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Biological Activities of a New Acrylamide Derivative from *Ipomoea turpethum*

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Abstract: The acrylamide derivative, 3-(4-hydroxy-phenyl)-N-[2-(4-hydroxy phenyl)-ethyl]-acrylamide (H-1) was screened against thirteen pathogenic bacteria for its antibacterial activities. The zones of inhibition produced by the test materials were found between 11 and 20 mm. The minimum inhibitory concentration (MIC) was also measured against *Bacillus subtilis* and *Shigella dysenteriae* and the value was found to be 128 µg/ml. The cytotoxic activity was also measured by brine shrimp lethality bioassay and the LC_{50} values of H-1 and standard ampicillin trihydrate were found to be 56.23 and 6.13 µg/ml.

Key words: *Ipomoea turpethum*, Convolvulaceae, 3-(4-hydroxy-phenyl)-N-[2-(4-hydroxy phenyl)-ethyl]-acrylamide, antibacterial activity, cytotoxicity

Introduction

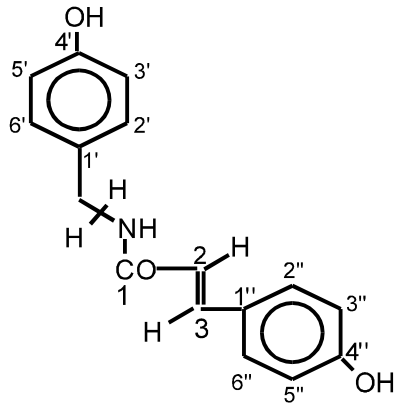
Making health care and medical facilities available to the people is now a major concern of a large number of countries. Due to the toxic and adverse effects of synthetic medicines being observed round the globe, herbal medicine has made a comeback to improve the fulfillment of our present and future health needs. Besides, herbal medicine can cope with the present economic conditions of our people who cannot afford to use the expensive medicine. Therefore our work is primarily concerned with the isolation of bioactive compounds from medicinal plants of Bangladesh. In continuation of our investigation (Abdur Rahman *et al.*, 2000) we selected a local plant "Dudhkalmi", botanical name: *Ipomoea turpethum* R. Brown, (Family-Convolvulaceae), as because the plant has folkloric reputation in native practice in the treatment of ascites, leucoderma, ulcer, constipation, piles, jaundice, inflammation and is also used as anthelmintic, purgative, antipyretic and alexeteric (Kirtikar and Basu, 1994; Ghani, 1998 and Khan, 1992). Previously we reported significant antibacterial and cytotoxic activities of extracts and three isolated compounds from this plant for the first time (Rashid *et al.*, 2002a). Herein we wish to report the antibacterial and cytotoxic activity of a new acrylamide derivative, 3-(4-hydroxy-phenyl)-N-[2-(4-hydroxy phenyl)-ethyl]-acrylamide isolated from the ethyl acetate extract by column and preparative thin layer chromatographic technique (CC and PTLC) and identified by spectral analysis (Beckett and Stenlake, 1986; Rashid, 2000). Previous phytochemical investigation revealed that the presence of acrylamide derivative is the new report in Convolvulaceae family (Rashid *et al.*, 2002b).

Materials and Methods

Plant materials: The matured stem of *Ipomoea turpethum* was collected from rural areas of Rajshahi, Bangladesh during December-January 1998.

Isolation of H-1: Air dried powdered stem (1050 g) was extracted with methanol in a Soxhlet apparatus at 65° 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 was isolated from ethyl acetate soluble fraction as yellowish white, crystalline powder (Rashid, 2000).

Antibacterial screening: *In vitro* antibacterial activity was performed with H-1 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. The compound was dissolved in methanol at a concentration of 100 µg per 10 µl. The activity was compared with the standard kanamycin disc (K-30 µg/disc) by the standard disc diffusion method (Srivastava, 1984; Bauer *et al.*, 1966).



3-(4-hydroxy phenyl)-N-[2-(4-hydroxy Phenyl)-ethyl] acryl amide

The MIC was determined against a Gram positive (*Bacillus subtilis*) and a Gram negative (*Shigella dysenteriae*) bacteria (10^7 cells/ml) by serial dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as bacteriological media.

Cytotoxic activity: The cytotoxic activity of H-1 was determined by the Brine shrimp lethality bioassay and was compared with standard antibiotic, ampicillin trihydrate (Persoone, 1988 and 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 (H-1 and standard ampicillin trihydrate) was dissolved in 1 ml dimethyl sulfoxide (DMSO) then 5, 10, 20, 40 and 80 µl of the test solution were taken in separate vials and 5 ml of the sea water was added to each vial containing 10 nauplii. A control group was used containing 20 µl of DMSO and 10 nauplii in 5 ml of seawater. After 24 hours, the number of survivors in each vial was counted. From this data the percentage mortality of the brine shrimp nauplii was calculated and the LC_{50} values were also determined.

Results and Discussion

A combination of column chromatography and preparative thin layer chromatography of the ethyl acetate extract of the stem of *Ipomoea turpethum* yielded a new metabolite 3-(4-hydroxy-phenyl)-N-[2-(4-hydroxy phenyl)-ethyl]-acrylamide that was identified by its extensive spectroscopic analysis.

The compound showed remarkable antibacterial activity against both Gram positive and Gram negative bacteria in comparison with standard Kanamycin disc (Table 1). The zone of inhibition was observed between 11 to 18 mm for Gram positive and 12 to 20 mm for Gram negative bacteria. Maximum inhibition was

Table 1: *In vitro* antibacterial activities of H-1 and Kanamycin

Species	H-1 (100 µg/disc)	Kanamycin (30 µg/disc)
Gram positive bacteria		
1. *Bacillus subtilis*	15	22
2. *B. megaterium*	11	20
3. *B. cereus*	15	22
4. *Psuedomonas aeroginosa*	15	23
5. *Staphylococcus aureus*	16	22
6. *Strepto-β -hemolyticus*	11	21
7. *Sarcina lutea*	18	30
Gram negative bacteria		
1. *Escherichia coli*	18	22
2. *Shigella dysenteriae*	12	21
3. *Shigella shiga*	20	25
4. *Shigella boydii*	12	19
5. *Shigella sonnei*	13	23
6. *Shigella flexneriae*	18	19

Table 2: The MIC value of the compound H-1

	Minimum inhibitory concentration in µg/ml	
Sample	*Bacillus subtilis*	*Shigella dysenteriae*
H-1	128	128

Table 3: Results of the brine shrimp lethality bioassay of H-1 and standard ampicillin trihydrate

Test samples	concentration (µg/ml)	Log of concentration	% of mortality	LC_{50} µg/ml
Control	20 µL DMSO	0.0	0.00	
AT	5	0.7	46.66	6.13
	10	1.00	53.33	
	20	1.301	66.66	
	40	1.602	76.66	
	80	1.903	80.00	
H-1	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: AT= Ampicillin trihydrate

observed against *Shigella shigae* so the compound might be useful against diseases caused by *Shigella* species.

The minimum inhibitory concentration (MIC) was done against *Bacillus subtilis* and *Shigella dysenteriae* and the value was 128 µg/ml against each organism (Table 2).

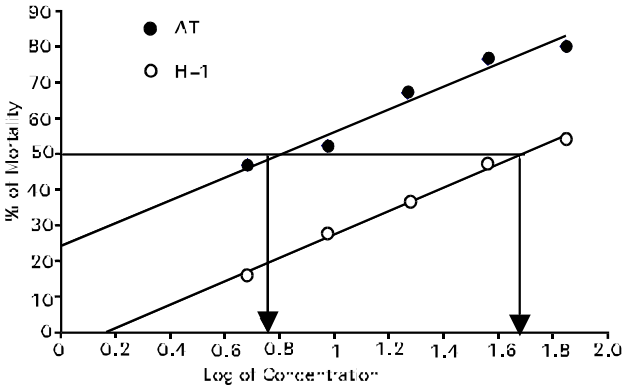


Fig. 1: Brine shrimp lethality bioassay of AT (Ampicillin trihydrate) and H-1

The median lethal concentration (LC_{50}) of H-1 and standard antibiotic was determined by extrapolation from the graph (Fig. 1) and the value was 56.23 and 6.13 µg/ml (Table 3).

From the antibacterial experimental results, it is evident that the compound H-1 showed significant antibacterial activity but were less potent than that of standard kanamycin. This antibacterial activity of 3-(4-hydroxy-phenyl)-N-[2-(4-hydroxy phenyl)-ethyl]-acrylamide is the new report from plant source.

In cytotoxicity experiment, it was shown that the compound H-1 is relatively less cytotoxic than standard antibiotic. 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 the compound could not be explained by these preliminary tests, it may be comment that the acrylamide derivative which is the new report from Convolvulaceae family may be used as a safe and effective chemotherapeutic agent. Thus the findings of this investigation and previous investigations (Abdur Rahman *et al.,* 2000 and Rashid *et al.,* 2002a) 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.

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