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Short Communication

Antimicrobial Activity of Crude Extract from *Calycopteris floribunsa*

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Petroleum ether, chloroform, ethyl acetate and ethanol extracts of *Calycopteris floribunda* leaves were investigated for their antimicrobial activities against ten human pathogenic bacteria and five phytopathogenic fungi using disc diffusion and poisoned food method respectively. The extracts showed good antibacterial and antifungal activity against the organisms tested herein. The chloroform extract exhibited the largest zone of inhibition (35 mm in diameter with 2 mg/disc extract) against *Pseudomonas aeruginosa*, and the highest inhibition of fungal radial mycelial growth (71.5% with 100 mg extract/ml medium) was recorded against *Alternaria alternata* using the same extract. The minimum inhibitory concentration (MIC) values were determined by broth macrodilution method and the lowest was recorded against *P. aeruginosa* (MIC 250 μg/ml) among the bacterial isolates and *A. alternata* (125 μg/ml) among the fungal isolates. The crude chloroform extract was found to be active against most of the fungal pathogens. So it may be used as a novel antimicrobial agent for controlling a number of pathogens.

Keywords: Antimicrobial activity, *Calycopteris floribunda*, leaf extract

Microorganisms have developed resistant to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases[1]. This situation forced scientists to search for new antimicrobial substances from various sources, such as medicinal plants. Various plant species have been serving as the best natural source of drugs and medicines from the beginning of civilization. Among the different plant derived, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity[2-5]. So, there is a continuing need for new antibacterial and antifungal agents since none of the available drugs is free from adverse effects and limitation. But medicinal plants possess various remedial properties with worthless materials and it is important to separate the worthless materials from the good. Much of the work has been done in India and other countries. But a very little work is done in this field from Bangladesh.

Calycopteris floribunda Lam. is a large diffuse or scandent shrub with yellowish green flowers belongs to the family Combretaceae, growing in Sylhet and Chittagong (Bangladesh). Leaves principally contain tannin, flavanol, calycopterin, calycopterin methyl ester and oxymethyl calycopterin[6]. Juice of young twig is used diarrhoea, dysentery, malarial fever and colic[6].

Leaves of *C. floribunda* were collected in fresh condition from Chittagong University campus, Chittagong, Bangladesh, in June 2006. The collected and cleaned samples were cut into small pieces (1-2 cm), dried in air to make it suitable for grinding. The samples were ground to fine powder mechanically and then 50 g of the dried powders were kept steeped 72 h in petroleum ether, chloroform, ethyl acetate and 95% ethanol. The extracts thus obtained were filtered, centrifuged at 5,000 rpm for 20 min and then concentrated to a gummy material under reduced pressure at 50°C by rotary vacuum evaporator. The crude extracts were then tested for antibacterial activity against ten human pathogenic bacteria, *viz.*, *Shigella dysenteriae* AE 14396, *S. sonnei* CRL.(ICDDR,B), *Salmonella typhi* AE 14612, *S. paratyphi* AE 14613, *Bacillus subtilis* BTCC 17, *B. cereus* BTCC 19, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* CRL(ICDDR'B), *Escherichia coli* ATCC 25922 and *Vibrio cholerae* AE 14748, and five phytopathogenic fungi, *viz.*, *Alternaria alternata* (Fr.) Kedissler., *Curvularia lunata* (Wakker) Boedijn, *Colletotrichum corchori* Ikata (Yoshida), *Fusarium equiseti* (Corda) Sacc. and *Macrophomina phaseolina* (Maubl) Ashby.

The *in vitro* sensitivity of the bacteria to the leaf extracts was done by disc diffusion method[7] using Mueller-Hinton agar medium and filter paper discs of 4 mm in diameter with 20 μl of 10% (2,000 μg/disc) the crude extracts. All the results were compared with the standard antibacterial antibiotic ampicillin (20 μg/disc, BEXIMCO Pharma Bangladesh Ltd, Dhaka). The *in vitro* antifungal activity of the crude extracts was determined by poisoned food technique[8] using 10% ethanolic solution (w/v) of the extracts mixed with sterilized melted potato dextrose agar (PDA) medium to obtain the desired concentration (100 μg/ml). All the results were compared with the standard antifungal antibiotic nystatin (100 μg/ml medium, BEXIMCO Pharma

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Bangladesh Ltd, Dhaka). The minimum inhibitory concentration (MIC) of the crude extracts was determined by broth macrodilution method[9]. The crude extracts were dissolved in 30% dimethyl sulfoxide (DMSO) to obtain 10% (w/v) solution.

The crude extracts (petroleun ether extract, chloroform extract, ethyl acetate extract and ethanol extract) obtained from *Calycopteris floribunda* were screened for their antibacterial activity against eleven human pathogenic bacteria and compared to that of standard antibacterial antibiotic ampicillin. The results of the sensitivity test and the MIC values are presented in Table 1. Highest zone of inhibition (14 to 35 mm) was observed with the chloroform extract using a concentration 2 mg/disc against all the bacterial strains tested herein and the largest zone of inhibition (35 mm) was found against *P. aeruginosa*. By comparison, the standard ampicillin disc (20 µg/disc) showed inhibitory activity against all the bacterial isolates with the zone of inhibition varied between 14 mm (against *E. coli* and *P. aeruginosa*) and 22 mm (against *S. dysenteriae*).

The chloroform extract also exhibited comparatively the lowest MIC values (250-1000 µg/ml) and the lowest MIC (250 µg/ml) was recorded against *P. aeruginosa* (Table 1). The crude ethanolic extract of leaves of *C. floribunda* exhibited moderate inhibitory activity (zone diameter 14 to 21 mm) with the largest zone of inhibition against *Shigella sonnei*. On the other hand, the petroleum ether extract and the ethyl acetate extract showed lower inhibitory effect against the test bacteria. Both extract were highly active against *Bacillus subtilis*. Similar antibacterial activity of other plant extracts has been reported previously[10-12].

The antifungal activity of the crude extract against five phyto-pathogenic fungi were studied and compared to that of standard antifungal antibiotic nystatin. The results of the inhibition of fungal radial mycelial growth and MIC values are summarized in Table 2. All the extracts inhibited the radial mycelial growth of all the test fungi at a concentration of 100 µg/ml medium to varying degrees, ranging from 13.0-30.5%, 40.0-71.5%, 22.5-49.5% and 29.0-48.0% respectively with petroleum ether extract, chloroform extract, ethyl acetate extract and methanol extract respectively. The chloroform extract exhibited comparatively the better activity against the fungi tested herein and the highest inhibition (71.5%) of fungal radial mycelial growth was recorded against *A. alternata*. Antifungal antibiotic nystatin (100 µg/ml medium) exhibited inhibitions (40.5-75.0%) of radial mycelial growth of all the five fungi, but it was much less active against *A. alternata* and *C. corchori* compared to that of chloroform extract. The MIC values of the chloroform extracts exhibited comparatively lower MIC values (125-750 µg/ml) and the lowest MIC value (125 µg/ml) was recorded against *A. alternate*. Similar antifungal activities on plant extracts of other plants have also been previously reported[13-15].

Table 1. *Antibacterial activity of crude leaf extracts from Calycopteris floribunda*

Bacterium	Diameter of zone inhibition (mm)[a] / MIC value (µg/ml)[b]			
	Petroleum ether	Chloroform	Ethyl actate	Ethanol
Bacillus subtilis	15 / 1,000	24 / 500	18 / 750	14 / 750
Bacillus cereus	12 / 1,500	14 / 1,000	14 / 1,000	14 / 1,000
Staphylococcus aureus	13 / 1,000	21 / 500	13 / 1,250	20 / 1,000
Escherichia coli	13 / 1,250	24 / 500	16 / 750	19 / 1,000
Vibrio cholerae	11 / 1,250	25 / 500	11 / 1,500	17 / 1,250
Shigella dysenteriae	11 / 1,500	20 / 500	11 / 1,500	20 / 1,000
Shigella sonnei	13 / 1,000	25 / 500	15 / 1,000	21 / 750
Salmonella typhi	13 / 1,500	17 / 750	12 / 1,250	20 / 750
Salmonella paratyphi	13 / 1,250	25 / 500	12 / 1,250	18 / 750
Pseudomonas aeruginosa	14 / 1,250	35 / 250	16 / 1,000	18 / 1,000

[a]Determined by disc diffusion method using crude extract (2 mg/disc); [b]The minimum inhibitory concentration (MIC) was determined by broth macrodilution method.

Table 2. *Antifungal activity of the crude extracts from Calycopteris floribunda*

Fungus	Inhibition of radial mycelial growth (%)[a] / MIC (µg/ml)[b]			
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol
Alternaria alternata	29.0 / 750	71.5 / 125	34.0 / 500	33.5 / 750
Curvularia lunata	30.5 / 750	62.0 / 250	32.0 / 750	48.0 / 250
Colletotrichum corchori	17.0 / 1,000	66.0 / 250	33.0 / 750	29.0 / 750
Fusarium equiseti	13.0 / 1,000	41.5 / 500	49.5 / 250	34.5 / 500
Macrophomina phaseolina	22.0 / 750	40.0 / 500	22.5 / 750	45.0 / 250

[a]Inhibition of radial mycelial growth was determined by poisoned food technique using crude extract (100 µg/ml culture medium); [b]The minimum inhibitory concentration (MIC) was determined by broth macrodilution method.

Our results could stimulate further pharmacological studies seeking pure antimicrobial agents from the plant resources. The present investigation confirms that there are antibacterial and antifungal properties in the crude extract of *C. floribunda* leaf. Further study is necessary to identify the active compound(s) and tested for bioactive and antimicrobial activities.

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