Pakistan Journal of Biological Sciences 5(5): 597-599, 2002

Antibacterial and Cytotoxic Activities of Extracts and Isolated Compounds of *Ipomoea turpethum*

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Abstract: Three compounds H-1 ($-sitosteryl-$-D glucoside), H-2 (22, 23-dihydro-"-spinosteryl glucoside) & CH-2 (salicylic acid) isolated from the chloroform extract of stem of *Ipomoea turpethum* and the crude petroleum ether, chloroform and ethyl acetate extracts were screened against thirteen pathogenic bacteria for their antibacterial activities. The minimum inhibitory concentration (MIC) of the isolated compound CH-2 was also measured against *Bacillus subtilis, Shigella dysenteriae, Sarcina lutea and Escherichia coli.* The values were found to be between 128 and 256 µg/ml. The cytotoxic activity of CH-2 and chloroform and ethyl acetate extracts was also measured by brine shrimp lethality bioassay and the LC_{50} values were found to be 56.23, 199.53 and 31.62 µg/ml, respectively.

Key words: *Ipomoea turpethum,* convolvulaceae, antibacterial activity, cytotoxicity.

Introduction

Ipomoea turpethum R. Brown, (Family-Convolvulaceae), Bengali name- "Dudhkalmi", is a large, climbing herb and perennial with milky juice, widely distributed in Bangladesh, India, Ceylon, Mauritania, Philippines, Africa and Australia (Kirtikar and Basu, 1994). The root of this plant is used as anthelmentic, purgative, antipyretic and alexeteric. It is also used in ascites, leucoderma, ulcer, constipation, piles, jaundice, and inflammation (Kirtikar and Basu, 1994; Ghani, 1998). The aqueous leaf extracts of this plant exhibited broad-spectrum antiviral activity (Khan, 1992).

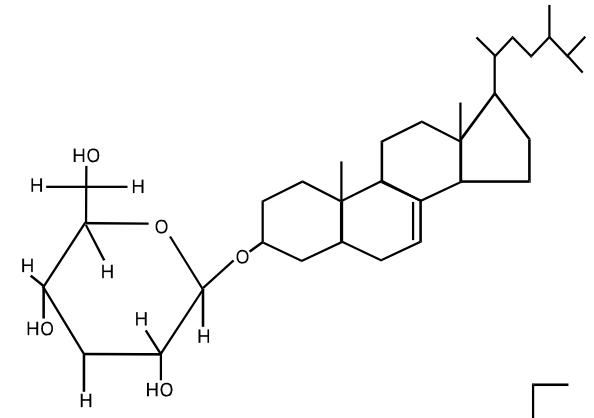
Although the plant *Ipomoea turpethum* is used for the above conditions, no antibacterial and cytotoxic study of this plant has previously been reported. As a part of continuing search for novel antibacterial and cytotoxic principles from the medicinal plants of Bangladesh, we studied *Ipomoea turpethum* and herein report the results of *in vitro* antibacterial and cytotoxic activity of the crude extracts and the isolated compounds.

Materials and Methods

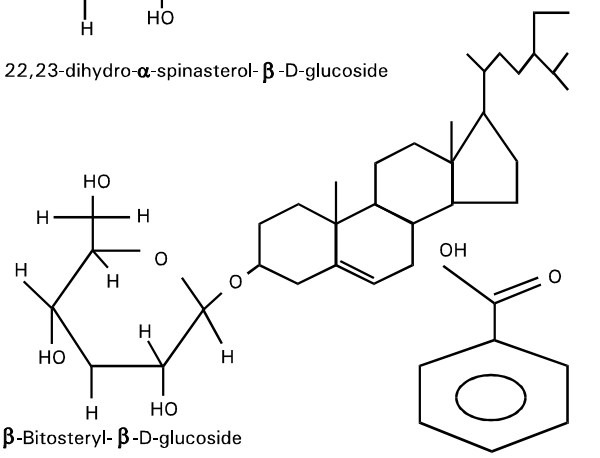
Plant materials: The matured stem of *Ipomoea turpethum* was collected from rural areas of Rajshahi and Naogaon, Bangladesh when it became mature (December - January 1998). The plant was taxonomically identified by Professor A.T.M. Nadiruzzaman, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh and the Bangladesh National Herbarium, Dhaka, Bangladesh.

The stem was cut into pieces, dried in an oven at 45°C and crushed. Then the crushed stem was extracted with methanol in a soxhlet apparatus at 65°C for 72 hrs in "Phytochemistry Research Laboratory, Department of Pharmacy, Rajshahi University, Bangladesh". The extract was filtered and concentrated with a rotary evaporator under reduced pressure at 50E C and the concentrated mass was successively fractionated with Petroleum ether, chloroform and ethyl acetate. By column chromatography (Beckett and Stenlake, 1986) and subsequently "Preparative thin layer chromatography" compound H-1, H-2 & CH-2 were isolated from chloroform fraction. The structures of these three compounds were elucidated by physical, chemical and spectroscopic analysis (Rashid, 2000).

Antibacterial screening: *In vitro* antibacterial activity was performed with crude extracts (petroleum ether, chloroform and ethyl acetate) and three isolated compounds (H-1, H-2 and CH-2) against thirteen pathogenic bacteria (7 gram positive and 6 gram negative) that were collected from the Institute of Nutrition and Food, University of Dhaka and ICDDRB. Nutrient agar and nutrient broth were used as bacteriological media. Petroleum ether, chloroform and ethyl acetate extracts were dissolved in sufficient amount of methanol, so that 10µl of solution contains 200µg of crude extract. Compound H-1, H-2 & CH-2 were also dissolved in methanol at a concentration of 100µg/10µl. The antibacterial activity of these samples was measured against all the test organisms and compared with the standard kanamycin disc (K-30µg/disc) by the standard disc diffusion method (Srivastava, 1984, Bauer *et al*., 1951).



22,23-dihydro-α-spinasterol-β-D-glucoside



β-Bitosteryl-β-D-glucoside

2-Hydroxy Benzoic acid

The MIC of pure compound CH-2 was determined against two gram positive (*Bacillus subtilis* & *Sarcina lutea*) and two gram negative (*Escherchia coli* and *Shigella dysenteriae*) bacteria (10^7cells/ml) by serial dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as bacteriological media.

Cytotoxic activity: The cytotoxic activity of the crude chloroform and ethyl acetate extract and the isolated compound CH-2 was

determined by the Brine shrimp lethality bioassay (Persoone, 1980; Mclaughlin and Anderson, 1988; Mayer *et al*., 1982).

Artemia salina Lech (Brine shrimp eggs) was allowed for 48 hours in seawater to hatch and mature as nauplii (Larvae). Five mg of each sample (crude chloroform, ethyl acetate and CH-2) was dissolved in 1ml dimethyl sulfoxide (DMSO). Test solutions 5, 10, 20, 40 & 80 µl were taken in separate vials and 5ml of the sea water was added to each vial containing 10 nauplii. A control group was used containing 80µl of DMSO and 10 nauplii in 5ml of seawater. After 24 hours, the number of survivors in each vial was counted. From this data the percentage of mortality of the Brine shrimp nauplii was calculated and the LC_{50} values were determined.

Results and Discussion

The crude Petroleum ether, chloroform & ethyl acetate extracts and compound CH-2 showed moderate activities and H-1 & H-2 showed little activities against some gram positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Sarcina lutea, Streptococcus- $- hemolyticus, Psuedomonas aureginosae, Sarcina sarcinaceae*) and some gram negative bacteria (*E. coli, Shigella dysenteriae, Shigella shiga, Shigella boydii, Shigella sonnei, Shigella flexneriae, Salmonella typhi*). The results of antibacterial activity of the extracts and the isolated compounds are shown in Table 1.

The minimum inhibitory concentration (MIC) of compound CH-2 against *Bacillus subtilis, Sarcina lutea, E. coli* and *Shigella dysenteriae* were 128, 128, 256 and 128µg/ml respectively (Table 2).

The cytotoxic activity of CH-2 and crude chloroform & ethyl acetate extract was determined by Brine shrimp lethality bioassay. The median lethal concentration (LC_{50}) was determined by extrapolation from the graph (Fig. 1) and the values were 56.23µg/ml, 199.53µg/ml and 31.62µg/ml for CH-2, crude chloroform & ethyl acetate extracts respectively (Table 3).

Owing to paucity of the sample, it was not possible to determine the MIC and cytotoxicity of H-I and H-2. However, the work is in progress that will be reported elsewhere.

From the antibacterial experimental results, it is evident that the crude extracts and the compound CH-2 showed significant antibacterial activity but were less potent than that of standard kanamycin whereas compound H-1 & H-2 showed little activities. The results of this study further justify the use of this plant in the management of microbial infection.

In cytotoxicity experiment, it was shown that the ethyl acetate extract is much more cytotoxic than chloroform extract. The cytotoxic action of a drug is exhibited by disturbing the fundamental mechanisms concerned with cell growth, mitotic activity, differentiation and function (Goodman *et al.,* 1980). Although the exact mechanism of cytotoxic action of these extracts could not be explaind by these preliminary tests, the results obtained indicate that the extracts may contain a safe and effective chemotherapeutic agent. Therefore there remains a possibility to isolate any important cytotoxic agent from the crude ethyl acetate extract of *Ipomoea turpethum*.

In recent years the pathogenic organisms are gaining resistance to existing antimicrobial agents hence the search for new, safe and more effective antimicrobial agents is a pressing need. Thus the findings of this investigation and previous investigation on other plants (Rahman *et al.,* 2000) would give valuable support to make clinical trial as well as toxicity studies of the isolated antibacterial and cytotoxic metabolites to get a more potent antimicrobial agent.

Table 1: *In vitro* antibacterial activities of extracts, H-1, H-2, CH-2 and Kanamycin.

Test organisms	Diameter of zone of inhibition in mm.						
	I	II	III	IV	V	VI	VII
Gram positive bacteria							
Bacillus subtilis	12	12	10	6	5	4	22
B. megaterium	8	9	8	0	0	13	20
Staphylococcus aureus	15	13	19	7	6	12	22
Sarcina lutea	15	12	13	8	7	14	23
Strepto-$ -hemolyticus	8	10	13	0	0	17	22
Psuedomonas aureginosa	14	12	12	7	6	15	21
Sarcina sarcinaceae	15	12	15	6	0	13	30
Gram negative bacteria							
Escherichia coli	15	9	6	9	5	12	22
Shigella dysenteriae	10	9	10	0	0	9	21
Shigella shiga	14	15	17	5	5	8	25
Shigella boydii	14	11	15	7	6	12	19
Shigella sonnei	15	14	12	10	7	14	23
Shigella flexneriae	10	10	11	9	6	12	19

NB: I = Petroleum ether extract (200 µg/disc), II = Chloroform extract (200 µg/disc), III = Ethyl acetate extract (200 µg/disc), IV = Compound H-1 (100 µg/disc), V = H-2 (100 µg/disc), VI = CH-2 (100 µg/disc) and VII = Kanamycin (K-30µg/disc)

Table 2: The MIC value of the compound CH-2 against *Bacillus subtilis*, *Sarcina lutea, Escherchia coli* and *Shigella dysenteriae*.

Sample	Minimum inhibitory concentration in µg/ml			
	Bacillus subtilis	*Sarcina lutea*	*E. coli*	*Shigella dysenteriae*
CH-2	128	128	256	128

Table 3: Results of the brine shrimp lethality bioassay of chloroform & ethyl acetate extract and isolated compound CH-2

Test samples	concentration (µg/ml)	Log of conc.	% of mortality	LC_{50} µg/ml
EA	5	0.7	25.00	31.62
	10	1.00	30.00	
	20	1.301	45.00	
	40	1.602	52.00	
	80	1.903	64.00	
CL	5	0.7	17.00	199.53
	10	1.00	28.00	
	20	1.301	30.04	
	40	1.602	35.00	
	80	1.903	43.00	
CH-2	5	0.7	16.00	56.23
	10	1.00	27.73	
	20	1.301	36.00	
	40	1.602	47.37	
	80	1.903	54.00	

NB: CL = Chloroform extract & EA = ethyl acetate extract

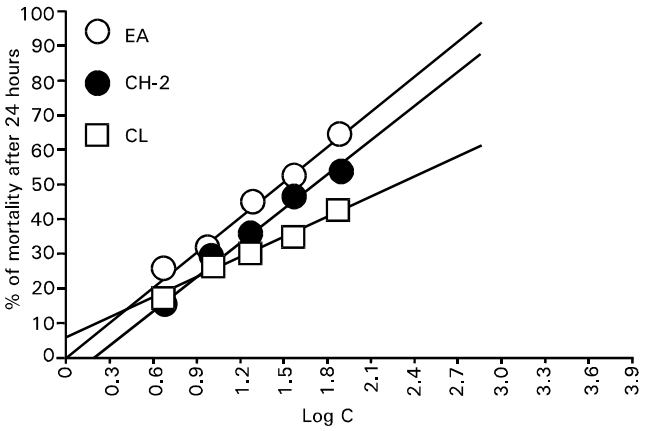


Fig. 1: Brine shrimp lethality bioassay of EA, CL & CH-2

Acknowledgements

The authors like to thank Dr. Naoki Sugimoto, National Institute of Health Sciences, Tokyo, Japan and Dr. Md. Abdur Rashid, Associate Professor, Department of Pharmacy, University of Dhaka, Bangladesh, for spectral analysis. The authors wish to thank Prof. A.T.M. Nadiruzzaaman, Department of Botany,

University of Rajshahi, Rajshahi, Bangladesh and the Bangladesh National Herbarium, Dhaka, Bangladesh, for identification of the plant.

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