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In vitro Antibacterial Screening of the Metabolite of a Monocillium Species Isolated from a Soil Sample of Bangladesh

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A *Monocillium* species isolated from a soil sample which grown optimally in Czapek's dox broth (acidic) medium yielded the metabolic compound HB identified as a mixture of novel compounds, Monocillinols A and B. Antibacterial activities of the compound were observed against fifteen Gram positive and Gram negative pathogenic organisms. The compound showed significant antibacterial activities against all pathogenic organisms tested except Gram negative *Shigella sonnei*. The minimum inhibitory concentration (MIC) values of the compound were determined against *Bacillus subtilis*, *Sarcina lutea*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Escherichia coli* and *Salmonella typhi* and found to be 128, 256, 128, 128, 128 and 128μg/ml, respectively.

Key words: *Monocillium* species, antibacterial activity, minimum inhibitory concentration

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Introduction

Infectious diseases like diarrhea, acute respiratory tract infection, tuberculosis and AIDS (UNDP report, 1994) are leading health problems with high morbidity and mortality in the developing countries (Black *et al.*, 1982, Walsh *et al.*, 1994). For these, various types of pathogenic organisms were responsible. Careless and indiscriminate use of antibiotics in many countries cause development of antibiotic resistant strains, which are increasing day by day. The phenomenon makes the research in anxiety and they have still been remaining the research to discover newer, safer and more effective antibiotics to solve the above mentioned problem. It was reported that soil organisms could provide a rich source of antibiotics (Arnold, 1992).

On this background attempts were taken to find highly antagonistic organisms from soil samples such as *Monocillium* species and a few number of these species were isolated and identified in the world. However, the isolated organism when grown in Czapek's dox broth (acidic) medium yielded the antibacterial substance HB, which was latter identified as a mixture of novel compounds, Monocillium A and B (Biswas *et al.*, 2000). In the present paper, we report that *in vitro* antibacterial screening of the compound against a wide variety of Gram positive and Gram negative organisms. Antibacterial screening is undertaken in two phases, a primary qualitative assay to detect the presence or absence of activity and a secondary assay which quantities the relative potency, expressed as minimum inhibitory concentration (MIC) value.

Materials and Methods

Collection of sample: To collect the compound HB, the *Monocillium* species was grown optimally in Czapek's dox broth (acidic) medium (Biswas, 1998). The cultured broth medium was filtered to separate the cultivated strain from the medium. Then the metabolites were isolated by solvent extraction method (Connors, 1982) and were condensed under reduced pressure in rotary evaporator to obtain condensed metabolites. From the condensed metabolites, the compound HB was isolated by different chromatographic methods like thin layer chromatography, column chromatography, preparative thin layer chromatography etc. Finally the compound HB was characterized by UV, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data Biswas *et al.*, 2000 in National Cancer Institute, Frederick, USA. This compound, HB was used to screen antimicrobial activity against various pathogenic organisms.

Antibacterial activity: To carry out the qualitative antibacteial activity of the compound HB, the primary assay method like agar diffusion technique (Beur *et al.*, 1996) was used. Fifteen pathogenic bacteria (six Gram positive and nine Gram negative) were selected. The test organisms were collected from the Department of Microbiology, University of Dhaka, Bangladesh. The test compound was dissolved in methanol to obtaine 500 μg/10 μL solution and 500 μg/disc was used to check the activity. Kanamycin 30 μg/disc was used as standard to compare the activity.

Minimum inhibitory concentration: Minimum inhibitory concentration called the secondary assay was carried out to determine quantitative antibacterial activity of the compound against various pathogenic organisms such as Gram positive *B. subtilis*, *Sa. Lutea* and *St.aureus* and Gram negative *Sh. dysenteriae*, *Es.coli* and *Sal.typhi*.It was carried out by serial broth - dilution technique (Reiner, 1982). The concentration of the organism was 10^{7} cells/ml.

Results and Discussion

The antibacterial activities of the compound HB against six Gram positive and nine Gram negative pathogenic bacteria have examined and the results are shown in Table 1. The compound showed significant activity against all tested pathogenic organisms except *Sh. sonnei*. Although the activity of test sample was comparable with that of Kanamycin, it was 16-17 fold less potent than the later. However, the activity profile of this compound was considerably interesting against *Sal. typhi*, *Es. coli*, *Ps. aeruginosa*, *Sa. lutea*, *St. aureus* etc. as compared to that of the standard Kanamycin.

The MIC values of the compound HB on three Gram positive and three Gram negative organisms are checked and presented in Table 2 and 3, respectively. The values were

Table 1: Results of antibacterial screening of the compound HB and Kanamycin standard Test bacteria Strain number Diameter of Zone of Inhibition (mm)

Test bacteria	Strain numbre	Diameter of zone of Inhibition (mm)	
		Test Sample 500 μg/disk	Kanamycin 03μ/disk
Gram positive			
Bacillus subtilis	QL-40	15	17
Sarcina lutea	QL-166	18	20
Staphylococcus aureus	ATCC-25923	20	25
Bacillus cereus	QL-29	20	22
Bacillus megaterium QL-38		19	18
Strep. β- haemolyticus	CRL	22	19
Gram negative			
Escherichia coli	FPFC-281	20	18
Salmonella typhi	AG-2631	29	26
Shigella dysenteriae AL-35587		9	11
Shigella sonnei	AJ-8992	Nil	19
Shigella flexneri	AL-30372	15	19
Klebsiella sp.		16	18
Pseudomonas aeruginosa	CRL	18	22
Shigella boydii	AL-17313	18	17
Shigella shiga	ATCC-26107	12	12

Table 2: Minimum inhibitory concentration of the compound HB against *B. subtilis*, *Sa. lutea* and *St. aureus*

No. of Test tube	Nutrient Broth medium added (ml)	Diluted solution of the compound HB	Inoculum added (μL)	Observation against *B. Subtilis*	Observation against *Sa. lutea*	Observation against *St. aureus*
1	1	512	10	NG	NG	NG
2	1	256	10	NG	NG	NG
3	1	128	10	NG	G	NG
4	1	64	10	G	G	G
5	1	32	10	G	G	G
6	1	16	10	G	G	G
7	1	8	10	G	G	G
8	1	4	10	G	G	G
9	1	2	10	G	G	G
C_s	1	512	0	NG	NG	NG
C_i	1	0	10	G	G	G
C_m	1	0	0	NG	NG	NG

C_s = Test tube with sample, C_i = Test tube with inoculum, C_m = Test tube with medium, G = Growth, NG = No Growth, No. of Cell = 10^7 cell/ml

Table 3: Minimum inhibitory concentration of the compound HB against *Sh. dysenteriae*, *Es. coli* and *Sal. typhi*

No. of Test tube	Nutrient Broth medium added (ml)	Diluted solution of the compound HB	Inoculum added (μL)	Observation against *Sh. dysenteriae*	Observation against *Es. coli*	Observation against *Sal. typhi*
1	1	512	10	NG	NG	NG
2	1	256	10	NG	NG	NG
3	1	128	10	NG	NG	NG
4	1	64	10	G	G	G
5	1	32	10	G	G	G
6	1	16	10	G	G	G
7	1	8	10	G	G	G
8	1	4	10	G	G	G
9	1	2	10	G	G	G
C_s	1	512	0	NG	NG	NG
C_i	1	0	10	G	G	G
C_m	1	0	0	NG	NG	NG

C_s = Test tube with samples, C_i = Test tube with inoculum, C_m = Test tube with medium, G = Growth, NG = No Growth, No. of Cell = 10^7 cell/ml

found to be 256 μg/ml against *B. subtilis*, *St. aureus*, *Sh. dysenteriae*, *Es. coli* and *Sal. typhi*. In short, our findings concluded that the novel compound monocillinois possess significant inhibitory activity against some pathogenic bacteria. However, the compound awaited further specific studies for better evaluation as an antibacterial agent.

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