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SHORT COMMUNICATION OPEN ACCESS

Antimicrobial and cytotoxic activities of the crude extracts of *Callistemon linearis*

Ayesha Haque[1], M. Mahboob Ali Siddiqi[2], A. F. M. Mustafizur Rahman[1], A. M. Sarwaruddin Chowdhury[1*], Choudhury M. Hasan[3]

[1]*Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering and Technology, University of Dhaka, Dhaka-1000, Bangladesh*

[2]*Institue of Natural Sciences, United International University, Dhaka-1209, Bangladesh*

[3]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh*

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Abstract

The petroleum ether (PE), carbon tetrachloride (CT) and ethyl acetate (EA) soluble fractions of a crude methanol extract of *Callistemon linearis* and crude methanol (ME) extract itself were subjected to antibacterial and antifungal activities and cytotoxicity against brine shrimp nauplii. Present investigation revealed that the PE, CT and ME extracts have the potent to moderate sensitivity against almost all the bacteria and fungi, whereas crude EA extract showed only mild antimicrobial activity. The brine shrimp lethality with LC_{50} values were 8.16, 14.21, 0.42, 54.07 and 0.33µg/mL for PE, CT, ME, EA extracts and Vincristine sulphate (VS), respectively. Compare to Vincristine sulphate (standard), the cytotoxicity exhibited by the crude methanol (ME) extract was promising and this clearly indicates the presence of potent bioactive compounds in *Callistemon linearis*.

* **Corresponding Author:** A. M. Sarwaruddin Chowdhury ✉ sarwar@du.ac.bd

Introduction

Antibacterial therapy was developed in the first half of the last century. The development of antimicrobial resistance in many bacterial and fungal organisms 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 (Osuala *et al.,* 2005). *Callistemon linearis* (Bengali name-Brushful; Family-*Myrtaceae*) is a beautiful evergreen shrubs and small trees with 34 species. They are commonly known as bottle brushes because of their cylindrical brush like flowers resembling a traditional bottle brush. They are found in the more temperate regions of Australia and seven species of *callistemon* have been introduced in India as an ornamental tree (Kanjilal and Das, 1992). Although several species of *Callistemon* have been investigated from phytochemical and pharmacological point of view, until now a little attention was given to *Callistemon linearis* and in particular, no study had been conducted yet in Bangladesh. Therefore, an attempt has been taken to investigate the chemical constituents and biological activities of *Callistemon linearis*. Previous phytochemical studies of different *callistemon* species revealed the presence of different monoterpenes, sesquiterpenes and flavonoids (Chowdhury *et al.,* 2012). However, the leaf extracts of *Callistemon linearis* were found to contain carbohydrate, glycoside, flavonoids, saponin, phytosterol, phenolic compounds and at the same time, four components namely *n*-Dec-3-ene; 3-carene; 1,8-cineol and gamaterpinine were isolated from the volatile oil of *Callistemon linearis* DC leaf (Das *et al.,* 2009). Antibacterial, antifungal and antioxidant activities of methanolic extract obtained from *Callistemon linearis DC* Leaf have been studied by an Indian researcher (Das *et al.,* 2008). Very recently, we have also reported on the isolation of betulinic acid and 2,3-dihydroxy Olean-12-en-28-oic acid from the methanol extract of the leaves of *Callistemon linearis* (Haque *et al.* 2013). As an extension of the previous study, present communication explores the antibacterial, antifungal and cytotoxic activities of leaves of *Callistemon linearis*.

Materials and methods

Plant materials

Fresh leaves of *Callistemon linearis* was collected from Chittagong BCSIR. A voucher specimen has been deposited in the Bangladesh National Herbarium, Dhaka (DACB-35514) for identification. The leaves of the plant were cut into small pieces and air dried for several days. The pieces were then dried in oven for 24 hours at 40 °C for better grinding. The oven dried leaves were then ground into a coarse powder.

Extraction and isolation

About 600 gm of the coarse powder was extracted with methanol (ME) at room temperature with occasional shaking and stirring, which was then filtered through Whatman No.1 filter paper. The filtrate was then concentrated at 50 °C in a rotary evaporator and a dry mass of methanol extract (40.6 gm) was obtained. A portion of the methanol extract (23 gm) was subjected to Vacuum Liquid Chromatography (VLC) for rapid fractionation with a number of organic solvents such as, petroleum ether, carbon tetrachloride and ethyl acetate of increasing polarity. As a result, crude extracts of petroleum ether (PE), carbon tetrachloride (CT) and ethyl acetate (EA) were obtained.

Antimicrobial tests

The antimicrobial activity of the crude extracts was determined by the disc diffusion method (Bauer, *et al.,* 1966) against ten bacteria (5 Gram positive and 5 Gram negative) and three fungi, collected from the stock cultures of the Institute of Nutrition and Food Science, University of Dhaka. The crude extracts (PE, CT, ME and EA) were dissolved separately in chloroform and applied to sterile filter paper discs at a concentration of 400µg/disc and carefully dried to evaporate the residual solvent. Standard disc of Ciprofloxacilin (400µg/disc) and blank discs (impregnated with chloroform followed by evaporation) were used as positive and negative

controls, respectively. The antimicrobial activity of the test samples was determined by measuring the diameter of zone of inhibition in millimeter and listed in Table 1.

Brine shrimp lethality test

Brine shrimp lethality bioassay technique of Meyer (Meyer *et al.,* 1982) was applied for the determination of cytotoxic property of the plant extracts of *Callistemon linearis*. The crude petroleum ether (PE), carbon tetrachloride (CT), methanol (ME) and ethyl acetate (EA) extracts were separately dissolved in DMSO. Four mg of each of the crude extracts (PE, CT, ME and EA) was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.78125 µg/mL were obtained by serial dilution technique. Vincristine sulphate (VS) and DMSO were used as the positive and negative control, respectively. The median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration and the results are shown in Table 2.

Table 1. Antimicrobial activity of the plant extracts of *Callistemon linearis*.

Test Microorganisms	Diameter of zone of inhibition (mm)				
	PE	CT	ME	EA	CIP
Gram positive bacteria					
Bacillus cereus	14	13	18	-	40
Bacillus megaterium	20	20	21	-	42
Bacillus subtilis	20	19	20	10	42
Staphylococcus aureus	20	20	20	10	42
Sarcina lutea	18	17	20	10	42
Gram negative bacteria					
Vibrio mimicus	19	18	21	9	41
Escherichia coli	20	13	20	10	40
Shigella dysenteriae	18	15	20	10	41
Pseudomonas aeruginosa	18	12	18	10	40
Shigella boydii	18	18	20	10	40
Fungi					
Sacharomyces cerevacae	18	17	20	10	43
Candida albicans	18	17	20	10	41
Aspergillus Niger	18	17	20	10	41

A diameter less than 9 mm was considered as inactive; PE: crude petroleum ether extract; CT: crude carbon tetrachloride extract; ME: crude methanol extract; EA: crude ethyl acetate extract; CIP: ciprofloxacin; "-" indicates no activity

Results and discussion

As plants produce secondary metabolites in order to protect themselves from microorganisms, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids and triterpenoid are producing a better opportunity for testing wide range of microorganism (Seyydnejad *et al.,* 2010).

In the present study a variety of gram positive and gram negative bacteria and fungi were selected for screening antimicrobial effects of *Callistemon linearis* leaf. The result of this study showed that

(Table-) the petroleum ether (PE), carbon tetrachloride (CT) and methanol (ME) extracts have the moderate to potent sensitivity against almost all the test bacteria and fungi, whereas crude ethyl acetate (EA) extract showed mild antimicrobial activity (except against *B. cereus* and *B. megaterium*). The average zones of inhibition produced by PE, CT, ME and EA crude extracts were found to be 14-20mm, 12-20mm, 18-21mm and 9-10mm, respectively at a concentration of 400µg/disc. The EA extract was found to show weaker antimicrobial effect than the other extracts, it could be because of the difference between extracted compounds in the various extracts. The petroleum ether extract showed the highest activity against the growth of *B. megaterium, B. subtilis, S. aureus* and *E. coli* having the zones of inhibition of 20mm. Besides this, other microorganisms were moderately inhibited (18-19mm) except *B. cereus* (14mm). The carbon tetrachloride (CT) extract showed potent activity against *B. megaterium and S. aureus* (20mm) and poor activity against *P. aeruginosa* (12mm), *B. cereus* and *E. coli* (13mm) whereas, moderate activity against rest of the test organisms (15-18mm). At the same time, the methanol (ME) extract showed promising activity against all the microorganisms (18-21mm), however, the highest activity of ME extract was against *B. megaterium* and *V. mimicus* (21mm). On the other hand, the ethyl acetate (EA) extract showed mild activity against all the test bacteria and fungi (9-10mm). Based on the above results it can be said that *Callistemon linearis* is an effective antimicrobial plant that can be used for folk medicine and will be a good source for finding new antimicrobial agents in order to treat and control infections.

Bioactive compounds are always toxic to living body at some higher doses. Thus *in vivo* lethality of a simple zoological organism can be used as a convenient monitor for screening in the discovery of new bioactive compounds (Hedrick, 1972). Such lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal and antitumor activities of any compound. Following the procedure of Meyer bioassy (Meyer *et al.,* 1982), the lethality of the petroleum ether (PE), carbon tetrachloride (CT), methanol (ME) and ethyl acetate (EA) crude extracts to brine shrimp were evaluated on *A. salina* (Meyer *et al.,* 1982) after 24 hours of exposure the samples and vincristine sulphate (VS). Test samples showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase of concentration of each sample. The LC_{50} were found to be 8.16, 14.21, 0.42, 54.07 and 0.33µg/mL for PE, CT, ME, EA extracts and VS, respectively. The cytotoxicity exhibited by the crude extracts was much significant (Table-2). However, the cytotoxicity exhibited by methanol (ME) extract was comparable to standard vincristine sulphate and indicates that it might have antitumour or pesticidal compounds.

Table 2. Brine shrimp lethality of the crude extracts of Callistemon linearis.

Sample	LC_{50} (µg/mL)
VS	0.33
PE	8.16
CT	14.21
ME	0.42
EA	54.07

VS: vincristine sulphate (Std.)

In conclusion, the present investigation showed that the leaf extracts of *Callistemon linearis* have significant antimicrobial and cytotoxic activities and further bioactivity guided chemical investigations are required to isolate the molecules that are responsible for bioactivities.

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