INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–5–757–759
http://www.ijab.org

Antimicrobial Screening of Some Acylated Derivatives of D-Glucose

ABUL K.M.S. KABIR[1], PIJUSH DUTTA AND M.N. ANWAR†
Department of Chemistry and †Microbiology, University of Chittagong, Chittagong-4331, Bangladesh
[1]Corresponding author's e-mail: kabir562000@yahoo.com

ABSTRACT

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside and thirteen of its substituted derivatives were employed as test chemicals for *in vitro* antimicrobial evaluation against four Gram-positive and seven Gram-negative human pathogenic bacteria. The same compounds were also screened for antifungal functionality test against six phytopathogenic fungi. The antimicrobial activities of two standard antibiotics, Ampicillin and Nystatin, were also measured for comparison. It was noticed that a good number of test chemicals showed moderate to good antimicrobial activities. It was also observed that the tested chemicals were more effective against the phytopathogenic fungi than those of the bacterial strains. Encouragingly, a number of test chemicals exhibited better antifungal activity than the standard antibiotic, Nystatin. A number of compounds, however, showed stimulation, rather than inhibition, against the fungal phytopathogens.

Key Words: Bacteria; Fungus; Antimicrobial activity; Ampicillin; Nystatin; Nutrient agar

INTRODUCTION

In the last few decades, considerable works have been done in the field of antimicrobial evaluation (Singh *et al*., 1990) of various chemical compounds. Different classes of chemical compounds have been screened for *in vitro* antimicrobial activities (Andary *et al*., 1982; Gupta *et al*., 1997) all over the world. Carbohydrates, especially acylated glycosides, are very important due to their effective biological activity (Andary *et al*., 1982; Kabir *et al.,* 1998; Kabir *et al*., 2001). Literature survey (Gupta *et al*., 1997) revealed that a large number of biologically active compounds possess aromatic and heteroaromatic nuclei. It was also revealed that if an active nucleus is linked to another active nucleus, the resulting molecule may possess greater potential for biological activity. From our ongoing works in this laboratory, it was observed that the substituted monosaccharide (Kabir *et al*., 2002; Kabir *et al*., 2003a, b) and uridine (Kabir *et al*., 2002; Kabir *et al*., 2003c) derivatives containing aromatic, heteroaromatic and acyl groups and also atoms like chlorine, bromine, sulfur enhances the biological activity many fold than the precursor compounds. Guided by the literature survey results and our own findings, we synthesized a series of acylated derivatives of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (1) containing a wide variety of substituents in a single molecular framework and evaluated their antimicrobial activities using various bacterial and fungal strains. The synthetic part of the project was earlier reported (Kabir *et al*., 2003d) and the antimicrobial evaluation results are reported here.

MATERIALS AND METHODS

Test chemicals. Some partially protected derivatives of D-glucose (1-14) (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*., 2003a). 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 fungal phytopathogens 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) *Maccrophomina phaseolina* (Maubi) Ashby.

Antibacterial activity assay. The *in vitro* antibacterial spectrums of the test chemicals including the standard antibiotic, Ampicillin, 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 under investigation were done by Poisons Food technique (Grover & Moore, 1962) and the technique with some modification by 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 percent mycelial growth inhibition following the formula given below:

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

Where, I = Percentage of inhibition.

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

T = Diameter of the fungal colony in treatment.

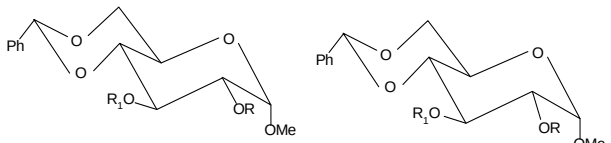
Control. Ampicillin (20 µg/disc) and Nystatin (100 µ/mL PDA) were used as standard antibacterial and antifungal control respectively; and chloroform, the solvent of the synthesized chemicals was used as negative control for comparison of results under identical condition.

RESULTS AND DISCUSSION

In this investigation thirteen acylated derivatives (2-14) of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (1) including the precursor (1) were selected as test chemicals for antimicrobial evaluation. Eleven human pathogenic bacteria and six plant pathogenic fungi were chosen as the test chemicals. For comparative studies, two standard antibiotic substances, Ampicillin and Nystatin, were also screened for antimicrobial activities.

The *in vitro* antibacterial screening results of the test chemicals (1-14) as well as the standard antibiotic, Ampicillin against the Gram-positive and Gram-negative bacterial strains are incorporated in Table I and II, respectively. From the results we observed that the test chemicals were more prone to inhibit the growth of Gram-negative bacteria in comparison to Gram-positive bacteria. Most of the chemicals of this series were insensitive towards the growth of bacterial strains. However, the zone of inhibition by the test chemicals 3 in case of *B. cereus* (10 mm) and *B. megatorium* (11 mm); by 3 in case of *S. typhi*

Fig. 1. Structure of compound 1-14



1 : R=R_1=H
2 : R=Stearoyl ; R_1=H
3 : R=Stearoyl ; R_1=Ac
4 : R=Stearoyl ; R_1=Ms
5 : R=Stearoyl ; R_1=Bz
6 : R=Stearoyl ; R_1=Ts
7 : R=Stearoyl ; R_1=Pv

8 : R=3,5$(NO_2)_2$.C_6H_3CO- ; R_1=H;
9 : R=3,5$(NO_2)_2$.C_6H_3CO- ; R_1=Ac
10 : R=3,5$(NO_2)_2$.C_6H_3CO- ; R_1=Ms
11 : R=3,5$(NO_2)_2$.C_6H_3CO- ; R_1=Bz
12 : R=3,5$(NO_2)_2$.C_6H_3CO- ; R_1=Pv
13 : R=R_1=PhO.CH_2.CO-
14 : R=R_1=Ms

Table I. Antibacterial screening studies against some Gram-positive bacteria Diameter of zone of inhibition in mm., sample 200µg.dw./disc

Compound no.	*B. cereus*	*B. subtilis*	*Stap.aureus*	*B. megaterium*
1	-	-	-	-
2	-	-	-	-
3	*10	9	-	*11
4	-	-	-	-
5	-	7	-	-
6	-	-	-	-
7	-	-	-	-
8	8	7	-	-
9	-	-	-	-
10	-	-	-	-
11	-	-	-	8
12	-	-	-	7
13	-	-	-	-
14	-	-	-	-
**Ampicillin (20µg.dw./disc)	16	16	20	15

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

(10 mm), *P. species* (10 mm) and *S. dysenteriae* (11 mm); by 8 in case of *V.cholerae* (16 mm) and by 12 in case of *S. dysenteriae* (10 mm) are worth mentionable. Although none of the test chemicals showed higher activity than the standard antibiotic, Ampicillin, some displayed comparable activity.

The *in vitro* antifungal evaluation results of the test chemicals (1-14) and the standard antibiotic, Nystatin is placed in Table III. From the results, it was revealed that the inhibition of *Colletotrichum corchori* by compound 5 (46.15%), of *Alternaria alternata* by compound 10 (56.82%), of *Curvularia lunata* by compound 10 (84.00%) and of *Macrophomina phaseolina* by compound 5 (84.82%) were better than that of the standard antibiotic, Nystatin. Besides these, 42.86% inhibition by 11 in case of *Curvularia lunata*, 41.54, 38.10 and 38.46% inhibition by *Macrophomina phaseolina* by the test chemicals 3, 10 and 11, respectively were also significant. Stimulation of the growth of *Alternaria alternata*y compound 13 (+14.29%), of *Curvularia lunata* by 2 (1.43%), by 3 (+5.71%), by 12 (+4.29%), by 13 (+4.69%) were also observed in the investigation. Stimulation of radial mycelial growth of some fungi with the test chemicals seems to be very interesting. This may be due to the utilization of the test chemical or/of

Table II. Antibacterial screening studies against some Gram-negative bacteria Diameter of zone of inhibition in mm. sample 200μg.dw./disc

Compound	*E. coli*	*V. cholerae*	*S. typhi*	*S. paratyphi*	*Pseudomonus species*	*S. sonnei*	*S. dysenteriae*
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	9	7	10	-	10	9	*11
4	-	-	-	-	-	-	-
5	-	6	-	-	-	-	7
6	-	-	-	-	-	-	-
7	-	-	-	7	-	-	6
8	6	*16	8	-	-	-	-
9	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-
12	-	7	-	-	-	-	*10
13	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-
**Ampicillin (20μg. dw./disc)	28	24	25	12	19	24	13

E. = Escherchia; V.= Vibrio; S.= Salmonella; * = Marked inhibition; **= Standard antibiotic; - = No inhibition; dw=Dry weight

Table III. Percent inhibition of fungal mycelial growth, sample 100 μg dw./mL PDA

Sample	*Coll. corchori*	*F. equiseti*	*A. alternata*	*C. lunata*	*B. theobromae*	*M. phaseolina*
1	4.00	-	3.70	22.86	-	15.38
2	3.85	4.92	-	+1.43	11.11	20.00
3	-	3.28	7.41	+5.71	5.56	41.54
4	11.54	12.00	-	10.00	22.22	17.86
5	*46.15	26.23	20.37	-	31.11	*84.62
6	5.45	15.55	11.43	12.50	12.00	10.26
7	-	1.64	14.81	4.29	28.89	23.08
8	5.77	14.75	9.26	21.43	16.67	18.46
9	11.54	13.33	20.37	4.00	20.00	17.86
10	-	20.00	*56.82	*84.00	26.67	38.10
11	26.92	26.23	16.67	42.86	11.11	38.46
12	5.77	6.56	9.26	+4.29	11.11	23.08
13	27.27	13.33	+14.29	+4.69	20.00	5.13
14	11.54	12.00	-	4.00	7.78	10.71
**Nystatin 100 μg dw./disc	41.00	45.00	51.00	70.00	70.00	76.00

Coll.= Colletotrichum; F.= Fusarium; A.= Alternaria; C.= Curvularia; B.= Botryodiplodia; M.= Macrophomina; * = Marked inhibition; **= Standard antibiotic; -= No inhibition; dw= Dry weight; += Stimulation

their degradative products (by fungal enzymes) by the fungus for their growth and development.

An important observation from this investigation was that these D-glucose derivatives were more active towards fungal organisms than towards bacterial strains. In general, a major number of the acylated D-glucose derivatives exhibited more inhibition towards the tested micro-organisms than the precursor compound 1. That is, with the introduction of various acyl substituents, the antimicrobial functionality of the glucopyranoside derivatives enhanced and in some cases, this functionality was greater than or comparable to the standard antibiotics employed.

Thus, a comparative study of antimicrobial evaluation of the test chemicals (1-14) was successfully carried out against a variety of human pathogenic bacteria and plant pathogenic fungi. This is the first report regarding the effectiveness of the selected chemicals against the selected pathogens. The results of this investigation may create an opportunity for further evaluation of these test chemicals against other micro-organisms. It is also expected that this piece of work employing monosaccharide derivatives as test chemicals will help further work on the development of pesticides and medicines for human disease control.

Acknowledgements. P. Dutta wishes to thank the university of Chittagong for a post-graduate scholarship and the Ministry of Education, Government of Bangladesh, for granting him deputation.

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(Received 15 March 2005; Accepted 20 June 2005)