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Biological Evaluation of Some Monosaccharide Derivatives

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ABSTRACT

Some acylated derivatives of benzyl 2,3-*O*-isopropylidene-α-D-mannofuranoside and methyl 6-*O*-triphenylmethyl-α-D-glucopyranoside, including the precursors, were employed as test chemicals for *in vitro* antimicrobial functionality test against six human pathogenic bacteria and four plant pathogenic fungi. For comparative study, biological activity of standard antibiotics, Ampicillin and Nystatin, were also carried out. The study revealed that the tested acylated derivatives exhibited moderate to good antibacterial and antifungal activities. It was also observed that the test chemicals were more effective against fungal phytopathogens than those of the bacterial strains. Encouragingly, a good number of test chemicals exhibited better antimicrobial activity than the standard antibiotics employed.

Key Words: Bacteria; Fungus; Standard antibiotic; Antimicrobial activity; Basal medium; Inhibition; Stimulation

INTRODUCTION

Acylated derivatives of monosaccharides are very important due to their effective biological activity (Andary *et al.,* 1982). Generally, it is known that the presence of aromatic and heteroaromatic nucleus in the carbohydrate molecules increases its biological activity markedly (Gupta *et al.,* 1997). Also, if an active nucleus or molecule is linked to another nucleus, the resulting molecule may possess greater potential for biological activity (Gupta *et al.,* 1997; Kabir *et al.,* 2001, 2003). Results of an ongoing research project on selective acylation and antimicrobial evaluation of monosaccharides, revealed that in many cases the combination of two or more heteroaromatic nuclei and acyl groups enhances the biological activity many fold than its parent nuclei. For example, some acylated derivatives of D-glucofuranose were found more active than those of the standard antibiotics (Kabir *et al.,* 2001). Encouraged by the above results, we deliberately synthesized some acylated derivatives of benzyl 2,3-*O*-isopropylidene-α-D-mannofuranoside (1) and methyl 6-*O*-triphenylmethyl-α-D-glucopyranoside (10) containing benzene moiety and various acyl groups (e.g. hexanoyl, octanoyl, myristoyl, palmitoyl, etc) in a single molecular framework. Antimicrobial activities of these compounds (Fig. 1) were evaluated using a variety of bacterial and fungal strains and the results are reported here.

MATERIALS AND METHODS

The test tube cultures of bacterial and fungal pathogens were collected from the Research Laboratory, Department of Microbiology, University of Chittagong. The names of the tested pathogens are given below:

Bacteria

i) *Bacillus cereus* BTCC 19
ii) *Bacillus megaterium* BTCC18 (Gram-positive).
iii) *Escherichia coli* ATCC 255922
iv) *Shigella sonnei* CRL (ICDDR,B)
v) *Pseudomonas species* CRL (ICDDR, B)
vi) *Salmonilla typhi* AE 14612 (Gram-negative).

Fungus

i) *Fusarium equiseti* (corda) Sacc,
ii) *Macrophomina phaseolina* (Maubl) Ashby
iii) *Curvularia lunata* (Wakker Boedign)
iv) *Botryodiplodia theobromae* (pat)

Test chemicals. A number of derivatives of benzyl 2, 3-*O*-isopropylidene-α-D-mannofuranoside (1) (2-9) and methyl 6-*O*-triphenylmethyl-α-D-glucopyranoside (10) (11-18) (Fig. 1) were employed as test chemicals for antimicrobial functionality test. The tested chemicals (Fig. 1, 1-18) were synthesized, isolated and purified in the Organic Research Laboratory, Department of Chemistry, University of Chittagong and reported earlier (Kabir *et al.,* 2001, 2003).

Antibacterial Studies

Preparation of nutrient agar (NA) medium. A suspension of agar powder (15.0 g), beef extract (3.0 g), peptone (5.0 g) and sodium chloride (0.5 g) in distilled water (1000 mL) in a beaker was heated gently with constant stirring. The mixture was boiled until agar powder and the other material were completely dissolved (~ 20 min). The medium was then transferred to a conical flask. The conical flask was then closed with a hand made cotton plug and rapped with aluminium foil. The medium was autoclaved for half an hour at 120°C and 15 psi to destroy the unexpected organisms. After autoclaving, the sterilized medium becomes ready for test. Stock culture of test organisms were

maintained on NA slants and preserved at 10°C. Occasional sub-culture (3/4 weeks intervals) system was also maintained to keep the culture in active condition with character unimpaired. Just before the use of test organism, suspension in sterile distilled water was prepared with 48 hour-old cultures. Inoculum concentration was determined through gradual dilution technique.

Sensitivity analysis. Sensitivity spectrum analysis was done by disc diffusion method (Bauer *et al.,* 1966) with little modification. Sterile paper disc of 4 mm in diameter and sterile petriplate of 90 mm in diameter were used throughout the experiment. Before use, paper discs were dried at 100°C in an oven. Then the discs were soaked with test chemicals (Fig. 1) at the rate of 20 μg (dry weight) per disc for antibacterial analysis. One drop of bacterial suspension was taken in a sterile petridish and then approximately 20 mL of sterilized melted NA (~45°C) was poured into the plate and mixed thoroughly. After solidification of the seeded NA medium, paper disc, after soaking with test chemicals (2% in CH_3OH), were placed at the center on the inoculated pour plate. A control plate was also maintained in each case with CH_3OH.

Firstly, the plates were kept for 24 h at low temperature (4°C) and the test chemicals diffused from disc to the surrounding medium by this time. The plates were then incubated at (35±2°C) for growth of test organisms and were observed at 24 h interval for two days. Antibacterial activity was expressed in terms of diameter of zone of inhibition in mm. Aseptic condition was maintained throughout the experiment. Each experiment was repeated three times. The standard antibiotic, Ampicillin, was used as a positive control and with test chemicals under identical conditions.

Antifungal Studies

Preparation of potato dextrose agar (PDA) medium. Glass petridishes (100 mm in diameter) were sterilized and the melted sterilized PDA medium was poured at the rate of 15-20 mL in each petridish. After solidification of the medium, the small portions of mycelium of each fungus were placed carefully at the center of each plate with the help of sterilized needles. Thus each fungus was transferred to a number of PDA plates.

Efficacy analysis. The antifungal activity (i.e. efficacy) of the test chemicals was assessed by food poisoned technique (Grover & Moore, 1962), in some modified conditions (Miah *et al.,* 1990). Required amount of medium was taken in conical flasks separately and was sterilized in an autoclave (at 120°C & 15 psi). After autoclaving, weighed amount of test chemical was added to this medium in conical flask at the point of pouring to obtain the desired concentration. The flask was shaken thoroughly to mix the chemical with the medium before pouring. The medium with definite amount of chemical (100 μg) was then poured into separate sterilized glass petridishes. Proper control was maintained separately with sterilized PDA medium without chemical and three replicates were prepared for each treatment.

After solidification of medium, the plates were of mycelial blocks (5 mm approx.) of individual test fungus, cut out from the outer margin of the growing cultures on PDA plates. The blocks were then placed at the center of each petridish in an inverted position. All the plates were inoculated at (25±2°C) for 3-5 days.

The linear mycelial growth of fungal colony was measured in two directions at right angle to each other after 3-5 days of incubation and average of three replicates was taken as the diameter of the colony in mm. The percentage inhibition of mycelial growth of test fungi was calculated as follows:

$$I = \frac{C - T}{C} \times 100$$

Where, I = percentage inhibition, C = diameter of the fungal colony in CH_3OH (control), T = diameter of the fungal colony in treatment. The antifungal results were compared with that of the standard antibiotic, Nystatin.

RESULTS AND DISCUSSION

Antibacterial activity studies. The results of antibacterial activity studies of the test chemicals are presented in Table I.

***Bacillus cereus* BTCC 19.** In case of *Bacillus cereus* it was found that chemicals 2, 3, 5 and 6 were more effective than other chemicals used. Inhibition zone of chemicals 10, 1, 4, 9, 11, 13 and 16 were found zero. However, none of the test chemicals showed higher inhibition than the standard antibiotic, Ampicillin.

***Bacillus megaterium* BTCC 18.** Chemicals 3, 6, 7, 15 and 17 showed marked inhibition than that of other chemicals tested. Chemicals 2 and 9 showed poor inhibition; whereas, chemicals 10, 4, 5, 8, 11, 13, 14 and 16 did not show any inhibition.

***Escherichia coli* ATCC 25522.** Compound 6 was found to be highly effective against this bacterium (inhibition zone 24 mm). Compounds 2 and 3 were moderately effective. Rest of the test chemicals did not show any inhibition at all. Encouragingly, in case of this bacterium, compounds 6 and 3 showed higher inhibition (24 mm and 18 mm) than Ampicillin (13 mm).

***Shigella sonnie* CRL (ICDDR,B).** Compound 15 showed maximum inhibition (12 mm) as compared to other compounds like 4, 16 and 17. The rest of the tested compounds did not show antibacterial functionality.

***Pseudomonas species* CRL (ICDDR,B).** Compounds 2 (18 mm) and 11 (16 mm) showed excellent inhibition against the tested organism, almost comparable to Ampicillin (18 mm); whereas, the rest of the synthesized chemicals have no effect against this organism.

Table I. Inhibition zone observed against bacteria

Compound No.	Diameter of inhibition zone in mm.					
	B.cereus	**B. megaterium**	**E. coli**	**S. sonnie**	**P. species**	**S.typhi.**
1	---	---	---	---	---	---
2	10	9	12	---	*18	*21
3	*20	13	*18	---	---	---
4	---	---	---	7	---	---
5	12	---	---	---	---	14
6	*18	12	*24	---	---	*27
7	8	12	---	---	---	---
8	7	---	---	---	---	---
9	---	8	---	---	---	12
10	---	---	---	---	---	---
11	---	---	---	---	*16	14
12	---	---	---	---	---	7
13	---	---	---	---	---	---
14	---	---	---	---	---	---
15	8	10	---	12	---	14
16	---	---	---	7	---	---
17	7	12	---	7	---	*21
18	---	---	---	---	---	---
**Ampicillin 20µg.dw/disc	*22	*19	13	*35	*18	*24

N.B:*= Marked inhibition;**= Standard antibiotic '---' = No inhibition; dw=Dry weigh

***Salmonella typhi* AE 14612.** The palmitoyl derivative (6) showed maximum inhibition (27 mm) against this bacterium which was higher than Ampicillin (24 mm). Compounds 2 (21 mm) and 17 (21 mm) were more effective than that of other compounds like 5 (15 mm), 9 (14 mm), 11 (14 mm), 12 (7 mm) and 15 (14 mm). The remaining tested compounds showed zero inhibition.

From this study we observed that some of the test chemicals showed moderate to marked inhibition against the bacterial pathogens employed. It was found that a good number of test chemicals were more effective than the standard antibiotic, Ampicillin and some showed comparable activity. It was also observed that some chemicals were unable to show any inhibition at all against the bacterial pathogens employed.

Antifungal Activity studies. The results of the percentage inhibition of mycelial growth due to treatment of chemicals are presented in Table II.

Fusarium equiseti. In case of this fungal strain, compounds 3 (48.34%), 7 (50.32%) and 15 (48.96%) were found to be more effective than the standard antibiotic, Nystatin (45.79%). The rest of the acylated derivatives showed moderate to good inhibition against this fungus.

Macrophonina phaseolina. In case of *Macrophonina phaseolina*, it was observed that the compounds 2 (85.93%), 6 (78.51%), 8 (72.43%), and 11 (82.35%) displayed marked toxicities even at low concentration. Rest of the compounds were less effective to the *Macrophonina phaseolina* as compared to the standard antibiotic, Nystatin.

Curvularia lunata. The screening data showed that the inhibition of the mycelial growth by chemicals 15 (52.36%), and 3 (50.22%) were much more than that by the other chemicals such as 1 (3.45%), 2 (15.33%), 4 (24.44%), 5 (20.34%), 6 (18.33%), 7 (31.11%), 8 (38.00%), 9 (40.00%), 10 (5.90%), 11 (26.32%), 12 (32.44%), 13 (36.62%), 14 (48.32%), 16 (46.41%), 17 (26.41%), and 18 (22.03%). However, all the acylated derivatives were less effective against this microorganism as compared to Nystatin (72.41%).

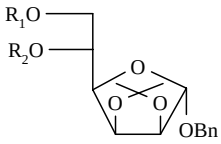
Botryodiplodia theobromae. Most of the tested chemicals showed stimulation rather than inhibition against this tested fungus. Compounds 10 (0.00%) and 18 (0.00%) did not show any inhibition or stimulation. Rest of the tested chemicals showed poor inhibition against this fungus.

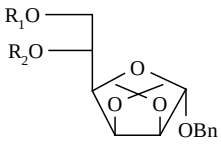
Table II. Percent Inhibition of mycelial growth due to treatment of synthesized chemicals

Compound No.	% Inhibition of mycelial growth, 100 µg, dw/ml PDA			
	Fusarium equseti	**Macrophonina phaseolina**	**Curvularia lunata**	**Botryodiplodia theobromae**
1	11.85	22.78	30.45	4.32
2	33.42	*85.93	15.33	+3.27
3	*48.34	40.22	50.22	15.49
4	*50.32	53.97	24.44	+4.30
5	31.97	20.63	20.34	+12.43
6	30.79	*78.51	18.33	+9.35
7	*48.96	60.03	31.11	16.67
8	36.35	*72.43	38.00	6.96
9	32.15	45.46	40.00	+8.37
10	5.15	20.36	5.90	0.00
11	26.32	*82.35	26.32	12.54
12	42.96	39.90	32.44	15.54
13	33.90	45.36	36.62	+8.92
14	26.67	40.63	32.54	+3.02
15	*52.93	30.93	48.32	+3.36
16	40.00	55.78	52.36	6.67
17	35.36	40.90	46.48	9.32
18	43.79	48.42	22.03	00
Nystatin	45.79	*70.78	*72.41	*70.00

N.B:* = Marked inhibition; ** = Standard antibiotic "+" = Stimulation; dw=Dry weight

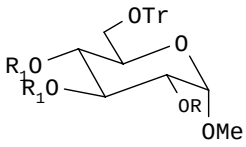
Fig. 1. Structure of compound 1-18

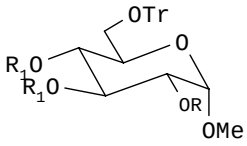




1. R_1=H; R_2=H
2. R_1=Myr; R_2=H
3. R_1=Myr; R_2=Ac
4. R_1=Myr; R_2=Bz
5. R_1=Myr; R_2=Ms

6. R_1=Pal; R_2=H
7. R_1=Pal; R_2=Ac
8. R_1=Pal; R_2=Bz
9. R_1=Pal; R_2=Ms





10. R=H; R_1=H
11. R=Hex; R_1=H
12. R=Hex; R_1=Ac
13. R=Hex; R_1=Bz
14. R=Hex; R_1=Ms

15. R=Oct; R_1=H
16. R=Oct; R_1=Ac
17. R=Oct; R_1=Bz
18. R=Oct; R_1=Ms

The overall results indicated that out of the four fungi, maximum average inhibition was observed in case of *Fusarium equiseti* whereas *Botryodiplodia theobromae* showed minimum average inhibition. Further studies in the synthesis of newer active antifungal ingredients will give us a new horizon in plant disease control. The selected chemicals have not been tested against the selected fungal pathogens before. This is the first report regarding the effectiveness of the selected chemicals against the selected pathogens. The result of the present investigation revealed that some of the newly developed chemicals may be tested against a wide range of phytopathogenic bacteria and fungi and the work is currently underway.

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