



Phytochemical and Pharmacological Evaluation of Methanolic Extract of *Euphorbia hirta* L

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ABSTRACT

Background: *Euphorbia hirta* is a plant mostly known as dudhiya in locally; belonging to the family Euphorbiaceae has been investigated for the presence of its secondary metabolites and evaluation of biological activities of the crude extractives with special emphasis to the antibacterial, anti-inflammatory and hypoglycemic activities. The phytochemical constituents of the dried powdered plant parts were extracted using organic solvents (methanol).

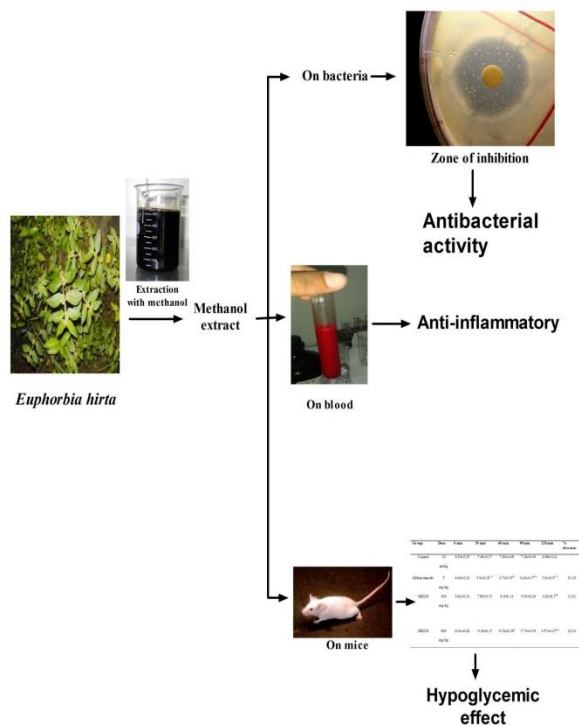
Methods: Primary phytochemical screening was accomplished by using established methods. Antibacterial activity tested on three gram-positive and three gram-negative bacteria by disk diffusion method. *In vitro* anti-inflammatory effect tested on the human RBC cell caused by the control group and comparing it with the positive and test group. The *in vivo* action was done using mice of both sexes. Hypoglycemic effect evaluated by oral glucose tolerance test (OGTT).

Results: In our study of the antibacterial assay, we have tried *Euphorbia hirta* preparations against some gram positive and gram-negative bacteria. The extract at two different concentrations 1000 µg/disc and 700 µg/disc showed significant as compared with standard Kanamycin 30 µg/disc showed zone of inhibitions against Gram-positive *Staphylococcus aureus* (Nil), *Bacillus subtilis* (10.0 ± 1.00, 7.0 ± 0.50), *Bacillus cereus* (Nil), *Salmonella typhi* (9.5 ± 0.50, 8.0 ± 0.29), *Salmonella paratyphi* (11.0 ± 0.50, 7.5 ± 0.50), *Escherichia coli* (12.5 ± 0.50, 8.5 ± 0.50) respectively. The percentage of membrane stabilization for methanolic extract of *Euphorbia hirta* is effective in inhibiting the heat-induced hemolysis of HRBC at different concentrations (1000, 500, 250, 125 µg/ml). Hence anti-inflammatory activity of the extracts was concentration dependent. In OGGT, the percentage of decrease of blood glucose level in glucose-induced mice after 2 hours with different treatment. MEEH at the highest dose of 800 mg/kg decreased blood glucose level (24.34%) than other treatments, accept standard Glibenclamide.

Conclusion: The present research suggests that that *Euphorbia hirta* leaf extract has significant antibacterial, anti-inflammatory and hypoglycemic activities. We can say that the obtained results support for the uses of this plant as traditional medicine.

Keywords: *Euphorbia hirta*, Methanol, Thrombolytic, Cytotoxic, Brine Shrimp, Acetic Acid.

GRAPHICAL ABSTRACT



INTRODUCTION

Bangladesh has a lot of medicinal plants and from the plants, many drugs and medicines were prepared from these plants [1, 2]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antibacterial, hypoglycemic and other properties [3,4]. Therefore, scientific studies have been carried out on the antibacterial, activities of plant extracts against different types of bacteria, which have resulted in the development of alternative plant-based antibacterial drugs and also hypoglycemic drugs [5].

Euphorbia hirta is widely used medicinal plants in Bangladesh. As per Unani system of medicine they are used in the treatment of gastrointestinal disorders (diarrhea, dysentery, intestinal parasitosis, etc.), bronchial and respiratory diseases (asthma, bronchitis, hay fever, etc.) [6-8]. The stem sap is used in the treatment of eyelid styes and a leaf poultice is used on swelling and boils. Decoction of dry herbs is used for skin

diseases. Decoction of fresh herbs is used as a gargle for the treatment of thrush. Species of this family are often used for several medicinal purposes.

E. hirta has been studied by various workers and some active constituents have been isolated. Afzelin, quercitrin, and myricitrin have been isolated from the methanolic extract of *E. hirta* [9]. The chemical investigation of *E. hirta* has led to the isolation of rutin, quercetin, euphorbin-A, euphorbin-B, euphorbin-C, euphorbin-D, 2,4,6-tri-O-galloyl- β -D-glucose, 1,3,4,6-tetra-O-galloyl- β -D-glucose, kaempferol, gallic acid, and protocatechuic acid [10,11]. *E. hirta* also contains β -amylin, 24-methylenecycloartenol, β -sitosterol, heptacosane, nonacosane, shikmic acid, tinyatoin, choline, camphol, and quercitol derivatives containing rhamnose and chtolphenolic acid [12,13]. As this plant contain several compounds, so there are possibilities of their potential activities.

So the principal aim of the study was to investigate the scientific basis of the traditional uses of the plant *Euphorbia*

hirta and in the same time find out the chemical groups present in the active parts to get preliminary idea about the active constituent. The main objective of the study was to investigate whether the Methanol extract of *Euphorbia hirta* possess

antimicrobial, cytotoxic and hypoglycemic activity or not.

MATERIALS AND METHODS

Plant materials

The plant material named as *Euphorbia hirta* was selected based on its medicinal uses. Using standard taxonomical methods, supplied by the Bangladesh Forest Research Institute (BFRI), Chittagong identified the plant's leaves. The plants were collected from Chittagong University's area. It was then separated and cleaned from impurities.

Preparation of sample

The leaves of the plant were dried without sunlight, through fan for 35 days. The leaves of the plant were cut into small pieces and ground into fine powder with the help of grinder. Then the powder of the plant stored in air tight container and placed in a cool, dry, dark place. 200 grams of dried powder was weighed and taken in an aspirator (5 L). Before placing powders into the aspirator, the jar was washed properly and then dried. 700 ml of solvent (methanol) was added gradually. The container with its content was sealed and kept for 20 days with occasional shaking & stirring. The major portion of the extractable compounds of the plant materials was dissolved in the solvent. Then the whole mixture was filtered through cotton wool, and the filtrate was concentrated by evaporation in dry and clean air. And finally, the methanol extract of *Euphorbia hirta* (MEEH) prepared.

Chemicals and reagents

All chemicals used were of analytical reagent grade. Methanol was purchased from Merck, Germany. Kanamycin (30 µg/disc, Oxoid, England) was used as a standard antibiotic disc. Dextrose, sodium citrate, citric acid and sodium chloride were purchased from Sigma-Aldrich and rested of the chemicals used were of BDH.

Phytochemical screening

The freshly prepared crude methanol extract was qualitatively tested for the presence of secondary metabolites especially Alkaloids, Glycosides, Cardiac glycosides, Steroids, Coumarin, Tannins, Flavonoids, Saponins and Reducing sugar through

established methods [14].

In vitro antibacterial activity

Antibacterial screening by disk diffusion technique

MEEH was screened at two concentrations (700 and 1000 µg/disc) against three gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) and three-gram negative bacteria (*Salmonella typhi*, *Salmonella paratyphi*, *Escherichia coli*) using the disc diffusion method [15-17]. Solutions of known concentration (33.3 mg/mL) of the test samples were prepared. Dried and sterilized filter paper discs (about 5 mm diameter) were then impregnated with known amounts (30 µl for 1000 µg/disc, 21µl for 700 µg/disc) of the test substances using a micropipette. Discs containing the test material were placed on nutrient agar medium (Merck, India) uniformly seeded with the pathogenic test microorganisms. The prepared inoculum size was approximately 10⁶ CFU/mL. Standard antibiotic discs (kanamycin, 30 µg/disc) and blank discs (impregnated with solvents) were used as positive and negative controls, respectively. These plates were then, kept at 4°C for a 1 h diffusion of the test material. There was a gradual change in concentration surrounding the discs. The plates were then, incubated at 37°C for 24 h to allow organism growth. The test materials having antibacterial activity inhibited microorganism growth, and a clear, distinct zone of inhibition surrounding the discs was visualized [18]. The antibacterial activity of the test agents was determined by measuring the diameter of the zone of inhibition expressed in millimeters (mm).

In vitro membrane stabilization activity

Anti-inflammatory activity of MEEH was evaluated by using *in vitro* human red blood cell stability method. A blood sample was collected from a fresh volunteer, who doesn't have anti-inflammatory or contraceptive drugs at least since a week. The collected blood was mixed with sterilized Alsever solution. Alsever solution was prepared by 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved

in distilled water. A blood sample was centrifuged at 3000 rpm and the packed cell was washed with isosaline and a 10% (V/V) suspension of isosaline was made. Five different concentration of the solution of MEEH was mixed with 1 ml phosphate buffer, 2 ml hyposaline and 0.5 ml HRBC

suspension. Diclofenac-Na was used as a contrastable drug and instead of hyposaline 2 ml water was used as a control. The hemoglobin content in the supernatant was calculated using Spectrophotometer at 560 nm spectrum. The result was estimated by following equations [19,20].

Hypoglycemic effect of glucose-induced hyperglycemic mice (OGTT)

Oral glucose tolerance test (OGTT) was performed according to the standard method with minor modification. Group I was treated as normal control group, Group II treated with glibenclamide (5 mg/ kg body weight), Group III-IV were treated with methanol extract of *Euphorbia hirta* leaves at 400 mg/kg and 800 mg/kg body weight respectively. Glucose solution (1 g/kg body weight) was administered at first [21]. Then drug and extract solutions were administered to the glucose-fed. Serum glucose level of a blood sample from tail vein was estimated by using glucometer at 0, 30min, 60min, 90 min and 120 min. Areas under the curves (AUC) for OGTT were calculated to evaluate glucose tolerance [22]. Percent decrease of blood glucose level after 120 min measured by

following equation,

$$\% \text{ decrease} = \frac{GL_{0 \text{ min}} - GL_{120 \text{ min}}}{GL_{0 \text{ min}}} \times 100$$

$GL_{0 \text{ min}}$ = Blood Glucose level at 0 min, $GL_{120 \text{ min}}$ = Blood Glucose level at 120 min

Statistical analysis

The results were expressed as Mean \pm SD from the triplicate experiment for the zone of inhibition from triplicate experiments for Antibacterial activity. Data were analyzed using one way ANOVA tests using SPSS Data Editor for Windows, Version 22.0 (SPSS Inc., USA) followed by Dennett's tests. $P < 0.05$, $P < 0.01$ and $P < 0.001$ was considered to be statistically significant in Dennett's tests. GraphPad PRISM[®] (version 6.00; GraphPad Software Inc., San Diego, CA, USA) was used for graphical presentation.

RESULTS

Results of *In vitro* phytochemical test

The results of various chemical tests for the detection and identification of chemical constituents are summarized in Table 1. The present phytochemical study indicates the presence of some chemical constituents in the plant parts which are responsible for the various pharmacological activity of the plant.

Table 1: Chemical constituents identified in the leaves extract

Examination	Test performed	Methanol
		extract
Alkaloids	Wagner's test	+
Glycosides	General test	+
Cardiac glycosides	Keller-killiani test	-
Steroids	Salkowski's test	+
Coumarin	General test	-
Tannins	Ferric chloride test	+
Flavonoids	Conc. HCl & alcoholic test	+
Saponins	Shake test (aq. Solution)	-
Reducing sugar	Fehling's test	+

Where, + = Present and - = Absent

In vitro antibacterial activity

Antibacterial activity results of *Euphorbia hirta* leaves extract are given in Table 2. The extract at two different concentrations 1000 µg/disc and 700 µg/disc showed significant as compared with standard Kanamycin 30 µg/disc showed zone of inhibitions against Gram-positive *Staphylococcus aureus* (Nil), *Bacillus subtilis* (10.0 ± 1.00, 7.0 ± 0.50), *Bacillus cereus* (Nil), *Salmonella typhi* (9.5 ± 0.50, 8.0 ± 0.29), *Salmonella paratyphi* (11.0 ± 0.50, 7.5 ± 0.50), *Escherichia coli* (12.5 ± 0.50, 8.5 ± 0.50) respectively. The extract showed the highest zone of inhibition against the Gram-negative *Escherichia coli* (12.5 ± 0.50, 8.5 ± 0.50) at

concentration 1000 µg/disc. However, *Staphylococcus aureus* and *Bacillus cereus* showed the no antibacterial activity to the extract *Euphorbia hirta* leaves.

In vitro membrane stabilization activity

The inhibition of hypotonicity induced HRBC membrane lysis i.e., stabilization of HRBC membrane was taken as a measure of the membrane stabilization activity. The percentage of membrane stabilization for methanolic extract of *Euphorbia hirta* is effective in inhibiting the heat-induced hemolysis of HRBC at different concentrations (1000, 500, 250, 125 µg/ml) as shown in Table 3. It showed the maximum % of protection *Euphorbia hirta* (36.4%) at 1000 µg/ml. Hence anti-inflammatory activity of the extracts was concentration dependent shown in Table 3.

Table 2: Results of antibacterial activity testing of *Euphorbia hirta* leaves

Name of the bacteria	Zone of inhibition			
	Negative control (Methanol)	<i>Euphorbia hirta</i>		Kanamycin
		1000 µg/disc	700 µg/disc	30 µg/disc
	30 µg/disc			
Gram-Positive				
<i>Staphylococcus aureus</i>	-	-	-	22.2 ± 0.76
<i>Bacillus subtilis</i>	-	10.0 ± 1.00	7.0 ± 0.50	18.2 ± 0.29
<i>Bacillus cereus</i>	-	-	-	25 ± 0.50
Gram Negative				
<i>Salmonella typhi</i>	-	9.5 ± 0.50	8.0 ± 0.29	25.3 ± 0.58
<i>Salmonella paratyphi</i>	-	11.0 ± 0.50	7.5 ± 0.50	20.3 ± 0.29
<i>Escherichia coli</i>	-	12.5 ± 0.50	8.5 ± 0.50	23.5 ± 0.50

Values are mean inhibition zone (mm) ± S.D of three replicates

Table 3: Percent of inhibition of methanol extract of *Euphorbia hirta*

Sample	Concentration (µg/ml)	Absorbance of Sample	% of hemolysis	% of protection	
				By <i>Euphorbia hirta</i>	By Diclofenac
1	1000	0.354	64.01447	36.4	47.42
2	500	0.465	84.0868	34.7	45.83
3	250	0.477	86.25678	28.6	45.05
4	125	0.478	86.43761	23.4	44.44
Control	0	0.553			

Hypoglycemic effect in oral glucose tolerance test mice (OGTT) in mice

Investigational induction of hyperglycemia resulted in increased glucose level in the blood (comparing the data of control of 0 h and 1 hour, Table 4). Both doses of leaves extract did not manifest any significant reduction in 30 min after administration. Most significant reduction ($P < 0.05$) was observed for 800 mg/kg dose of methanol extract of *Euphorbia hirta* at 120 min. At 120 min this dose also showed a significant reduction. Standard glibenclamide (5 mg/kg) showed a significant reduction in 30, 60, 90 and 120 min. These findings suggest that evidently, 800 mg/kg dose is more potent than 400 mg/kg dose. Time interaction with each

specific hour in this experiment was also found significant. Percentage of decrease in blood glucose level in glucose-induced mice after 2 hours with different treatment are also presented in Table 4. MEEH at the highest dose of 800 mg/kg decreased blood glucose level (24.34%) than other treatments, accept standard Glibenclamide.

Values with different superscripts in the same column are significantly different from control at each specific hour after the administration of different groups. For ^a $P > 0.05$, ^b $P < 0.01$ and ^c $P < 0.001$. One-way ANOVA followed by Dunnett's multiple comparisons was performed to analyze this comparison. “-” means no decrease.

Table 4: Effect of *Euphorbia hirta* leaves extract on glucose-induced hyperglycemia (mmol/L) in normal mice

Group	Dose	0 min	30 min	60 min	90 min	120 min	% decrease
Control	10 ml/kg	6.52 ± 0.35	7.46 ± 0.37	7.80 ± 0.49	7.28 ± 0.39	6.69 ± 0.21	-
Glibenclamide	5 mg/kg	6.46 ± 0.24	5.6 ± 0.18 ^c	4.7 ± 0.19 ^a	4.24 ± 0.17 ^b	3.8 ± 0.15 ^c	41.18
MEEH	400 mg/kg	5.62 ± 0.34	7.98 ± 0.33	6.9 ± 0.14	5.63 ± 0.28	4.82 ± 0.3 ^a	14.23
MEEH	800 mg/kg	6.04 ± 0.28	9.46 ± 0.2 ^a	6.52 ± 0.38 ^a	5.74 ± 0.39	4.57 ± 0.27 ^a	24.34

Values are presented in mean ± SEM (n=6). MEEH= methanol extract of *Euphorbia hirta*

DISCUSSION

The present study has been approached to demonstrate the phytochemical investigation, Antibacterial, Anti-inflammatory and *in vivo* hypoglycemic activities of methanol extract of the leaves of *Euphorbia hirta*. The leaves of the plant were extracted by cold extraction process using Methanol as a solvent. The extract was then tested for the

presence of some chemical constituents by different identification technique, which gave positive result about the presence of Glycoside, Alkaloid, Steroid, Tannin, Flavonoid and Reducing sugar in both extracts of leaves.

In our study of the antibacterial assay, we have tried *Euphorbia hirta* preparations against some gram positive and gram negative bacteria. The extract at two different

concentrations 1000 µg/disc and 700 µg/disc showed significant as compared with standard Kanamycin 30 µg/disc showed zone of inhibitions against Gram-positive *Staphylococcus aureus* (Nil), *Bacillus subtilis* (10.0 ± 1.00 , 7.0 ± 0.50), *Bacillus cereus* (Nil), *Salmonella typhi* (9.5 ± 0.50 , 8.0 ± 0.29), *Salmonella paratyphi* (11.0 ± 0.50 , 7.5 ± 0.50), *Escherichia coli* (12.5 ± 0.50 , 8.5 ± 0.50) respectively. The extract showed the highest zone of inhibition against the Gram-negative *Escherichia coli* (12.5 ± 0.50 , 8.5 ± 0.50) at concentration 1000 µg/disc. However, *Staphylococcus aureus* and *Bacillus cereus* showed the no antibacterial activity to the extract *Euphorbia hirta* leaves.

The inhibition of hypotonicity induced HRBC membrane lysis, stabilization of HRBC membrane was taken as a measure of the membrane stabilization activity. The percentage of membrane stabilization for methanolic extract of *Euphorbia hirta* is effective in inhibiting the heat-induced hemolysis of HRBC at different concentrations (1000, 500, 250, 125 µg/ml). Hence anti-inflammatory activity of the extracts was concentration dependent.

Investigational induction of hyperglycemia resulted in increased glucose level in the blood (comparing the data of control of 0 h and 1 hour, Table 3). Both doses of leaves extract did not manifest any significant reduction in 30 min after administration. Most significant reduction ($P < 0.05$) was observed for 800 mg/kg dose of methanol extract of *Euphorbia hirta* at 120 min. At 120 min this dose also showed a significant reduction. Standard glibenclamide (5 mg/kg) showed a significant reduction in 30, 60, 90 and 120 min. These findings suggest that evidently 800 mg/kg dose is more potent than 400 mg/kg dose. Time interaction with each specific hour in this experiment was also found significant.

Percentage of decrease in blood glucose level in glucose-induced mice after 2 hours with different treatment. MEEH at the highest dose of 800 mg/kg decreased blood glucose level (24.34%) than other treatments, except standard Glibenclamide.

CONCLUSIONS

The overall results of the study indicated significant Antibacterial, Anti-inflammatory and Hypoglycemic activities of methanol extracts of leaves of *Euphorbia hirta*, it can be expected that distinctive dynamic auxiliary metabolites were available in this concentrate and maybe some of these mixes may work synergistically. On the other hand, further studies are important to illustrate the component lying with these impacts. On the other hand, this is the first write about this example and it may serve as a stride concerning the natural and pharmacological exercises of this specimen. But further studies are also required to identify the phytoconstituents responsible for these bioactivities and to establish the mechanism of action of such activities.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

EMPLOYMENT OR LEADERSHIP: None declared.

HONORARIUM: None declared.

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