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Biological Evaluation of Extracts and Triterpenoids of *Euphorbia hirta*

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Abstract. Antibacterial and antifungal activities of crude extracts and two triterpenoids, taraxerone (EH-1) and 11α, 12α-oxidotaraxerol (EH-2) isolated from the plant *Euphorbia hirta* were tested against fourteen pathogenic bacteria and six fungi. Crude extracts and pure compounds exhibited significant activity against most of the bacteria tested. On the other hand, all the crude extracts and pure compounds were active, but not significant enough, against most of the tested fungi. The minimum inhibitory concentrations (MICs) of the isolated compounds were also determined against the tested organisms (10^7 cells/ml) and the effective values were found to be between 64-128 μg/ml. In the brine shrimp lethality bioassay, the compounds were screened for their probable cytotoxic activity, and the LC_{50} values of EH-1 and EH-2 were found to be 17.78 and 10 μg/ml, respectively.

Keywords: *Euphorbia hirta*, triterpenoids, brine shrimp lethality bioassay, antibacterial activity, antifungal activity, taraxerone, 11α,12 α-oxidotaraxerol

Introduction

Many microorganisms can cause several diseases and now, in this world of modern science, human beings are capable of facing any challenge against these diseases. In spite of the tremendous advancement of medical science and technology, nevertheless, diseases are the leading health problem, particularly in the under-privileged populations in the remote rural areas in the developing countries. In Bangladesh, a very poor country with poor hygiene, diarrhoea, cholera, typhoid, malaria, diptheria, etc., are major causes of morbidity and mortality.

Euphorbia hirta, Family Euphorbiaceae, is commonly grown in almost all the districts of Bangladesh, hotter parts of India, and other tropical and subtropical countries, where the plant is used in traditional medicine for several illnesses since the time immemorial. Juice of the plant is given in the treatment of aphthae, dysentery and colic diseases, and is used by women to increase the flow of milk. A decoction is used to treat asthma and chronic bronchial affections. The plant is chiefly prescribed in the treatment of cough, gonorrhoea and bowel complaints. On the Gold Coast, it is ground and mixed with water and used as an enema (Kirtikar and Basu, 1996).

Due to the fact that this plant is very useful, as found by previous reports and the fact that little information is available on its biological activity, there is a need to find out more about the potential of this plant as an antimicrobial agent. The present study is, therefore, designed to assess the potency of the plant extracts and triterpenoids isolated therefrom on selected microorganisms and also to determine the cytotoxic effect of these compounds.

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Materials and Methods

Source of the plant and microbiological cultures. The plant *Euphorbia hirta,* was collected from the Rajshahi University Campus, Bangladesh. Organisms used in the present studies were obtained from the Department of Pharmacy, Rajshahi University, pure cultures of which were previously procured from the Institute of Food and Nutrition, University of Dhaka, and also from ICDDR, Bangladesh. All solvents used during this study were redistilled and purified. Other chemicals, including the culture media used, were of analytical grade unless otherwise specified.

Plant material preparation, extraction procedure, and compound identification. The whole plant of *E. hirta* was cut into small pieces, dried in an oven at 40 °C to a constant weight, pulverized into fine powder in a grinding machine, and stored in an airtight container. One kg of the powdered plant material was exhaustively extracted with rectified spirit at room temperature. Solvent of the extract was evaporated under reduced pressure and the extract was fractionated with petroleum ether

(40-60 °C), chloroform and methanol. Compounds EH-1 and EH-2 (Fig .1) were isolated from the petroleum ether extract by column chromatography (Beckett and Stenlake, 1986), followed by TLC and preparative TLC (Egon, 1969). The compounds were identified as taraxerone (EH-1) and 11α, 12α-oxidotaraxerol (EH-2) on the basis of spectral data coupled with physical and chemical evidences (Bhattacharjee, 2002),

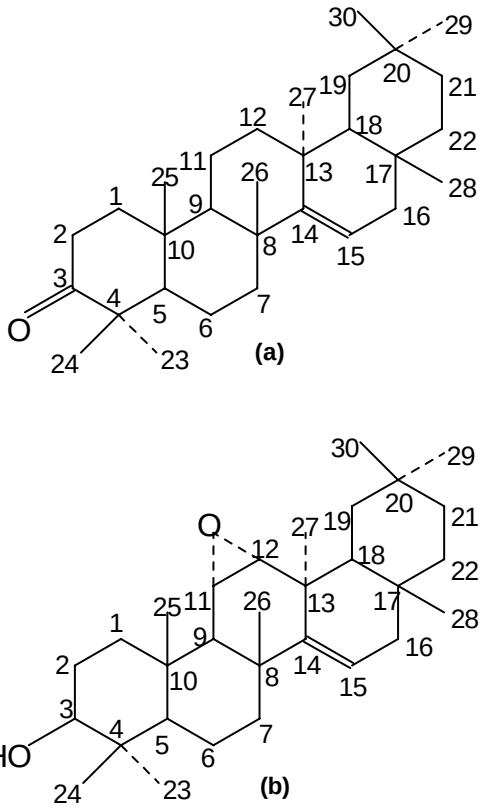

Fig. 1. Compounds isolated from the petroleum ether extract of *Euphorbia hirta,* identified as (a) taraxerone (EH-1) and (b) 11α, 12 α-oxidotaraxerol (EH-2).

and also by comparison with the previously reported values (Kuo *et al.,* 1996; Tanaka and Matsunaga, 1988).

Antimicrobial screening. *In vitro* antibacterial and antifungal screenings were performed with the crude petroleum ether, chloroform and methanol extracts, as well as the isolated pure compounds (EH-1 and EH-2) against 14 pathogenic bacteria, 5 of which were gram-positive and 9 were gram-negative, and 6 pathogenic fungi by the standard disc diffusion method (Barry, 1980; Bauer *et al.,* 1966). Nutrient agar medium was used for determining the antibacterial activity, whereas potato dextrose agar medium (PDA) was selected for antifungal screening. Standard antibiotic discs of kanamycin (30 µg/disc) and fluconal (50 µg/disc) were used for the comparison of antibacterial and antifungal efficiency tests, respectively.

The crude extracts were dissolved in sufficient amounts of the respective solvents so that each 15 µl solution contained 400 µg of the test material. On the other hand, compounds EH-1 and EH-2 were dissolved separately in sufficient volume of chloroform to get a concentration of 200 µg/10 µl. The antimicrobial activities were determined by measuring the diameter of the inhibitory zones in mm using a transparent scale. The diameters of the zones of inhibition produced by the tested samples were then compared with the diameters of the zones of inhibition produced by the standard antibiotic discs used.

Minimum inhibitory concentration (MIC). The MIC values of compound EH-1 were determined against the gram-positive *Sarcina lutea* and the gram-negative *Shigella dysenteriae* (10^7 cells/ml), while the MIC values of compound EH-2 were evaluated against the gram-positive *Streptococcus aureus* and the gram-negative *Shigella dysenteriae* (10^7 cells/ml) by the serial dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as bacteriological media.

Brine shrimp lethality bioassay. Cytotoxic effects of compounds EH-1 and EH-2 were evaluated by determining LC_{50}, using the brine shrimp lethality test (Mayer *et al.,* 1982; Persoone, 1980). The test sample was dissolved in DMSO and specific volumes were transferred to the different vials containing 10 living shrimp nauplii to which seawater was added to make the volume upto 5 ml in each vial. The final concentrations of the test sample in the vials was 6, 12, 24, 48 and 96 µg/ml. Three replicates were done for each concentration. A control was also run similarly, by taking 10 living shrimps nauplii in 5 ml seawater, but without any test sample isolated from the plant. The same assay procedure was performed with standard ampicillin trihydrate for comparison of the efficiency of the test samples.

After incubation for 24 h, the vials were observed and the number of shrimp nauplii deaths in each vial was counted, using a magnifying glass. From these data, the mean percentage of mortality of the nauplii was calculated for each concentration of the test sample used.

Results and Discussion

As shown in Table 1, all the extracts (petroleum ether, chloroform, and methanol) displayed mild to moderate activity against most of the tested bacteria. The results were compared with those of kanamycin as the standard antibiotic. Of the three extracts, chloroform extract did not show any activity against gram-negatives, *Pseudomonas aeruginosa* and

Escherichia coli, whereas methanol extract was inactive only against the gram negative, *Pseudomonas aeruginosa*. On the other hand, the purified compounds, EH-1 and EH-2, isolated from *E. hirta,* were found to be active against all the tested bacteria. The compound EH-1 exhibited strong activity against the gram-positive *Sarcina lutea* (16 mm) and gram-negative *Shigella dysenteriae* (17 mm), whereas the compound EH-2 showed strong activity against the gram-positive *Streptococcus aureus* (18 mm) and the gram-negative *Shigella dysenteriae* (16 mm). Results depicted in Table 2 demonstrate that all the extracts and the pure compounds, EH-1 and EH-2, were active but not significantly enough, against most of the fungi tested. Furthermore, they did not have any activity against *Aspergillus niger* and *Trichoderma viride*.

During the preliminary screening work, it was found that the compound EH-1 was strongly active against the gram-positive *Sarcina lutea* and the gram-negative *Shigella dysenteriae,* whereas compound EH-2 displayed strong activity against the gram-positive *Streptococcus aureus* and the gram-negative *Shigella dysenteriae*. An attempt was, therefore, made to determine the minimum inhibitory concentrations (MICs) of these compounds against the above mentioned organisms. As shown in Table 3, MIC values of the compound EH-1 were found to be 64 µg/ml and 128 µg/ml against *Sarcina lutea* and *Shigella dysenteriae,* respectively, and those of EH-2 were the same, i.e., 128 µg/ml against *Streptococcus aureus* and *Shigella dysenteriae*. It is expected that further work on the plant may yield clinical success for controlling diseases caused by pathogenic bacteria.

Compounds EH-1 and EH-2 exhibited positive results on brine shrimp lethality bioassay, indicating toxic biological activity. The mortality rate of brine shrimp nauplii was found to increase with the increase in concentration of the compounds and a plot of log of concentration against percentage of mortality gave almost linear correlation (Fig. 2). From the graph, the LC_{50} (concentration at which 50 % mortality of the nauplii occured), as estimated by extrapolation, was found to be 17.78 µg/ml for EH-1, and 10.00 µg/ml for EH-2 (Table 4).

Table 1. Antibacterial activities of different extracts and the purified compounds, EH-1 and EH-2, isolated from *Euphorbia hirta*

	Diameter of zone of inhibition (mm)					
Test organisms	A	B	C	D	E	F
Gram-positive bacteria						
Bacillus subtilis	10	12	14	10	11	29
B. cereus	12	9	13	13	10	28
B. megaterium	10	12	11	9	14	30
Sarcina lutea	14	16	13	16	12	25
Streptococcus aureus	13	10	14	10	18	23
Gram-negative bacteria						
Escherichia coli	9	0	10	13	10	20
Shigella dysenteriae	12	10	14	17	16	22
S. sonnei	10	11	12	12	11	25
S. shiga	11	10	13	11	10	22
S. boydii	13	9	11	14	13	24
S. flexneriae	10	11	13	12	11	22
Pseudomonas aeruginosa	10	0	0	10	12	30
Salmonella typhi	10	10	12	12	14	24
Klebsiella sp.	13	10	12	14	13	23

A= petroleum ether extract (400 µg/disc); B = chloroform extract (400 µg/disc); C = methanol extract (400 µg/disc); D = EH-1 (200 µg/disc); E = EH-2 (200 µg/disc); F = kanamycin (30 µg/disc)

Table 2. Antifungal activities of different extracts and the purified compounds, EH-1 and EH-2, isolated from *Euphorbia hirta*

	Diameter of zone of inhibition (mm)					
Test organisms	A	B	C	D	E	F
Aspergillus flavus	10	8	12	12	10	17
Aspergillus niger	0	0	0	0	0	0
Penecillum sp.	7	8	10	8	10	11
Trichoderma viride	0	0	0	0	0	10
Candida albicans	10	8	12	13	10	16
Botryodiplodia theobromae	7	9	10	10	9	14

A = petroleum ether extract (400 µg/disc); B = chloroform extract (400 µg/disc); C = methanol extract (400 µg/disc); D = EH-1 (200 µg/disc); E = EH-2 (200 µg/disc); F = fluconal (50 µg/disc)

Table 3. Minimum inhibitory concentration (MIC) values of purified compounds, EH-1 and EH-2, isolated from *Euphorbia hirta* against the tested bacterial organisms

Test organisms	Tested sample	Concentrations of the tested sample (µg/ml)									
		512	256	128	64	32	16	8	4	2	1
Sarcina lutea	EH-1	-	-	-	-	+	+	+	+	+	+
Shigella dysenteriae		-	-	-	+	+	+	+	+	+	+
Strept.aureus	EH-2	-	-	-	+	+	+	+	+	+	+
Shigella dysenteriae		-	-	-	+	+	+	+	+	+	+

- = no growth; + = growth

Table 4. Cytotoxicity of purified compounds, EH-1 and EH-2, isolated from *Euphorbia hirta,* using brine shrimp lethality bioassay

Test samples	Conc (µg/ml)	Log conc (Log C)	Number of shrimps died* vial 1	vial 2	vial 3	Average number of deaths	Mortality (%)	LC_{50} (µg/ml)
Ampicillin trihydrate	6	0.7781	4	5	4	4.333	43.33	
	12	1.0791	6	6	7	6.333	63.33	
	24	1.3802	7	8	8	7.666	76.66	
	48	1.6812	9	9	10	9.333	93.33	
	96	1.9822	10	10	9	9.666	96.66	7.49
EH-1	6	0.7781	3	3	3	3.000	30.00	
	12	1.0791	4	5	4	4.333	43.33	
	24	1.3802	6	6	5	5.666	56.66	
	48	1.6812	7	7	6	6.666	66.66	
	96	1.9822	8	8	8	8.000	80.00	17.78
EH-2	6	0.7781	4	4	4	4.000	40.00	
	12	1.0791	5	5	6	5.333	53.33	
	24	1.3802	6	7	7	7.000	70.00	
	48	1.6812	8	8	9	8.333	83.33	
	96	1.9822	9	10	10	9.666	96.66	10.00

*10 shrimp nauplii were taken in each vial; control comprised of no test sample added, in which no deaths occurred

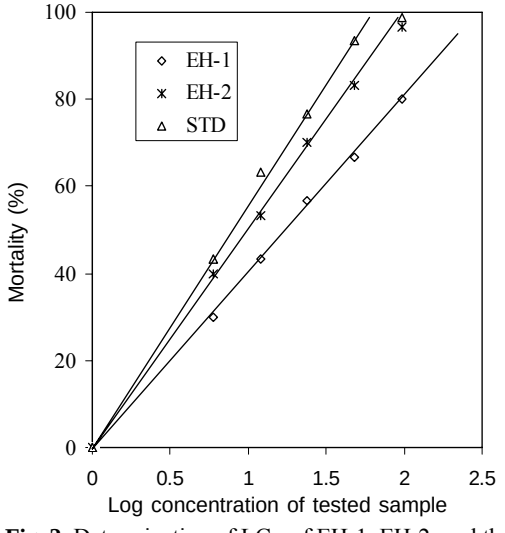

Fig. 2. Determination of LC_{50} of EH-1, EH-2, and the standard ampicillin trihydrate (STD) against brine shrimp nauplii.

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