

**CYTOTOXICITY AND ANTIBACTERIAL ACTIVITY OF *ANDROGAPHIS PANICULATA*,
EUPHORBIA HIRTA AND *URGINIA INDICA***

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ABSTRACT

Based on the traditional uses, petroleum etheric, ethanolic and aqueous extracts of *Andrographis paniculata* leaves, *Euphorbia hirta* leaves and *Urginea indica* bulbs were subjected to cytotoxicity and antibacterial activity test. In brine shrimp lethality test, the LC₅₀ of aqueous extract of *Andrographis paniculata* leaves was found to be 600 µg/ml and that of ethanolic and aqueous fractions of *Euphorbia hirta* leaves were 800 and 80 µg/ml respectively. Similarly, the ethanolic and petroleum etheric extracts of *Urginea indica* bulbs showed significant mortality in the test shrimp and LC₅₀ were found to be 600 and 100 µg/ml respectively. Again, in disc diffusion test aqueous extract of *A. paniculata* leaves showed significant inhibition against three bacteria, *Bacillus megaterium* (6 mm), *Shigella dysenteriae* (9 mm) and *Vibrio cholera* (9 mm). Similarly, the ethanolic extract of *E. hirta* significantly inhibited the growth of *Bacillus subtilis* (6 mm), *Bacillus megaterium* (7 mm) *Shigella dysenteriae* (13 mm) and *V. cholera* (9 mm). However, the aqueous extract of the plant showed no effect on the growth of the test bacteria. And in case of *U. indica* bulb extracts, ethanolic fraction produced remarkable zone of inhibition against *Bacillus subtilis* (7 mm), *Bacillus megaterium* (5 mm), *Staphylococcus aureus* (12 mm), *Salmonella typhi* (9 mm) and *Vibrio cholera* (11 mm). On the other hand petroleum etheric fraction of it produced zone of inhibition only against *Bacillus subtilis* (7 mm) and *Bacillus cereus* (9 mm).

KEYWORDS: *Andrographis paniculata*, *Euphorbia hirta*, *Urginea indica*, Antibacterial, Cytotoxic.

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INTRODUCTION

Medicines derived from plants have pivotal role in health care of ancient and modern cultures. Almost 60% of drugs approved for cancer treatment are of natural origin like vincristine, vinblastine, etoposide, taxanes and camptothecins are all examples of plant-derived anticancer compounds¹. But most of the anticancer drugs have inevitable severe adverse reactions. Therefore, it is an imperative need of the day to develop alternative therapeutic measures with low risk of adverse effects against this deadly disease. There is always a hope that the traditional medicinal plants may provide potent and safe remedies. Among the recent advances in cancer chemotherapy, phytochemicals play an important role. Similarly, plants are one of the natural sources of antimicrobial agents. A wide range of plants those possess various medicinal properties are used in the preparation of herbal dugs. The different parts like root, stem, flower, fruit, twigs, exudates and modified plant organs are used as drugs. While some of these raw drugs

are collected in smaller quantities by the local communities and folk healers for local use. A vast majority of these plants have not been yet adequately evaluated by using standard scientific methods². The people of Bangladesh especially of rural areas are dependent on herbal treatment. Different Ayurvedic preparations available in market throughout the country which are used topically for skin infections and benign tumor contain *A. paniculata*. Moreover, the villagers in different areas of the country use the latex of *E. hirta* against the warts and infection by injury like cuts or burns. The traditional practitioners use different parts of the plant in treatment of asthma, bronchitis, worm infection, conjunctivitis and dysentery. In Yunani medicines *U. indica* is used as one of the active ingredients and these are used for the treatment of skin diseases as well as internal pains and scabies. Some herbal products, which are used as tumor suppressants also contain *U. indica*.

Considering the above magnitude of the plants as one of the natural sources for anticancer and antimicrobial drugs a systematic exploration was undertaken to screen different fractions of *Andrographis paniculata* (Burm.f.) Wall. (Family- Acanthaceae), *Euphorbia hirta* Linn. (Family-Euphorbiaceae) and *Urgenia indica* Kunth. (Family-Liliaceae) for cytotoxicity and antibacterial activity.

MATERIALS AND METHODS

Plant materials

The fresh plant parts were collected from Chittagong, Bangladesh. Then those were kept in the sun under a shed till dried. Taxonomical identification of the plants was done by the taxonomist, botanist of Bangladesh Council for Scientific and Industrial Research Herbarium, Chittagong, Bangladesh. And the herbarium sheets signed by the taxonomist Mohammad Mohiuddin and were preserved in Pharmacognosy Laboratory of University of Science and Technology.

Preparation of extract

For the preparation of water extract the dried *A. paniculata* and *E. hirta* leaves were ground and the powder was packed in the thimble of a Soxhlet apparatus and extracted with 1000 ml of distilled water at 100°C for 48 hrs. Again, the ethanol and petroleum ether extract of *E. hirta* leaves and *U. indica* bulbs were prepared by simple maceration in an air tight container. Fine powder materials were merged in the respective solvents and the homogenates were kept for 2 weeks at room temperature (25±2°C). The entire extracts were filtered twice. At first, using sterile cotton and then Whatman-41 filter paper. Petroleum ether and ethanol were completely evaporated at room temperature by using an electric fan facilitating the evaporation of the solvents. While aqueous extracts were dried by using rotary vacuum evaporator at 100°C, 1 atmospheric pressure in a flask with 80 rpm.

Shrimp eggs and Microorganisms

The brine shrimp eggs for cytotoxicity assay and bacterial strains - *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella sonnei*, *Shigella dysenteriae*, *Salmonella typhi* and *Vibrio cholerae*, for antibacterial sensitivity assay were collected from Bangladesh Council of Scientific and Industrial Research, Bangladesh.

Media

Nutrient agar, Nutrient broth media manufactured by HiMedia Laboratories Pvt. Ltd. India were used as bacterial culture media.

Chemicals

Ethanol (≥99.5%; Merck KGaA, Germany) was used as solvent in maceration extraction of the plant material. Sea salt (Sodium Chloride Crystal GR; Merck Ltd., Mumbai, India) was used as medium for hatching the eggs of shrimp. In cytotoxicity test dimethyl sulfoxide (≥99.9%, BioReagent, for molecular biology; Sigma-Aldrich, India) was used as solvent to dissolve the extracts. In antibacterial sensitivity assay amoxicillin (Ultrafen, Tab. 50mg, Beximco Pharmaceuticals Ltd., Bangladesh) was used as reference standard. The water used for hot percolation and other purposes was prepared in laboratory by distillation.

Growth and Maintenance of Test Bacteria

Bacterial cultures of *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella sonnei*, *Shigella dysenteriae*, *Salmonella typhi* and *Vibrio cholerae* were obtained from the Microbiology Laboratory of Bangladesh Council of Scientific and Industrial Research, Chittagong. The bacteria were maintained on nutrient broth (NB) at 37°C³.

Preparation of Inoculums

Preliminary culture of the bacterial strains *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella sonnei*, *Shigella dysenteriae*, *Salmonella typhi* and *Vibrio cholerae* were prepared in nutrient broth and kept overnight in a rotary shaker at 37°C, then centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610 nm})³.

Brine shrimp lethality test

In vitro lethality assay of the different fractions of *A. paniculata*, *E. hirta* and *U. indica* were used to detect cell toxicity⁴. Brine shrimp eggs were placed in seawater (3.8% w/v sea salt in distilled water) and incubated at 24-28°C in front of a lamp. Eggs were hatched for 48 hrs and a large number of larvae (nauplii) were born in the water. Then extract solutions (50 µg/ml) were prepared by using the solvent, dimethyl sulfoxide (DMSO). Ten sample concentrations (20 – 1000 µg/ml) were used; 3 test tubes for each concentration. With the help of a Pasteur pipette 10 living shrimps were taken in ten test tubes. In control test tubes seawater and DMSO were taken. Alive nauplii were counted after 16 hrs and the lethal concentrations (LC₅₀) were calculated from mean percent mortality. The plot of percent mortality versus log concentration of the extract produced an approximate linear correlation between them on graph. From the graph (Figure 1.1-1.5) the concentration at which 50%

mortality (LC₅₀) of brine shrimp nauplii occurred were obtained. The resulting data are summarized in table 1.

Antibacterial sensitivity test

Antibacterial activity of different fractions of *A. paniculata*, *E. hirta* and *U. indica* were tested by disc diffusion method⁵. Here 100 µl of suspension of entire bacteria containing 2.0×10^6 cfu/ml were taken in sterile Petri-dishes (9 mm in diameter) containing sterile sabouraud dextrose agar medium (15 ml). The filter paper discs (5 mm in diameter) were individually impregnated with the crude extracts at the dose of 100 µg/disc and then placed onto the agar medium in the Petri-dishes which were previously inoculated with the test bacteria. After that the Petri-dishes were kept at 4°C for 2 hrs in a refrigerator and subsequently incubated at 37°C for 24 hrs in an incubator. The diameter of the zone of inhibition was measured in millimeter. Amoxicillin (30 µg/disc) was used in positive control and ethanol (solvent) was used in negative control.

The resulting data are summarized in the table 2.

Statistical analysis

The results of brine shrimp lethality and antibacterial sensitivity test were statistically evaluated by using one way ANOVA. Values with $p < 0.5$ were considered significant.

RESULTS AND DISCUSSION

In this project different fractions of *Andrographis paniculata*, *Euphorbia hirta* and *Urgenia indica* were subjected to brine shrimp lethality and antibacterial sensitivity test. In cytotoxicity test by using brine shrimp the extracts of the plants produced significant cell toxicity. From table 1 it was observed that aqueous extract of *A. paniculata* leaves was produced 50% mortality (LC₅₀) at the concentration of 600 µg/ml. And that of the ethanolic and aqueous fractions of *E. hirta* were found to be 800 and 80 µg/ml respectively. Similarly, ethanolic and petroleum etheric extracts of *U. indica* were also found to be cytotoxic with LC₅₀ of 600 and 100 µg/ml respectively. Thus the study depicts that the extract may contain bioactive compounds having antitumor, anticancer or pesticide properties. However, this cannot be confirmed without further higher and specific tests.

On the other hand, in antibacterial disc diffusion test different fractions of *Andrographis paniculata*, *Euphorbia hirta* and *Urginea indica* were found to be active but not against all of the test bacteria and showed various degree of zone of inhibition. From table 2 it was

observed that aqueous extract of *A. paniculata* leaves produced significant inhibition against three species such as; *Bacillus megaterium* (6 mm), *Shigella dysenteriae* (9 mm) and *Vibrio cholerae* (9 mm). But there was no zone of inhibition in entire Petri-dishes containing aqueous extract of *E. hirta* while observed in naked eyes. Nevertheless, the ethanolic extract of the plant showed distinct zone of inhibition against *Bacillus subtilis* (6 mm), *Bacillus megaterium* (7 mm), *Shigella dysenteriae* (13 mm) and *Vibrio cholerae* (9 mm). And among the two fractions of *U. indica* bulbs the ethanolic one produced notable zone of inhibition against *Bacillus subtilis* (7 mm), *Bacillus megaterium* (5 mm), *Staphylococcus aureus* (12 mm), *Salmonella typhi* (9 mm) and *Vibrio cholera* (11 mm). But the petroleum etheric extract of the plant was active only against two bacteria - *Bacillus subtilis* (7 mm) and *Bacillus cereus* (9 mm). Thus the results depicted that the extracts may contain some biologically active compounds which have antibacterial activity.

The results of present investigation clearly indicated that cytotoxicity as well as antibacterial activity varied with the species of the plant and the solvents used for extraction. Thus the study ascertained the importance of plants as traditional remedies of Ayurvedic, Yunani and Herbal medicine systems which could be of considerable interest to the development of new drugs.

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REFERENCES

1. Grever MCB. Cancer drug discovery and Anticancer therapy targeting the apoptotic development. In: De Vita VHS and Rosenberg SA, editors. Cancer: Principles and Practice of Oncology. 8th ed. Lippincott Williams & Wilkins; 2009. p.328-339.
2. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of Industrial and Medicinal materials. Science 1985; 228:1154-1160.
3. Mahesh B, Satish S. Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. World Journal of Agricultural Sciences 2008; 4 Suppl 1:839-843.
4. Meyer BN, Ferrigni NR, Putnam JE, Jacobson JB, Nicholas DE, McLaughlin JL. Brine shrimp A convenient general bioassay for active plant constituents. Planta Medica 1982; 45:31-34.
5. Latha L, Yoga S, Sasidharan Z, Zuraini S, Suryani L, Shirley S et al. Antimicrobial activities and toxicity of crude extract of the *Psophocarpus tetragonolobus* pods. Afr J Trad CAM 2007; 4(1): 59-63

Table 1: Cytotoxic activity of different fractions of *A. paniculata*, *E. hirta* and *U. indica*

Sample	% Mortality										LC ₅₀ (µg/ml)
	Concentration (µg/ml)										
	20	40	60	80	100	200	400	600	800	1000	
	Log concentration										
	1.3	1.6	1.8	1.9	2.0	2.3	2.6	2.8	2.9	3.0	
AEAP	30	30	40	40	40	40	40	50	50	60	600
EEEH	30	40	40	40	40	40	40	40	50	50	800
AEEH	30	40	40	50	50	50	60	60	60	60	80
EEUI	20	30	30	30	40	40	40	50	50	50	600
PEEUI	20	30	30	40	50	50	50	50	60	60	100
Mean	26	34	36	40	44	44	46	50	54	56	
SD	5.48	5.48	5.48	7.07	5.48	5.48	8.94	7.07	5.48	5.48	

p<0.0001; calculated by using one way ANOVA where Fisher F-value = 11.08; SD = Standard deviation

UIEE = *Urgenia indica* ethanolic extract, UIPEE = *Urgenia indica* petroleum etheric extract, EHEE = *Euphorbia hirta* ethanolic extract, EHAE = *Euphorbia hirta* aqueous extract and APAE = *Andrographis paniculata* aqueous extract

Table 2: Antibacterial activity of different fractions of *A. paniculata*, *E. hirta* and *U. indica*

Sample (0.1 mg/disc)	Zone of Inhibition(mm)									
	B.su	B.ce	B.me	E. co	P.ae	S.au	S.so	S. dy	S.ty	V.ch
AEAP	nd	nd	6	nd	nd	nd	nd	9	nd	11
AEEH	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
EEEH	6	nd	7	nd	nd	nd	nd	13	nd	9
EEUI	7	nd	5	nd	nd	12	nd	nd	9.5	11
PEEUI	7.0	9.0	nd	nd	nd	nd	nd	nd	nd	nd
AM	nd	nd	37	nd	8	12	8.0	22	7	6

p<0.5; calculated by using one way ANOVA where Fisher F-value = 1.35; nd = Not detected

AM = Amoxicillin, B.su = *Bacillus subtilis*, B.ce = *Bacillus cereus*, B.me = *Bacillus megaterium*, E.co = *Escherichia coli*, S.au = *Staphylococcus aureus*, S.so = *Shigella sonnei*, S.ty = *Salmonella typhi*, P.ae = *Pseudomonas aeruginosa*, S.dy = *Shigella dysenteriae* and V.ch = *Vibrio cholera*

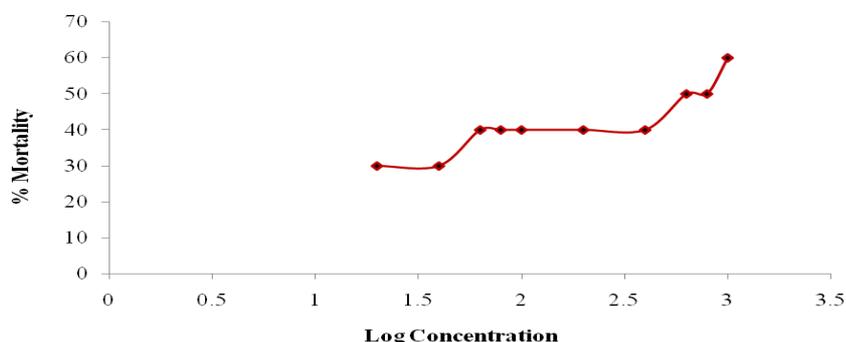


Figure 1.1: The effect of aqueous extract of *Andrographis paniculata* in brine shrimp lethality assay

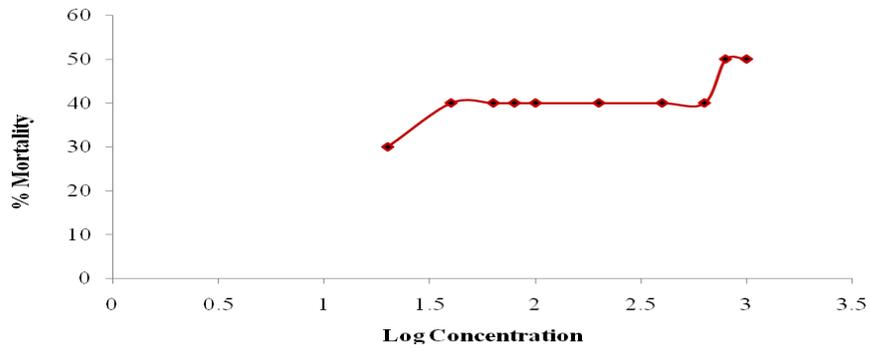


Figure 1.2: The effect of ethanolic extract of *Euphorbia hirta* in brine shrimp lethality assay

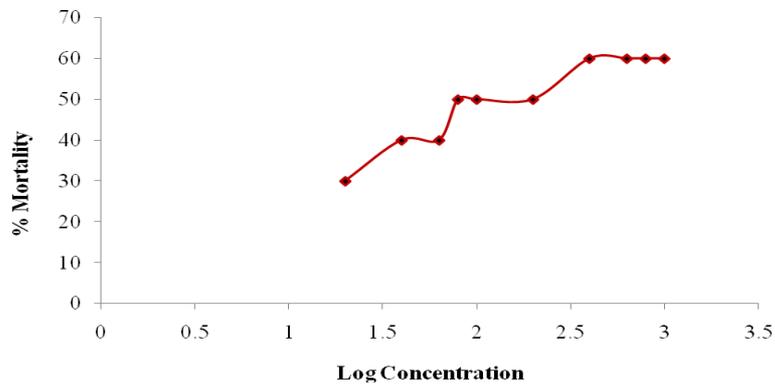


Figure 1.3: The effect of aqueous extract of *Euphorbia hirta* in brine shrimp lethality assay

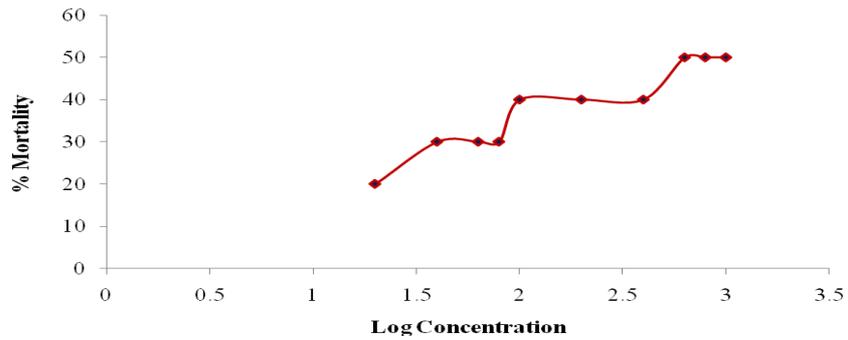


Figure 1.4: The effect of ethanolic extract of *Urgenia indica* in brine shrimp lethality assay

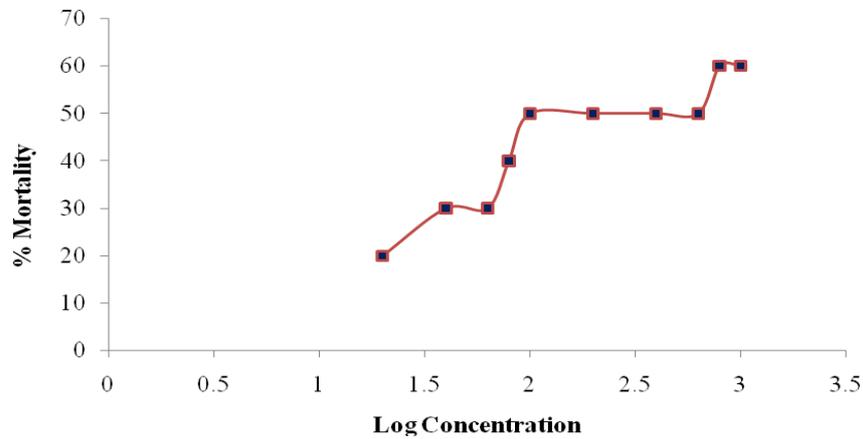


Figure 1.5: The effect of petroleum etheric extract *Urgenia indica* in brine shrimp lethality assay

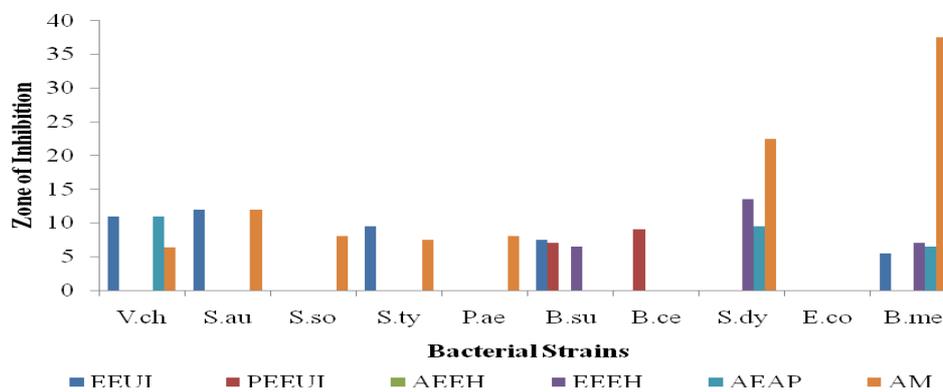


Figure 2: Antibacterial activities of different fractions of *A. paniculata*, *E. hirta* and *U. indica*

UIEE = *Urgenia indica* ethanolic extract, UIPEE = *Urgenia indica* petroleum etheric extract, EHEE = *Euphorbia hirta* ethanolic extract, EH aqueous extract and APAE = *Andrographis paniculata* aqueous extract; AM = Amoxicillin, V.ch = *Vibrio cholera*, S.au = *Staphylococcus c sonnei*, S.ty = *Salmonella typhi*, P.ae = *Pseudomonus aeruginosa*, B.su = *Bcillus subtilis*, B.ce = *Bacillus cereus*, S.dy = *Shigella dysenteriae*, and B.me = *Bacillus megaterium*

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