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Antimicrobial Screening of Some Derivatives of Methyl α-D-Glucopyranoside

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Abstract. *In vitro* antimicrobial functionality test of methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside and its twelve acylated derivatives against ten human pathogenic bacteria and six phytopathogenic fungi comparative to Ampicillin and Nystatin revealed the tested chemicals to possess moderate to good antibacterial and antifungal activity and to be more effective against fungal phytopathogens. Many of these chemicals exhibited better antimicrobial activity than the standard antibiotics. Minimum Inhibition Concentration (MIC) of methyl 4,6-*O*-cyclohexylidene-2-*O*-myristoyl-3-*O*-palmitoyl-α-D-glucopyranoside against *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus* was 25, 12.5 and 25 μg/disc, respectively.

Keywords: antibacterial activities, antifungal activities, methyl glucopyranoside derivatives

Introduction

Carbohydrates, especially acylated glycosides, are very important due to their effective biological activity. A large number of biological compounds possess aromatic, heteroaromatic and acyl substituents. Nitrogen, sulphur and halogen containing substituents enhance the biological activity of the parent compound (Ghorab *et al*., 1988).

Over the last few years, a wide variety of acylated monosaccharide derivatives were prepared and their biological evaluation was carried out at the laboratory of the University of Chittagong (Kabir *et al*., 2005; 2004; 2003; 2002). It was found that the combination of two or more acyl substituents in a single molecular framework enhances the biological activity manifold than their parent compounds. For example, some acylated derivatives of D-glucopyranose were found more active than those of the standard antibiotics (Kabir *et al*., 2007; 2005).

Later, some derivatives of methyl α-D-glucopyranoside containing a cyclohexane moiety and various acyl groups (e.g. octanoyl, decanoyl, lauroyl, myristoyl, palmitoyl, acetyl, benzoyl, 2-chlorobenzoyl, 4-chlorobenzoyl, mesyl, brosyl and pivaloyl) were synthesized in a single molecular framework, and their antimicrobial aganist bactria and fungal screening was carried out using a variety of bacterial and fungal strains. The synthetic part of this piece of work has been reported earlier (Kabir *et al*., 2006); the results of *in vitro* antimicrobial screening experiments are reported here.

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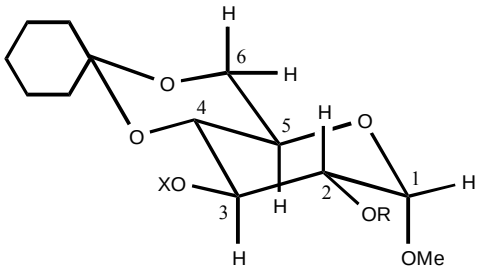
Materials and Methods

Methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside (**1**) and its acylated derivatives (**2-13**) were used as test chemicals for the determination of antimicrobial activity. The test chemicals **1-13** (Fig. 1) were synthesized, isolated and purified at the Organic Research Laboratory of the Department of Chemistry, University of Chittagong. Test tube cultures of bacterial and fungal pathogens were collected from the Microbiology Research Laboratory, Department of Microbiology, University of Chittagong, Bangladesh.

Test bacteria. Test chemicals **1-13** were subjected to antibacterial screening against four gram-positive and six gram-negative bacterial strains viz., *Bacillus subtilis* BTCC 17, *Bacillus cereus* BTCC 19, *Bacillus megaterium* BTCC 18, *Staphylococcus aureus* ATCC 653, *Escherichia coli* ATCC 25922, *Salmonella typhi* AE 14612, *Shigella dysenteriae* AE 14396, *Salmonella paratyphi*, *Shigella sonnei* CRL (ICDDR,B) and INABA ET (Vibrio).

Test fungi. Test compounds **1-13** were screened for their antifungal activities against six phytopathogenic fungi viz., *Colletotrichum corchori* (Ikata Yoshida), *Macrophomina phaseolina* (Tassi) Goid, *Curvularia lunata* (Wakker Becdijin), Fusarim *equiseti (Corda) Sacc., Alternaria alternata* (Fr.) Kedissler and *Botryodiplodia theobromae* (pat).

Antibacterial studies. *In vitro* antibacterial activities of the test chemicals were studied by disc diffusion method (Bauer *et al.,* 1966) and nutrient agar (NA) was used for culture of



1. R = X = H
2. R = Myristoyl; X = H
3. R = Myristoyl; X = Ac
4. R = Myristoyl; X = Bz
5. R = Myristoyl; X = 2-Cl.Bz
6. R = Myristoyl; X = 4-Cl.Bz
7. R = Myristoyl; X = Ms
8. R = Myristoyl; X = Bs
9. R = Myristoyl; X = Pv
10.R = Myristoyl; X = Octanoyl
11.R = Myristoyl; X = Decanoyl
12.R = Myristoyl; X= Lauroyl
13.R = Myristoyl; X = Palmitoyl

Fig. 1. Structure of compounds.

bacteria. Throughout the experiment, paper disc of 4 mm diameter and petridish of 70 mm diameter were used which were sterilized in autoclave and dried at 60 °C in an oven. Pour plates were made with sterilized melted NA (45 °C). After solidification of pour plates, the test organisms (suspension) were seeded uniformly over them. Paper discs after soaking with test chemicals (2% in $CHCl_3$) were placed at the centre of the inoculated pour plates. A control plate was also maintained in each case with chloroform. The plates were kept for 4 h at low temperature (4 °C). Test chemicals diffused from discs to the surrounding medium by this time. The plates were then incubated at 35± 2 °C and the growth of test organisms was observed at 24 h intervals for two days. The activity was expressed in terms of inhibition zone diameter in mm. Each experiment was repeated thrice. Standard antibiotic, Ampicillin (for bacteria) from FISONS (Bangladesh) Ltd. was used as a positive control and compared with test chemicals under identical conditions.

Antifungal studies. *In vitro* antifungal activities were determined by poisoned food technique (Grover and Moore, 1962) in some modified conditions (Miah et al., 1990) and Potato Dextrose Agar (PDA) medium was used for culture of fungi. Specific amount of medium was taken in conical flasks separately and was sterilized in autoclave at 120 °C and 15 psi. Weighed amount of test chemical was added to the medium in conical flask at the time of pouring to obtain the desired concentration. The flask was shaken thoroughly for uniform mixing of the chemical with the medium. The medium with definite amount of chemical (100 mg) was then poured into separate sterilized petridishes. Proper control was maintained separately with sterilized PDA medium without chemical and three replicates were prepared for each treatment. The media were allowed to solidify.

Mycelial blocks (5 mm approx.) of individual test fungus were cut out from the outer margin of the cultures growing on PDA plates. The blocks were then placed at the center in all document of each petridish in an inverted position. All the plates were inoculated at 25±2 °C and incubated 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 of inhibition, C = diameter of the fungal colony on control ($CHCl_3$) dish and T = diameter of the fungal colony on the test dish.

Results were compared with that of the standard antibiotic, Nystatin.

Results and Discussion

Aim of the present study is directed towards investigation of the antibacterial and the antifungal activities of the synthesized D-glucose derivatives (**1-13**). Test chemicals (**2-13**) were prepared from a common precursor, namely, methyl 4,6-*O*-cyclohexylidene-α-D-glucopyranoside (**1**) and contain a wide variety of substituents. The test chemicals (**1-13**) were screened for their antimicrobial activity against ten human pathogenic bacteria and six phytopathogenic fungi. For

Table 1. Zone of inhibition of the test chemicals against gram-positive test organism

Chemical	Diameter of inhibition zone in mm (100 µg dw./disc)			
no.	*B. subtilis*	*B. cereus*	*B. megaterium*	*S. aureus*
1	00	01	02	04
2	-	-	08	08
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	-	-	-	-
7	-	-	08	18
8	06	10	10	18
9	-	-	-	-
10	15	09	12	-
11	08	12	*22	15
12	-	-	-	-
13	08	*25	11	*25
Ampicillin	25	22	19	20

N.B.: * =Marked inhibition; - = No inhibition; dw = Dry weight

Table 2. Zone of inhibition of the test chemicals against gramnegative test organism

Chemical no.	Diameter of zone of inhibition in mm (100 µg dw./ disc)					
	E. coli	*S. typhi*	*S. paratyphi*	*S.dysenteriae*	*S. sonnei*	*Vibrio* (Inaba et)
1	01	02	-	01	05	03
2	10	-	-	-	10	-
3	-	-	-	-	-	-
4	-	-	-	08	-	-
5	-	-	-	-	-	-
6	-	-	10	-	-	-
7	10	-	-	-	15	10
8	-	-	-	06	10	10
9	-	-	-	-	-	-
10	10	08	-	10	12	15
11	12	10	11	08	16	14
12	-	-	-	-	-	-
13	*14	06	-	-	08	-
Ampicillin	13	24	17	35	35	25

* = marked inhibition; - = no inhibition; dw = dry weight

comparative study, the antimicrobial activity of two standard antibiotics, viz. Ampicillin and Nystatin, were also evaluated against these microorganisms. The results of antibacterial activity of the test chemicals (**1-13**) were expressed in terms of zone of inhibition in mm (Table 1 and 2).

The test chemicals exhibited promising inhibitory activity against a number of gram-positive as well as gram-negative bacterial strains. The inhibition data indicated that the chemical **11** was more active (zone of inhibition, 22 mm) against *B. megaterium* and chemical **13** was more active against *B. cereus* (T=25 mm) and *S. aureus* (T=25 mm) than the standard antibiotic, Ampicillin. On the other hand, chemical **13** was more effective against *E. coli,* than the standard drug wheras, chemicals **1-6** displayed little inhibition against the bacterial strains. It was also observed that chemical **13** was very effective against *B. cereus, B. subtilis* and *S. aureus* as compared to Ampicillin; result of minimum inhibition concentration (MIC) test of chemical **13** against these bacterial strains are presented in Table 3.

It was found that selectively acylated derivatives **8, 10, 11** and **13** showed moderate to marked inhibition against gram-positive bacteria while chemicals **7, 10, 11** and **13** were very active against gram-negative bacteria. It was also observed that the acylated derivative **11** was reasonably effective against both the gram-positive and gram-negative organisms.

MIC is the minimum concentration of the antibacterial agent in a given culture medium below which bacterial growth is not inhibited. MIC methods are widely used in the comparative testing of new agents. In clinical laboratories MIC method is used to establish the susceptibility of organisms that give equivocal results in disc tests, in cases where disc tests are

Table 3. Minimum inhibition concentration (MIC) values of test chemical **13**

Test chemical	Bacteria	Sample concentration (µg/ disc)	Zone of Inhibition (mm)	MIC (µg/disc)
13	*B. cereus*	100	25	25
		50	18	
		25	12	
		12.5	-	
		6.25	-	
	B. subtilis	100	27	12.5
		50	*19*	
		25	14	
		12.5	07	
		6.25	-	
	S. aureus	100	25	25
		50	17	
		25	10	
		12.5	-	
		6.25	-	

Table 4. Antifungal activity of the test chemicals and Nystatin

Chemical no.	% Inhibition of fungal mycelial growth, 100 µg (dw) sample/ ml PDA					
	F. equiseti	*M. phaseolina*	*C. corchori*	*B. theobromae*	*Curvularia Iunata*	*Alternaria alternata*
1	8.15	5.01	7.11	3.26	1.5	6.35
2	30.7	34.2	*44.4	20.0	-	15.4
3	30.7	28.5	33.3	32.5	35.7	+6.6
4	38.4	50.0	33.3	+6.6	40.0	+4.7
5	35.3	35.7	33.3	20.6	38.5	20.0
6	35.3	31.4	37.7	28.8	38.5	21.4
7	35.3	50.0	*66.6	40.0	42.8	23.8
8	30.7	*71.4	*66.6	15.0	42.8	28.5
9	30.7	42.8	33.3	15.3	-	*64.2
10	*46.1	42.8	55.5	35.6	48.5	16.6
11	*45.0	28.5	37.7	35.6	44.2	+4.7
12	33.8	35.7	37.7	10.6	37.1	*59.0
13	38.4	35.7	*51.1	+4.4	55.1	40.4
Nystatin	44.70	70.78	40.51	70.0	70.0	51.0

* = marked inhibition; - = no inhibition; dw = dry weight; + = stimulation

unreliable and when a more accurate result is required for clinical management. The MIC value of the chemical **13** was 25, 12.5 and 25 mg /disc against *B. cereus, B. subtilis* and *S. aureus*, respectively. Considering the remarkable inhibitory activity of compound **13** among the tested chemicals against three potential pathogenic bacteria, further experiments are required for evaluating the efficacy of this compound.

The antifungal activity of the test chemicals were evaluated against six phytopathogenic fungi and compared with the similar activity of the antifungal antibiotic, Nystatin. Results of the inhibition of fungal mycelial growth are presented in Table 4.

Fusarium equiseti. Among the chemicals tested, chemicals **10** (46.1%) and **11** (45.0%) showed the highest inhibition, which were more than the standard antibiotic Nystatin employed (44.7%). The rest of the chemicals were, however, less toxic to this fungus as compared to Nystatin.

Macrophomina phaseolina. Most of the test chemicals displayed moderate to marked toxicity towards plant pathogenic fungus *M. phaseolina*. Chemical **8** showed relatively higher inhibition (71.4%) than the standard antifungal drug Nystatin. (70.78%). The rest of the chemicals were either less effective than Nystatin or did not show any inhibition or stimulation.

Colletotrichum corchori. Most of the D-glucose derivatives displayed marked toxicity towards *C. corchori*. Among these, chemicals **2** (44.4%), **7** (66.6%), **8** (66.6%), **10** (55.5%) and **13** (51.1%) showed relatively higher inhibition than the standard antibiotic, Nystatin (40.51%).

Botryodiplodia theobromae. Chemicals **3** (32.5%), **7** (40.0%), **10** (35.6%) and **11** (35.6%) showed reasonable toxicity against *B. theobromae*, but were not as toxic as the antifungal drug Nystatin (70.0%). Here again, chemicals **4** (+6.6%) and **13** (+4.4%) showed stimulation rather than inhibition against this fungus.

Curvularia lunata. Chemicals **4-8** and **10-13** were very effective in the inhibition of mycelial growth of *C. lunata* though not as effective as Nystatin (75.00%); whereas chemicals **2** and **9** were inactive against this plant pathogenic fungus.

Alternaria alternata. Chemicals **9** (64.2%) and **12** (59.0%) displayed very effective inhibition, though not as effective as Nystatin (51.55%). Chemicals **3** (+6.6%), **4** (+4.7%) and **11** (+4.7%), showed stimulation whereas the rest of the chemicals displayed moderate to poor inhibition against this phytopathogen.

Conclusion

The synthesised chemicals (**1-13**) had not been tested as yet against the selected bacterial and fungal pathogens; this is the first report on the subject. The antimicrobial screening data indicated the tested compounds to possess promising biologi-

cal activities and can be used as good source of antimicrobial agents at least in the field of agriculture. It is concluded that some of the synthesised acylated derivatives of methyl 4,6-*O*-cyclohexyli-dene-α-D-glucopyranoside (**1**) may be further tested against a wide range of bacteria and phytopathogenic fungi. Afterwards, these could be sent to pesticide manufacturers for further tests. It is also expected that this piece of work employing carbohydrate derivatives as test chemicals will contribute towards the development of pesticides and medicines for human and plant disease control with minimum environmental hazards.

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