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Antibacterial and Antifungal Evaluation of Some Derivatives of Methyl α-D-Mannopyranoside

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ABSTRACT

Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside and a number of its acylated derivatives were screened for *in vitro* antibacterial activity against eleven human pathogenic bacteria. These compounds were also screened for *in vitro* antifungal activity against six phytopathogenic fungi. For comparative study, antimicrobial evaluation of standard antibiotics, Ampicillin and Nystatin were also performed. The experimental results acertained that the tested D-mannose derivatives showed moderate to good antimicrobial activities. It was interesting to observe that the selected compounds were more sensitive against fungal phytopathogens than those of the bacterial strains. Encouragingly, a good number of test compounds exhibited better biological activity than the standard antibiotics employed.

Key Words: Antibacterial; Antifungal; Antibiotics; Nutrient agar; Potato dextrose agar

INTRODUCTION

Over the years, considerable works have been done in the field of antimicrobial screening studies of chemical compounds (Singh *et al*., 1990). Different classes of chemical compounds have been screened for *in vitro* antimicrobial activities all over the world. Carbohydrates, especially acylated glycosides, are very important due to their effective biological activity (Andary *et al*., 1982). Literature survey revealed that a large number of biologically active compounds possess aromatic and heteroaromatic nuclei (Gupta *et al*., 1997). It was also revealed that if an active nucleus is linked to another nucleus, the resulting molecule may possess greater potential for biological activity. Results of our previous works on synthesis and antimicrobial evaluation of monosaccharide derivatives, it was noticed that in many cases the combination of two or more acyl substituents enhanced the antimicrobial activity considerably as compared to the parent molecule (Kabir *et al*., 1999; Kabir *et al*., 2002; Kabir *et al*., 2004; Kabir *et al*., 2005). Encouraged by our past findings and in continuation of the project, we synthesized a series of D-mannose derivatives (Kabir *et al*., 2004) containing a wide variety of acyl substituents and evaluated their antimicrobial functionalities. The results thus obtained are reported here.

MATERIALS AND METHODS

Test chemicals. Some partially protected derivatives of D-mannose (1-11) (Fig. 1) were used as test chemicals. The chemicals were synthesized, isolated, purified and characterized in the Organic Research Laboratory, Department of Chemistry, University of Chittagong and reported earlier (Kabir *et al*., 2004). In all the cases, a 1% solution (w/v) in chloroform of the chemicals were used.

Biological evaluation of the test chemicals. The antimicrobial assay of the chemicals was done in the laboratory of microbial enzymes and antagonistic microbes and their antimicrobial agents, Department of Microbiology, Chittagong University. The test micro-organisms (Bacteria and Fungi) for antimicrobial assay were collected from this Laboratory. Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were used as basal medium for antibacterial and antifungal test, respectively.

Bacterial Cultures. The following Gram-positive and Gram-negative bacterial cultures were used as test organisms.

Gram-positive bacteria. i) *Bacillus cereus* BTCC 19, ii) *Bacillus subtilis* BTCC 17, iii) *Staphylococcus aureus* BTCC 43 and iv) *Bacillus megaterium* BTCC 18.

Gram-negative bacteria. v) *Escherichia coli* BTCC 12, vi) *Vibrio cholerae* CRL(ICDDR,B), vii) *Salmonella typhi* AE 14612, viii) *Salmonella paratyphi*-A CRL(ICDDR,B), ix) *Pseudomonas species* CRL (ICDDR,B), x) *Shigella sonnei* CRL (ICDDR,B) and xi) *Shigella dysenteriae* AE 14396.

Fungal cultures. The following phytopathogenic fungi were used as test fungi.

i) *Colletotrichum corchori* ii) *Fusarium equiseti* (Corda) Sacc., iii) *Alternaria alternata* Savulescu and Sandu Ville, iv) *Curvularia lunata* Wakker boedijn, v) *Botryodiplodia theobromae* Pat. and vi) *Macrophomina phaseolina* (Maubi) Ashby.

Antibacterial activity assay. The *in vitro* antibacterial spectra of the synthesized chemicals were done by disc

diffusion method (Bauer *et al.,* 1966) using 200 µg(dw) of chemical per disc (4 mm) and NA as basal medium. Antibacterial activities were indicated by clear zone of growth inhibition around the disc. The inhibition zones were recorded after 24 to 48 h of incubation at 37±1ºC.

Antifungal activity assay. The *in vitro* antifungal activity of the test chemicals were done by Poisons Food technique (Grover & Moore, 1962) and the technique with some modification (Miah *et al*., 1990) using 100 µg of chemical per mL of PDA medium. The diameter of radial growth of the test fungi was measured after 3 to 5 days of incubation at 27±1ºC and expressed as % mycelial growth inhibition following the formula given below:

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

Where, I = Percentage of inhibition.

C = Diameter of the fungal colony in control ($CHCl_3$)

T = Diameter of the fungal colony in treatment.

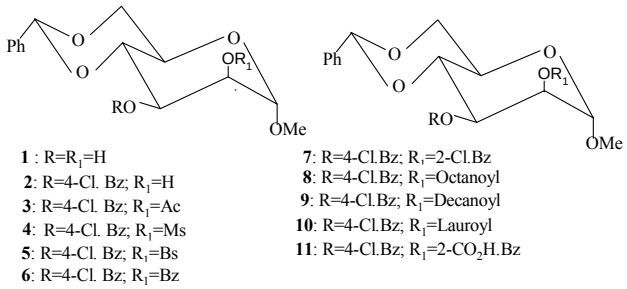
Control. Ampicillin (20 µg/disc) and Nystatin (100 µg/mL PDA) were used as standard antibacterial and antifungal control, respectively and chloroform, the solvent of the test chemicals was used as negative control for comparison of results under identical condition.

RESULTS AND DISCUSSION

In the present investigation, some acylated derivatives of D-mannose (1-11) were selected as probable test chemicals and screened *in vitro* for their antibacterial and antifungal activities against eleven human pathogenic bacteria and six phytopathogenic fungi. These test chemicals contained a wide variety of substituents such as phenyl, 4-chlorobenzoyl, acetyl, methanesulphonyl, benzenesullphonyl, benzoyl, 2-chlorobenzoyl, octanoyl, decanoyl, lauroyl, and 2-carboxybenzoyl. These test chemicals were prepared from a common precursor namely, methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (1). In fact, we deliberately incorporated the above mentioned substituents into the D-mannose molecule in order to study their effectiveness against the micro-organisms tested. Since various acylated carbohydrate derivatives are known to have effective biological activity, these acylated derivatives are expected to exhibit such an activity. For comparative study, the biological activity of the precussor compound (1) and two standard antibiotic substances, Ampicillin and Nystatin were also determined.

The antibacterial activity of the test chemicals (1-11) against the Gram-positive bacteria and Gram-negative bacteria are shown of Table I and II, respectively. Few of the synthesized compounds exhibited antibacterial activities in different degrees. Compound 11 was, however, very effective against both Gram-positive and Gram-negative bacteria. Both Gram-positive and Gram-negative bacteria were found insensitive towards compounds 3, 4, 5 and 9. Compound 6, however, showed little sensitivity towards the tested human pathogenic micro-organisms.

Fig. 1. The structure of compounds 1-11



1 : R=R_1=H
2: R=4-Cl. Bz; R_1=H
3: R=4-Cl. Bz; R_1=Ac
4: R=4-Cl. Bz; R_1=Ms
5: R=4-Cl. Bz; R_1=Bs
6: R=4-Cl. Bz; R_1=Bz
7: R=4-Cl.Bz; R_1=2-Cl.Bz
8: R=4-Cl.Bz; R_1=Octanoyl
9: R=4-Cl.Bz; R_1=Decanoyl
10: R=4-Cl.Bz; R_1=Lauroyl
11: R=4-Cl.Bz; R_1=2-CO_2H.Bz

Table I. Antibacterial screening of the test chemicals against Gram-positive bacteria

Compound no.	Zone of inhibition (mm) Test bacteria			
(200µg dw/disc)	*B. cereus*	*B. subtilis*	*Stap. aureus*	*B. megaterium*
1	-	-	-	-
2	8	-	-	8
3	-	-	-	-
4	-	-	-	-
5	-	-	-	-
6	9	8	8	9
7	-	-	-	-
8	-	-	-	9
9	-	-	-	-
10	8	-	-	-
11	*15	7	-	*15
**Ampicillin (20µg.dw./disc)	16	16	20	15

* = Marked inhibition; **= Standard antibiotic; - = No inhibition; dw= Dry weight

The *in vitro* antifungal evaluation results of the eleven test chemicals (1-11) and the standard antibiotic, Nystatin is presented in Table III. From the results, it was indicated that compound 11 was very highly sensitive towards all the used fungal phytopathogens except in the case of *Alternaria alternata* and Botryodiplodia theobromae, where its activity was almost comparable to the standard antibiotic, Nystatin. Compound 2 was observed to be very sensitive against *Botryodiplodia theobromae*. Compound 8 inhibited the radial mycelial growth against *Fusarium equiseti* better than the standard antibiotic, Nystatin. It was encouraging to notice that most of the test chemicals were more or less sensitive towards the tested fungal phytopathogens.

An interesting observation was that some of the test chemicals showed stimulation rather than inhibition. Stimulation of radial mycelial growth of fungi with test chemicals may be due to utilization of the chemicals or/of their degradative products (by fungal enzymes) by the fungus for their growth and development. From the results of this investigation in (Table I, II, III), it was observed that compound 11 containing a 2-carboxybenzoyl group was very effective against the human pathogenic bacteria as well as the phytopathogenic fungi.

The results of this investigation was quite encouraging in that some of the newly prepared D-mannose derivatives were found to be very effective against some

Table II. Antibacterial screening of the test chemicals against Gram-negative bacteria

Compound no. (200 µg dw/disc)	Zone of inhibition (mm) Test bacteria						
	E. coli	*V. cholerae*	*S. typhi*	*S. paratyphi*	*Pseudomonus species*	*S. sonnei*	*S. dysenteriae*
1	-	-	-	-	7	-	-
2	-	-	7	-	-	-	9
3	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-
6	8	-	8	8	-	-	*10
7	-	-	-	-	-	-	7
8	-	-	8	-	-	8	-
9	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
11	-	*15	*14	*15	7	8	*14
**Ampicillin (20µg.dw./disc)	28	24	25	12	19	24	13

* = Marked inhibition; ** = Standard antibiotic; - = No inhibition; dw = Dry weight.

Table III. Percent inhibition of fungal radial mycelial growth by the test chemicals

Compound no. (100µg dw/mL PDA)	% inhibition of radial mycelial growth Test fungi					
	C. corchori	*F. equiseti*	*A. alternata*	*C. lunata*	*B. theobromae*	*M. phaseolina*
1	23.08	1.64	8.00	+9.09	-	7.69
2	27.27	15.91	21.62	*46.67	*62.65	*40.00
3	23.64	13.64	16.62	6.67	25.30	30.67
4	5.45	-	10.81	15.56	19.28	36.00
5	1.82	9.09	5.41	6.67	10.67	14.67
6	-	11.36	2.70	4.44	15.66	17.33
7	5.45	9.09	5.41	8.89	+2.41	+13.33
8	+1.82	*52.27	-	11.11	15.66	36.00
9	-	2.27	+5.41	-	19.28	+6.67
10	1.82	22.73	16.92	11.11	21.69	26.67
11	*60.00	*52.27	*48.65	*80.00	*69.88	*77.33
**Nystatin (100µg dw/ml PDA)	41.00	45.00	51.00	70.00	70.00	76.00

* = Marked inhibition; ** = Standard antibiotic; - = No inhibition; dw =Dry weight; + =Stimulation

phytopathogens and further study will be needed before sending them to pesticide producing companies for further tests. It is also expected that this piece of work employing monosaccharide derivatives as test chemicals will open the scope for further work on the development of pesticides/medicines for human disease control with minimum environmental hazards.

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