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Original Article

In vitro Antibacterial and Antifungal Activity of *Borreria articularis*

Razia Sultana[1], M Shafiqur Rahman[1], M Nazrul Islam Bhuiyan[2], Jaripa Begum[2] and M Nural Anwar[1*]

[1]Department of Microbiology, University of Chittagong, Chittagong 4331, Bangladesh, [2]Bangladesh Council of Scientific & Industrial Research (BCSIR), Chittagong Cantonment, Chittagong 4220, Bangladesh

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Petroleum ether extract, chloroform extract, ethyl acetate extract, ethyl alcohol extract and a pure compound 6-methyl-5-cyclodecen-1-ol obtained from aerial parts of *Borreria articularis* were studied for their antimicrobial activities against eleven human pathogenic bacteria and four human pathogenic fungi using disc diffusion and poisoned food method respectively. Ethyl acetate extract, ethanol extracts and the pure compound 6-methyl-5-cyclodecen-1-ol exhibited good antibacterial and antifungal activity against all the pathogens tested herein. The ethanol extract and 6-methyl-5-cyclodecen-1-ol exhibited the largest zone of inhibition (20 mm) at a concentration of 2,000 µg/disc against *Escheichia coli* and *Vibrio cholerae* respectively. In case of fungi, the crude extract of ethanol and the pure compound exhibited the highest inhibition 53.5 and 52.0% of fungal radial mycelial growth (with 100 µg/ml medium) against *Aspergillus ustus* and A. ochraceus respectively. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the pure compound 6-methyl-5-cyclodecen-1-ol were determined by broth macrodilution method. The lowest MIC (500 µg/ml) and MBC (1,000 µg/ml) were determined against *V. cholerae*. However, for fungi, the lowest MIC (750 µg/ml) and MFC (1,500 µg/ml) were recorded against *A. ochraceus*. The results suggest that active antimicrobial agent(s) present in the extracts of *B. articularis* may have potential for the treatment of bacterial and fungal infections.

Keywords: Antimicrobial activity, *Borreria articularis*, Crude extracts, 6-Methyl-5 cyclodecen-1-ol

Introduction

Plants are still widely used in ethnomedicine around the world. In modern China, the traditional medicine based principally on the use of herbs is used widely as a significant instrument of health care. Microorganisms have developed resistance 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. Secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity[2-4]. 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. Now-a-days, the natural products have been accepted as important sources of biologically active (antimicrobial) substances and the major sources of which are still left undiscovered. But a few works have been done in this field in Bangladesh.

Borreria articularis (Linn. f.) Will. (Beng. Madnabata kadu) is an annual, procumbent herb with opposite leaves, small flowers and dehiscent fruits of two coriaceous mericarps, which belongs to the family Rubiaceae. It grows everywhere in Bangladesh as a weed in cultivated fields. Plant principally contains alkaloids, glycosides, sterols, D-mannitol and ursolic acid[5]. The plant is used in ophthalmia, inflammation of eye and gums, blindness, carache, fever, spleen complaints, sore, conjunctivitis, haemorrhage, gallstones, dysentery and diarrhoea[5]. In the light of the above information, the present investigation was undertaken which deals with the studies of the extracts and pure compound of *B. articularis* against various pathogenic bacterial and fungal strains, the results of which are being reported in the present communication.

Materials and Methods

Collection and extraction of plant material

Arial parts of *Borreria articularis* were collected in fresh condition from Chittagong University campus, Chittagong, Bangladesh. 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 20 g of the dried powder were kept steeped 72 h in petroleum ether, chloroform and ethyl acetate separately. On the other hand 250 g of the dried powder were kept steeped 72 h in ethyl alcohol. The extracts thus obtained separately were filtered, centrifuged at 2,000 rpm for 20 min and concentrated to a gummy material under reduced pressure at 50°C by rotary vacuum evaporator. The gummy materials were then collected in a small vial, dried as usual. Thus crude extracts were obtained.

Purification of crude extract

Solvent-solvent partitioning of concentrated crude ethanol extract was done using the protocol designed by Kupchan and modified

* ***Corresponding author:***
Dr M Nural Anwar, Professor, Department of Microbiology, University of Chittagong, Chittagong 4331, Bangladesh
Tel (Office): (031) 682031-39/4464; Tel (Home): (031) 681688; Fax: +880 (031) 726310; E-mail: anwarmn54@yahoo.com

by Wagenen[6]. All the fractions were collected separately, dried as usual and tested for any of their antimicrobial efficacy. Of these, only the chloroform extract fraction showing high antimicrobial activity was subjected to fractionation by column chromatography using silica gel (200-300 mesh) as the adsorbent. From it a total of 21 fractions were collected separately using mixtures containing different proportions of ethyl acetate and methanol with increasing polarity as the eluants. The column-separated fractions thus obtained were examined using thin layer chromatography (TLC) technique and ultraviolet light (254 and 366 nm) to detect the presence of any fluorescent compound. The R_f-values for each were measured as usual. Fractions showing same R_f-value were mixed together and grouped into six fractions (A-F).

Selection and identification of active fraction

All the fractions (A to F) were tested against three sensitive bacteria (*Escherichia coli, Bacillus cereus* and *Salmonella typhi*) and a fungus (*Aspergillus ochraceus*). Of these, the highly antibacterial and antifungal fraction B, eluted in petroleum ether and ethyl acetate mixture of 20: 80, yielded 1.3 g of brownish material.

GC-MS analysis

The pure compound B isolated from ethanolnic extract of *B. articularis* was analyzed by gas chromatography-mass spectrometry (GC-MS) electron impact ionization (EI) method on GC-17A gas chromatograph (Shimadzu, Japan) coupled to a GC-MS QP 5050A Mass spectrometer (Shimadzu, Japan); fused silica capillary column (30 m x 2.5 mm; 0.25 µm film thickness), coated with DB-5 ms (J&W); column temperature 100°C (2 min) to 250°C at the rate of 3°C/min; carrier gas, helium at constant pressure of 90 kPa. Acquisition parameters full scan; scan range 40-350 amu. Sample was injected by splitting and the split ratio 1:20.

Identification of the compounds

Compound identification was done by comparing the NIST library data of the peaks with those reported in literature, mass spectra of the peaks with literature data . Percentage composition was computed from GC peak areas on DB-5 ms column without applying correction factors.

Test organisms

The crude extracts and pure compound obtained from *Borreria articularis* were tested for their antibacterial activity against eleven 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, *B. megaterium* BTCC 18, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* CRL(ICDDR,B), *Escherichia coli* ATCC 25922 and *Vibrio cholerae* AE 14748, and four human pathogenic fungi, *viz*., *Aspergillus niger* BTCC 504, *A. ochraceus* BTCC 515, *A. ustus* BTCC 503 and *Candida albicans* BTCC 493.

Determination of antimicrobial activity

The *in vitro* antibacterial and antifungal activities of the plant materials were determined by disc diffusion method[7] and poisoned food technique[8] respectively. Mueller-Hinton (agar and broth) medium was used for culture of bacteria and Sabouraud (agar and broth) medium was used for culture of fungi. 5% ethanolic solution of the crude extracts and pure compound were used as the test material. All the results were compared with the standard antibacterial antibiotic Ampicillin and antifungal antibiotic nystatin. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values of the crude extracts and pure compound were determined by macrodilution broth technique[9].

Results and Discussion

The crude extracts (petroleum ether extract, chloroform extract, ethyl acetate extract and ethanol) obtained from *Borreria articularis* were screened for their antibacterial activity against eleven human pathogenic bacteria. The results of the sensitivity test are presented in Table 1. All the crude extracts except petroleum ether extract exhibited good antibacterial activity against at least seven bacterial strains tested. But the ethyl acetate and ethanol extracts showed comparatively the better antibacterial activity against all the eleven bacterial pathogens tested herein. The ethanol extract exhibited the largest zone of inhibition (20 mm) with 2,000 µg/disc extract against *E. coli*. Similar antibacterial activity of other plant extracts has been reported previously[10-12].

Table 1. *Antibacterial activity of crude extracts of Borreria articularis*

Bacterium	Diameter of inhibition zone in mm (Crude extract, µg/disc)							
	Petroleum ether		Chloroform		Ethyl acetate		Ethanol	
	1,000	2,000	1,000	2,000	1,000	2,000	1,000	2,000
Staphylococcus aureus	-	-	-	-	7	9	8	14
Bacillus cereus	-	-	6	7	9	11	10	14
Bacillus megaterium	-	-	-	-	8	9	7	11
Bacillus subtilis	-	7	8	10	10	11	7	10
Escherichia coli	-	-	-	-	9	10	15	20
Vibrio cholerae	-	-	10	12	8	9	9	10
Shigella dysenteriae	-	6	10	11	8	10	8	10
Shigella sonnei	-	-	8	9	10	11	10	11
Salmonella typhi	-	-	-	-	8	10	9	11
Salmonella paratyphi	-	-	7	8	6	7	6	7
Pseudomonas aeruginosa	-	-	7	8	6	7	6	9

After spectroscopic (GC-MS) analysis, the pure compound B isolated from ethanolic extract of *B. articularis* was identified as 6-methyl-5-cyclodecen-1-ol ($C_{11}H_{200}$, molecular weight 168) shown in Figure 1. The antibacterial activity, MIC and MBC values of the pure compound (6-methyl-5-cyclodecen-1-ol) isolated from *B. articularis* are presented in Table 2. It appeared that the pure compound exhibited good antibacterial activity against all the bacterial strains. The largest zone of inhibitions (15 mm and 20 mm in diameter) were recorded against *V. cholerae* with the pure compound at the concentration of 1,000 and 2,000 µg/disc respectively. The pure compound exhibited the MIC and MBC values from 500 to 2,000 µg/ml and 1,000 to 3000 µg/ml respectively. The lowest MIC (500 µg/ml) and MBC (1000 µg/ml) were found against *V. cholerae*. The standard antibacterial antibiotic ampicillin (20 µg/disc) was also found to be active against all the bacteria tested herein. The antibacterial activity of the pure compound from other plants has been reported by other workers[13-15].

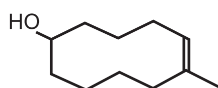

Figure 1. *Structure of 6-methyl-5-cyclodecen-1-ol.*

The antifungal activity of the crude extracts (100 µg/ml medium) against four human pathogenic fungi was studied and the results of the inhibition of radial mycelial growth of fungi are summarized in Table 3. It appeared that the chloroform, ethyl acetate and ethanol extract of *B. articularis* inhibited the radial mycelial growth of all the test fungi at the concentration of 100 µg/ml medium. But petroleum ether extract exhibited inhibition only against *A. ochraceus* and *A. ustus*. The ethyl acetate and ethanol extract exhibited comparatively better antifungal activity than the others. The highest inhibition (53.5%) of fungal radial mycelial growth was recorded against *A. ustus* with ethanol extract at a concentration of 100 µg/ml medium. Similar antifungal activities on plant extracts of other plants have also been previously reported [8,12,16].

The antifungal activity, MICs and MBCs of the pure compound (6-methyl-5-cyclodecen-1-ol) isolated from *B. articularis* are presented in Table 4. From the results, it appeared that the pure compound exhibited good antifungal activity against all the fungal pathogens tested herein. The highest inhibition (52.0%) of fungal radial mycelial growth was recorded against *A. ochraceus* with 6-methyl-5-cyclodecen-1-ol at a concentration of 100 µg/ml medium. The lowest MIC (750 µg/ml) and MFC (1,500 µg/ml) were determined against *A. ochraceus*. Standard antifungal antibiotic nystatin (100 µg/ml) exhibited inhibitions of radial mycelial growth of all the six fungi. The antifungal activities of the pure compound from other plants have also been reported previously[17-20].

From this study, it was found that *B. articularis* has antibacterial and antifungal properties. The pure compound 6-methyl-5-cyclodecen-1-ol isolated from *B. articularis* may be used as a novel natural antibacterial and antifungal agent against a wide variety of infectious pathogens after confirming its cytotoxicity. Earlier Mukherjee *et* ***al.***[21] reported a new triterpene, 3á-acetoxy-oleana-12-en-29-oic acid along with â-amyrin from chloroform extract of the aerial parts and roots of *B. articularis.* Therefore, further phytochemical studies are needed to identify other constituents responsible for antimicrobial activity.

Table 2. *Antibacterial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of pure compound (6-methyl-5-cyclodecen-1-ol) isolated from Borreria articularis*

Bacteriium	Diameter of zone of inhibitions in mm			MIC of pure compound (µg/ml)	MBC of pure compound (µg/ml)
	Pure compound (µg/disc)		Ampicillin (20 µg/disc)		
	1,000	2,000			
Staphylococcus aureus	10	13	22	1,500	2,500
Bacillus cereus	12	15	18	750	1,500
Bacillus megaterium	11	13	16	1,000	2,000
Bacillus subtilis	11	14	19	750	1,500
Escherichia coli	6	9	10	2,000	3,000
Vibrio cholerae	15	20	15	500	1,000
Shigella dysenteriae	6	8	22	2,000	3,000
Shigella sonnei	13	16	20	750	2,000
Salmonella typhi	10	12	20	1,500	2,000
Salmonella paratyphi	7	9	17	1,500	2,500
Pseudomonas aeruginosa	14	17	12	750	2,000

Table 3. *Antifungal activity of the crude extracts from Borreria articularis*

Fungus	Percentage inhibition of fungal mycelial growth (100 µg/ml medium)			
	Petroleum ether	Chloroform	Ethyl acetate	Ethanol
Aspergillus niger	-	28	37	34.5
Aspergillus ochraceus	13.5	32.5	42.5	49
Aspergillus ustus	11	30.5	38.5	53.8
Candida albicans	-	29	45	50

Table 4. *Antifungal activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the pure compound (6-methyl-5-cyclodecen-1-ol) isolated from Borreria articularis*

Fungus	Percentage inhibition of fungal mycelial growth[a]		MIC (mg/ml) of 6-methyl-l-5-cyclodecen-1-ol	MFC (mg/ml) of 6-methy 5-cyclodecen-1-ol
	Pure compound (100 µg/ml)	Nystatin* (100 µg/ml)		
Aspergillus niger	43.5	50.5	1,500	2,500
Aspergillus ochraceus	52	62	750	1,500
Aspergillus ustus	37.5	58	1,500	3,000
Candida albicans	48	42.5	1,000	2,000

**Standard antifungal activity*

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