IN VITRO ANTIMICROBIAL ACTIVITY OF CALLISTEMON SALIGNUS LEAVES

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Received: 22 March 2013, Revised and Accepted: 2 April 2013

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. 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. Plants can possess antimicrobial natural products to protect themselves from microbial infection and deterioration. 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. In recent years, concern over pathogenic and spoilage microorganisms in foods has increased due to the increase in outbreaks of food borne disease. 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. 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 anti-bacterial, anti mycobacterium tuberculosis properties and insecticidal activities. The phytochemical studies revealed the presence of C – methyl flavonoids, lipid and butilnic acid. Therefore, the present 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-TechI/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 Sabouraund agar medium in the laboratory and maintained on the same medium. R. oligoporus 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. 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 x 10^8 cfu/ml. Sugar tubes containing molten agar (10 ml) were sterilized and cooled to about 40-42°C. The tubes were then inoculated with 0.1 ml of the appropriate culture suspension of bacteria 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 and filled with different Callistemon Salignus leaf extracts and allowed to diffuse for 45 minutes. The solvents used for extraction were analyzed similarly as control. Ciprofloxaclin (10µl/disc) and amphotericin-B (100units/disc) were used as standards for bacteria and fungi, respectively. The plates were incubated at 37°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

The in vitro antimicrobial activity of water, methanol, ethyl acetate and hexane extracts of *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 (12.33 mm) and hexane extracts (15.33 mm) showed significantly (p<0.05) higher activity compared to methanol extract. In the case of bacterial 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**