}± 0.33	10.33^{b}± 0.33	16.67^{c}± 0.33	866.00*
P. valgaris	23.67^{c}± 0.88	- -	9.33^{a}± 0.33	10.67^{ab}± 0.33	12.00^{b}± 0.58	267.42*
Yeast	14.33^{c}± 0.33	- -	8.33^{a}± 0.33	10.00^{b}± 0.58	35.33^{d}± 0.88	654.87*
R. oligosporus		- -	8.67^{a}± 0.33	11.00^{b}± 0.58	22.00^{d}± 0.58	295.75*
A. niger	14.00^{c}± 0.58	- -	10.00^{a}± 0.58	8.33^{b}± 0.33	16.33^{d}± 0.33	222.94*

Values are the mean of three tests ± SEM

a-d Mean values with the same superscript within a row do not differ significantly (p>0.05).

*** Indicate significant difference (p<0.05).**

The in vitro antimicrobial activity of water, methanol, ethyl acetate and hexane extracts of dried Callistemon Salignus leaves are shown in Table 1. The solvents used to prepare extracts showed no activity. Water extract showed activity against B.subtilis (8.33 mm) and S. aureus (9.33 mm) only whereas other three extracts showed active against all tested microorganisms. All the four extracts did not show significantly different activity against B. subtilis. Ethyl acetate (12.33mm) and hexane extracts (15.33 mm) showed

significantly ($p<0.05$) higher activity compared to the water (9.33 mm) and methanol (8.33 mm) extracts against S. aureus. Activity of methanol, ethyl acetate and hexane extracts against M.luteus, S. marcenscens and P. aeruginosa were found to be 8.67, 8.67 and 13.67; 10.00, 11.67 and 20.67; 11.00, 12.00 and 19.67 mm,respectively. In the case of M. luteus, S.marcenscens and P. aeruginosa, methanol and ethyl acetate extracts

activity did not differ significantly but these were found to be significantly lower ($p<0.05$) compared to activity of hexane extract. Methanol, ethyl acetate and hexane extracts zones of inhibition for B.megaterium and E. coli were 10.67, 13.00 and16.33; 8.67, 10.33 and 16.67 mm, respectively,which were significantly ($p<0.05$) different for

each other. P. valgaris, ethyl acetate extract (10.67 mm) activity was not significantly different compared to methanol extract (9.33 mm) and hexane extract (12.00 mm) but hexane extract gave significantly higher activity compared to methanol extract. In the case of bacterial species, standard ciprofloxacin (10 µl/disc) showed significantly higher ($p<0.05$) activity compared to all the extracts except hexane extract against M. luteus. Zones of inhibition was found to be 8.33, 8.67 and 10.00 mm for methanol extract, 10.00, 11.00 and 8.33 mm for ethyl acetate extract and 35.33, 22.00 and 16.33mm for hexane extract against yeast, R.oligosporus and A. niger, respectively. Hexane extract showed a significantly higher ($p<0.05$) activity compared to methanol and ethyl acetate extract as well as standard amphotericin B (100units per disc) against yeast and fungi.

DISCUSSION

Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots, or fruits have been reported by many workers [3,6,11]It is worthwhile to note that there are no data in the literature to indicate previous investigation of the antimicrobial activity of Callistemon Salignus leaves for comparison. Water extract showed activity against only two microorganisms among the selected microorganisms. Methanol and ethyl acetate extracts have more or less similar activities but hexane extract showed significantly higher activity compared to other extracts against most microorganisms tested. The results of present study indicate that the Callistemon Salignus leaf extracts have inhibitory activities against microorganisms, although their antibacterial activities are lower than that of the standard (ciprofloxacin). However, in the case of fungi, hexane extract had significantly higher activity than the standard (amphotericin-B). The results of the present work indicate that Callistemon Salignus leaf extracts may be an ideal for further research into their uses for food preservation as well as pharmaceutical and natural plant-based products.

REFERENCES

1. M.J.Kao, "Encyclopedia of Chinese Material Medica". Shin Wen Feng Press, Taibei, China, 1980.
2. N. Tomoko, A. Takashi, T. Hiromu, I .Yuka, M. Hiroko, I. Munekaju, T. Totshiyuki, I. Tetsuro,A. Fujio ,I. Iriya,N.Tsutomu and W. Kazuhito, J. Health Sci,Vol.48.Year 2002, Page- 273.
3. M.M .Cowan, Clin. Microbiol. Rev. Vol.12.Year 1999,Page-564.
4. Z.U.Shariff , "Modern Herbal Therapy for Common Ailments". Nature Pharmacy Series,Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited, United Kingdom,Vol.1. 2001, pp 9-84.
5. R.V. Tauxe, Dairy Food Environ. Sanit.Vol.17.Year1997,Page-788.
6. G.J Nychas , Natural antimicrobials from plants. In New Methods of Food Preservation; Gould, G. W., Ed.;Blackie Academic and Professional: London,United Kingdom, 1995; pp 58-59.
7. E.J Smid and L.G.M Gorris,In "Handbook of Food Preservation". Rahman, M. S., Ed.; Marcel Dekker: New York, 1999, pp.285.
8. T.Y Stead and G. Butler, Your Australian Garden, No 5 – Callistemon and other bottle brushes D. G. Stead memorial wildlife research foundation, 1983.
9. W.RElliot and D.L Johnes, Encyclopedia of Australian plants, Vol. 2. Year 1982.
10. 10.P.R. Murray ,E.J Baron , M.A Pfaller ,F.C Tenover and H.R Yolken , "Manual of Clinical microbiology", 6 th Ed, ASM Press, Washington DC, 1995,pp. 15-18.
11. 11.J.L. Mau, C.P.Chen and P.C. Hsieh , J. Agric. Food Chem.Vol.49.Year 2001,Page- 183-188.