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In vitro Antibacterial Activity of the Extracts and a Glycoside from *Sida rhombifolia* Linn

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The research work was conducted to investigate the *in vitro* anti-bacterial activity of a glycoside, phenyl ethyl β-D-glucopyranoside, isolated from the stem of the plant *Sida rhombifolia*. The petroleum ether (C_2H_5-O-C_2H_5), chloroform ($CHCl_3$) and ethylacetate (CH_3-CO-O-C_2H_5) extracts of the plant were screened against eleven pathogenic bacteria for their antimicrobial activities. The test materials were found to be significant *in vitro* antibacterial activities, against most of the test bacteria. The zones of inhibition produced by the test materials were found to be between 8 and 24 mm. The minimum inhibitory concentration (MIC) values of the isolated compound was also determined against *Bacillus subtilis, Sarcinia lutea, Escherichia coli* and *Shigella shiga* which were 128, 64, 64 and 128 μg ml^{-1}, respectively.

Key words: Glycoside, pathogenic bacteria, antibacterial activity, zone of inhibition

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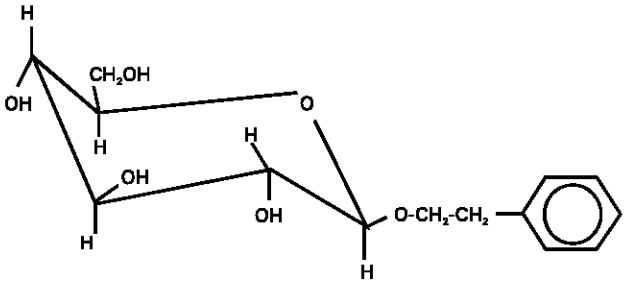
Introduction

Malvaceae is a cosmopolitan family of herbs, shrub and trees (Heywood, 1978). Modern research carried out on the malvaceous plants revealed that most of the plants belonging to this family are medicinally important as they contain biologically active compounds. *Sida rhombifolia* locally known as Berela in Bangladesh belongs to the family Malvaceae. It has considerable reputation for its medicinal value in traditional medicine. The roots and stems are useful in fever, heart disease, piles and all kinds of inflammation. An infusion of the root is given in dysentery. Stem is also employed as demulcent and emollient (Kirtikar and Basu, 1993). The plant is also useful in tuberculosis and used in skin diseases and as a diuretic (Colonel, 1986). From the literature survey, it is revealed that the preliminary screening of methanolic extract (CH_3OH) of the leaves of the plant *Sida rhombifolia*, showed anti-tumour and anti-HIV activity (Muanza *et al.*, 1995). The ethanolic (C_2H_5OH) extracts of this plant showed inhibitory activity on NF-kB cell line as an anti–inflammatory model (Bork *et al.*, 1996).
Although *Sida rhombifolia* is locally used for the above conditions, no antibacterial study of this plant has previously been reported. As a part of continuing search for novel antibacterial principles from the medicinal plants of Bangladesh, studied, *Sida rhombifolia* and herein report the results of *in vitro* antibacterial investigation.

Materials and Methods

Plant materials: Matured stems of the plant were collected from Rajshahi University Campus, Rajshahi, Bangladesh, during the month of November-December 1999. The plant was taxonomically identified by the Department of Botany, Rajshahi University as well as Bangladesh National Herbarium, where a voucher specimen is kept.

Extraction, isolation and characterization: The sun dried stems were put in an oven at 45 ^{0}C, crushed into power with a crushing machine and extracted in soxhlet apparatus with methanol (CH_3OH) for 72 hours at it's boiling point (64.5 ^{0}C) (Morrison and Boyd, 1996). The concentrated methanolic extract was diluted with distilled water and solvent-solvent partitioning was successfully carried out by Kupchan method (Grode *et al.*, *1983*) using petroleum ether (C_2H_5-O-C_2H_5), chloroform ($CHCl_3$) and ethylacetate (CH_3-CO-O-C_2H_5) (Islam, 2000). Each of the extract was concentrated at reduced pressure using rotary evaporator and thus ready for antibacterial screening.
The compound, phenyl ethyl β-D-glucopyranoside was isolated from the chloroform fraction by column chromatography (Beckett and Stenlake, 1986) followed by preparative thin layer chromatography (PTLC) (Egon and Stahl, 1969).



Phenyl Ethyl β-D-glucopyranoside

This compound was characterized on the basis of it's ^{1}H-NMR, ^{3}C-NMR, ^{1}H-^{1}H COSY 90^0 (Islam, 2001). Then this compound was subjected to antibacterial screening.

Antibacterial Screening: Eleven pathogenic bacteria (four gram positive and seven gram negative) were selected for the test and collected from the Department of Microbiology, Dhaka University, Dhaka, Bangladesh. Nutrient agar was used as a bacteriological medium. The petroleum ether (C_2H_5-O-C_2H_5), chloroform ($CHCl_3$) and ethylacetate (CH_3-CO-O-C_2H_5) extracts were dissolved separately in sufficient amount of methanol to get a concentration of 500μg 10 μl^{-1}. The compound, Phenyl ethyl β-D-glucopyranoside was also dissolved separately in methanol in the same way to get a concentration of 300μg 10μl^{-1}. Then *in vitro* antibacterial activity of these samples were carried out by the standard disc diffusion method (Barry, 1980; Berghe and Vlietnck, 1991; Bauer *et al.*, 1996; Rios *et al.*, 1988) against selected test organisms. The diameter of zone of inhibition produced by the extracts and pure compound, Phenyl ethyl β-D-glucopyranoside was then compared with those produced by the standard antibiotic (Kanamycin 30 μg disc^{-1}).

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of the compound, Phenyl ethyl β-D-glucopyranoside was determined against two gram positive (*Bacillus subtilis* and *Sarcina lutea*) and two gram negative (*Escherichia coli* and *Shigella shiga*) bacteria. The tests were carried out by serial dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as bacteriological media.

Results and Discussion

The antibacterial activities of the petroleum ether (C_2H_5-O-C_2H_5), chloroform ($CHCl_3$) and ethylacetate (CH_3-CO-O-C_2H_5) extracts and the pure compound, phenyl ethyl β-D-glucopyranoside, isolated from the chloroform extract of *Sida rhombifolia* stem against eleven pathogenic bacteria (Table 1). The zone of inhibition of petroleum ether, chloroform and ethylacetate extract were found to be 11-18, 11-22 and 12-24 mm at a concentration of 500 μg disc^{-1}, respectively. The compound, Phenyl ethyl β-D-glucopyranoside produced zone of inhibition between 9 and 14 mm, at a concentration of 300 μg disc^{-1}.

Table 1: Antibacterial activities of extracts, pure compound, Phenyl ethyl *β-D*-glucopyranoside and Kanamycin standard

	Zone of Inhibition (Diameter in mm)				
Name of bacterial strains	A	B	C	D	E
Gram positive					
Bacillus subtilis	18	20	17	10	29
Bacillus megaterium	11	14	13	9	25
Staphylococcus aureus	16	21	15	9	28
Sarcina lutea	15	16	24	13	27
Gram negative					
Escherichia coli	16	16	15	12	22
Klebsillea species	10	12	14	10	23
Pseudomonas aeruginosa	11	15	24	10	30
Shigella dysenteriae	15	20	17	7	21
Shigella shiga	14	16	24	10	29
Shigella sonnei	13	22	14	9	33
Shigella boydii	14	11	12	7	32

A = Petroleum ether extract (C_2H_5-O-C_2H_5), 500μg disc^{-1}
B = Chloroform extract ($CHCl_3$), 500μg disc^{-1}
C = Ethylacetate extract (CH_3-CO-O- C_2H_5), 500μg disc^{-1}
D = Phenyl ethyl β-D-glucopyranoside, 300μg disc^{-1}
E = Standard Kanamycin, 30μg disc^{-1}

The crude extracts and the glycosidal compound, phenyl ethyl β-D-glucopyranoside showed significant activity against the bacteria tested. Chloroform extract showed more activity than ethylacetate extract against most of the test bacteria and in particular against *Bacillus subtilis* and *Staphylococcus aureus* (Gram positive) and *Shigella dysenteriae* and *Shigella sonnei*. Ethylacetate extract showed highest activity against *Sarcina lutea*, *Shigella shiga* and *Pseudomonas aeruginosa* but petroleum ether extract has shown the lowest activity in most of the test bacteria.

Table 2: The MIC values of the isolated compound against test organisms

	Minimum inhibitory concentration in μg ml^{-1}			
Sample	*Bacillus subtilis*	*Sarcina lutea*	*Escherichia coli*	*Shigella shiga*
Phenyl ethyl β-*D*-glucopyranoside	128	64	64	128

Minimum inhibitory concentration (MIC) values of the compound, phenyl ethyl β-*D*-glucopyranoside were 64 μg ml^{-1} against *Sarcina lutea* and *E. coli* and 128 μg ml^{-1} against *Bacillus subtilis* and *Shigella shiga* (Table 2).

From minimum inhibitory concentration (MIC) values, it was found that this glycosidal compound was more potent against *Sarcina lutea* and *Escherichia coli* possessing less minimum inhibitory concentration (MIC) values against *Bacillus subtilis* and *Shigella shiga*.

In conclusion, the study reports for the first time the antibacterial activity for the crude extracts and the isolated compound from the stem of *Sida rhombifolia*. However, further and specific studies are needed to better evaluate the potential effectiveness of other isolated compound from this plant.

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