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Phytochemical Screening and Evaluation of Antioxidant, Antibacterial and Cytotoxicity Activities of Methanolic and Ethanolic Extracts of *Enhydra fluctuans* Lour (Helencha) (Family: Asteraceae) Stems and Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Author NAH carried out the laboratory tests and prepared the plant extracts and managed the literature searches. Author MSI prepared the draft of the manuscript and made necessary corrections after peer review process. Author SS designed the study, wrote the protocol and managed the analyses of the study. Authors SJM and FIS performed the graphical evaluations and checked the manuscript. Author MB reviewed the scientific contents of the manuscript. All the authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the presence of various phytochemicals and to evaluate DPPH scavenging activity, total flavonoid content, antimicrobial and cytotoxic activities of the different extracts of *Enhydra fluctuans* lour.
Place and Duration of Study: The study was carried out between June 2017 to November 2017 in the Department of Pharmacy, Daffodil International University, Dhaka, Bangladesh.

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Methodology: The leaves and stems of *Enhydra fluctuans* lour were extracted with ethanol and methanol. By qualitative analysis phytochemical evaluation was carried out using DPPH scavenging activity and total flavonoid content determined in vitro antioxidant activity of the crude extract. Cytotoxic activity was determined by using brine shrimp lethality bioassay and antimicrobial activity was determined by using disc diffusion method.
Results: The presence of flavonoids, steroids, saponins, carbohydrates, vitamin C, alkaloids, diterpenthees, and amino acids are influence by phytochemical study. The DPPH radical scavenging activity of methanolic and ethanolic extract were mild to moderate than ascorbic acid (standard). The IC_{50} value of methanolic and ethanolic extract were respectively 619.55,115.67 µg/ml compared with the standard ascorbic acid with IC_{50} value of 17.81 µg/ml. The total flavonoid contents were 67 mg (QE/gm extract) and 21 mg (QE/gm extract) for 500 µg/ml concentration for two extract. The ethanolic extract of 400 µg/disc exhibited the highest zone of inhibition of 12 mm against *Escherichia coli.* The LC_{50} value of methanolic and ethanolic extract were 3.29, 27.446 µg/ml respectively compared with the standard vincristine sulphate with LC_{50} value of 0.632 µg/ml.
Conclusion: All of those study of a methanolic and ethanolic extract of *Enhydra fluctuans* prove that it has an antioxidant, antimicrobial and cytotoxic activity.

Keywords: Phytochemical; antioxidant; Enhydra fluctuans Lour; cytotoxic activity; antibacterial activity.

ABBREVIATIONS

DMSO : Dimethyl sulphooxide
LC_{50} : Lethal concentration 50
E. fluctuans Lour: *Enhydra fluctuans* Lour
ASA : Ascorbic acid
CA : Catechin

1. INTRODUCTION

Medicinal plants have played an important role in treating various kinds of diseases, increased drug resistance and side effects of pharmaceutical drugs have led to a more research-based study on traditionally available plants [1]. The use of medicinal plants can represent the best solution for different diseases [2]. *Enhydra fluctuans* Lour (Family Asteraceae) commonly Bengali name as Hingcha Sag, helencha, hinche, hingcha, hincha. It has potential as a medicinal plant and also has numerous beneficial chemical constituents such as rich in protein and is a good source of β-carotene. It also contains saponins, flavonoids, tannins, phenols, carbohydrates, myricyl alcohol, kaurol, cholesterol, sitosterol, glucoside, sesquiterpene lactones including germacranolide, enhydrin, fluctuanin and fluctuandin, a number of diterpenoid acids and their isovalerate and angelate derivatives, stigmasterol, cholesterol, sitosterol, glucoside, other steroids and gibberellins A_9 and A_{13} have been isolated from this plant [1-3]. The stems are somewhat fleshy, 30 centimeters or more in length, branched, rooting at the lower nodes, and somewhat hairy, leaves are stalkless, linear-oblong, 3 to 5 centimeters in length, pointed or blunt at the tip, usually truncate at the base. The flowering heads are without stalks, are borne singly in the axils of the leaves, and excluding the bracts, are less than 1 centimeter in diameter. The outer pair of the involucral bracts is ovate, and 1 to 1.2 centimeters long; the inner pair is somewhat smaller. The flowers are white or greenish-white. The acheness is enclosed by rigid receptacle-scales [4]. *E. fluctuans* leaves which are used to treat inflammation, skin diseases, laxative, bronchitis, nervous affection, neuralgia, leucoderma, gonorrhea, biliousness and smallpox [5], also is used as a cooling agent which is applied cold to the head by paste formulation [6]. *E. fluctuans* Lour has multiple pharmacological activities that include antimicrobial activity [7-9], anti-inflammatory activity [1], anti-oxidant activity [10], cytotoxic activity [7], CNS depressant activity [11], hepatoprotective activity [12], antidiarrheal activity [13], analgesic activity [14], cytotoxic activity [7]. So the aim of the present work was to investigate the antioxidant, cytotoxic and anti-bacterial activity of the methanolic and ethanolic extract of leaves and stems of *E. fluctuans* lour.

2. METHODS AND MATERIALS

2.1 Collection of Sample and Preparation of Extract

For this present investigation the plant of *Enhydra fluctuans* was collected from Naryangonj, Sonargaon on December 2016. For the identification of plant parts leaves and stems were sent to Bangladesh National Herbarium

Mirpur, Dhaka on 30 January 2017.The specimen of plant was taxonomically identified at the Bangladesh National Herbarium, Mirpur, Dhaka and its accession number is 43114. About 140gm of dried and powdered plant material was soaked in 500ml of methanol in an amber glass container for about 14 days at room temperature with occasional shaking. After 14 days, the solution was filtered using cotton filter and Whitman's filter paper number 1. The filtrate was concentrated to solid mass by using a rotary evaporator [15,16].

2.1.1 Drugs and chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St. Louis, MO, USA); ethanol, methanol, Quercetin, Sodium hydroxide and ascorbic acid were purchased from Merck, Germany Other chemicals were obtained from local sources and were of analytical grade.

2.1.2 Phytochemical screening

Phytochemical screening of the fruit extract was tested for the presence of active principles such as alkaloids, flavonoids, tannins, proteins, etc. using the standard procedures by Rohit Kumar Bargah [17,18].

2.1.3 Test for steroids (Salkowski Test)

In this steroid test, the 2 ml of the extract was mixed with 2 ml of acetic anhydride and then boiled and colored. 2 ml concentrated sulphuric acid was added from the sides of the test tube. Observation: The formation of reddish brown ring at the junction which indicate a positive test for steroids.

2.1.4 Test for saponins (Foam Test)

In this saponin test, the 5 ml of the extract was shaken vigorously with 5 ml distilled water in a test tube and the mixture was warmed. Observation: The formation of stable foam was taken as an indication of the presence of saponins.

2.1.5 Test for glycosides (Keller Killiani Test)

Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

2.1.6 Test for alkaloids (Dragendroff's Test)

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

2.1.7 Test for tannins (Braymer's Test)

In this tannin test, the 2 ml of the extract was mixed up with 2 ml of distilled water and 2-3 drops of $FeCl_3$ (5%) solution were added. The formation of green color precipitate indicates the presence of tannins. Observation: Green coloration precipitate which indicates the presence of tannins.

2.1.8 Test for vitamin C (DNPH Test)

Test solution was treated with dinitrophenylhydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

2.1.9 Test for amino acids (Xanthoproteic Test)

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

2.1.10 Test for flavonoids

In this, the 1 ml of the flavonoid test extract and 1ml of lead acetate Pb $(OAc)_4$ (10%) solution is added [19]. Observation: Yellow coloration precipitate was taken as a positive result for flavonoids.

2.1.11 Test for carbohydrates (Molisch's Test)

In this carbohydrate test, the 2 ml of the extract was added with10ml H_2O, treated with 2 drops of ethanolic αnaphthol (20%) solution and added concentrated sulphuric acid in a test tube. Observation: The formation of the reddish violet ring at the junction which indicates the presence of carbohydrates.

2.1.12 Test for carbohydrates (Benedict's Test)

Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in the water

bath, observed for the formation of reddish-brown precipitate to show a positive result for the presence of carbohydrate.

2.1.13 Test for diterpenes (copper acetate Test)

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

2.2 Antioxidant Evaluation

2.2.1 DPPH radical scavenging assay

DPPH was used to evaluate the free radical scavenging assay as described by Abdel-Rahman et al. [20]. About 2 ml of the methanol solution of plant extract or standard at different concentration was taken in a test tube and 3ml of methanol solution of DPPH was added into the test tube. The test tube was incubated at room temperature for 30 minutes in dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against blank. Blank solution contained all reagents except plant extract or standard solution. The percentage (%) inhibition activity was calculated from the following equation:

% DPPH radical scavenging activity = $\{(A_0 - A_1)/A_0\} \times 100$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. The experiment was repeated three times at each concentration. The experiment was repeated three times at each concentration.

2.2.2 Determination of total flavonoids

The total flavonoids contents of methanolic and ethanolic extracts of *E. fluctuans* were estimated by aluminium chloride colorimetric method as described by Zhisen, et al. [21] with some modification. Quercetin was used as standard and the flavonoid content of the extracts were expressed as mg of Quercetin equivalent / gm of dried extract. To 0.5 ml of the sample solution, 2.5 ml of distilled water was added. Then 0.15 ml of sodium nitrate solution was added and mixed together. All the test tubes were kept in the dark place for 6 minutes. Then 0.3 ml 10% aluminium chloride hydrated solution was added into the test tube and wait for 5 minutes in the dark place for complete reaction. Finally, 1 ml sodium hydroxide solution and 0.55 ml distilled water were added. Then the solution was mixed together very well. The absorbance was measured of all the sample at fixed wavelength 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared using 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.81 µg/ml, 3.90 µg/ml, 1.95 µg/ml, 0.97 µg/ml solution of Quercetin in methanol or ethanol. Flavonoid compounds in plant extracts in Quercetin equivalents was calculated by the using equation:

$C = (c \times V)/m$

Where, C = total content of flavonoid compounds, mg/g plant extract, in Quercetin equivalent (QE); c = the concentration of Quercetin established from the calibration curve, mg/ml; V = the volume of extract, ml; m = the weight of pure plant extracts, gm. The experiment was repeated three times at each concentration.

2.3 Antibacterial Test

The antibacterial activity of ethanolic of extract of *E. fluctuans* leaves were determined by disc diffusion technique of Abdel-Rahman, et al. [22,23] against four bacteria. The bacterial strains used for the experiment were collected from the Daffodil International University, Dhaka, Bangladesh. Each of the extract was again dissolved in respective solvents to be applied on sterile filter paper at 200 µg/disc, 250 µg/disc, 300 µg/disc, 400 µg/disc and cautiously dried to evaporate the remaining solvent. Standard antibiotic, ciprofloxacin (30 µg/disc) was used as a positive control. The extract was tested against one Gram-positive and three Gram negative bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis).* The antibacterial activities of the extract were ascertained by measuring the respective zone of inhibition in millimeters.

2.4 Cytotoxicity Evaluation

The brine shrimp lethality bioassay was determined by the method reported by Meyer and Zhao et al. [24-25]. 4 mg of methanolic and ethanolic extracts were dissolved in DMSO and solutions of various concentrations such as 400 µg/ml to 0.0390 µg/ml were obtained by the

serial dilution technique. Standard Vincristine Sulfate was used as the positive control and DMSO was used as the control, respectively. Next ten matured shrimps were taken to each of the experimental vials and the control vial. The number of the nauplii that died after 24 hours was counted and the LC_{50} was calculated from the regression equation, obtained from the logarithm of sample concentration versus percentage mortality of the shrimp nauplii. The experiment was repeated three times at each concentration.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The chemical group tests were performed and the results are mentioned in the Table 1. Here (+) means the component is present and (-) means the component is absent. The phytochemical screening of ethanolic extract of *E. fluctuans* indicated the qualitative presence of flavonoids, steroid, saponins, carbohydrate, vitamin C, alkaloid, diterpenes, amino acids and the absences of tannins, glycosides. Methanolic extract of *E. fluctuans* indicated the qualitative presence of steroids, saponins, tannins, glycosides, carbohydrates, alkaloids, flavonoids, diterpenes, amino acids and the absences of Vitamin C.

3.2 DPPH Radical Scavenging Activity

The ethanolic and methanolic extract of leaves and stems of *E. fluctuans* lour were subjected to free radical scavenging activity. Here, ascorbic acid (ASA) was used as reference standard. The percentage of inhibition of methanolic and ethanolic extract of *E. fluctuans* lour were 58.96, 72.34 where percentage of inhibition of ascorbic acid was 92.54 at concentration 500 µg/ml. The IC_{50} value of *E. fluctuans* lour found very mild to moderate. The antioxidant activity of IC_{50} values in DPPH method of methanolic and ethanolic extract of *E. fluctuans* lour and ascorbic acid were 619.55, 115.67, 17.81 µg/ml are given in Table 2 and in Fig. 1.

Table 1. Phytochemical test results phytochemical screening of *E. fluctuans* lour

Chemical test	Ethanolic extract	Methanolic extract
Steroid	+	+
Saponins	+	+
Tannins	-	+
Glycosides	-	+
Carbohydrate	+	+
Vitamin C	+	-
Alkaloid	+	+
Flavonoid	+	+
Amino acid	+	+
Diterpens	+	+

+= Present; - = Absent.

Table 2. IC_{50} value of standard and different extract of *Enhydra fluctuans* lour

Test sample	IC_{50} (µg/ml)
Methanolic extract of *E. fluctuans* lour	619.55
Ethanolic extract of *E. fluctuans* lour	115.67
Ascorbic acid (standard)	17.81

Table 3. IC_{50} value of ascorbic acid (ASA)

Absorbance of control	Concentration (µg/ml)	Absorbance of ascorbic acid	Inhibition (%)	IC_{50}
	500	0.050	92.54	
	250	0.052	92.24	
	125	0.054	91.94	
	62.5	0.056	91.64	
0.670	31.25	0.208	68.96	17.81
	15.625	0.313	53.28	
	7.813	0.541	19.25	
	3.906	0.588	12.24	
	1.953	0.613	8.51	
	0.977	0.622	7.16	

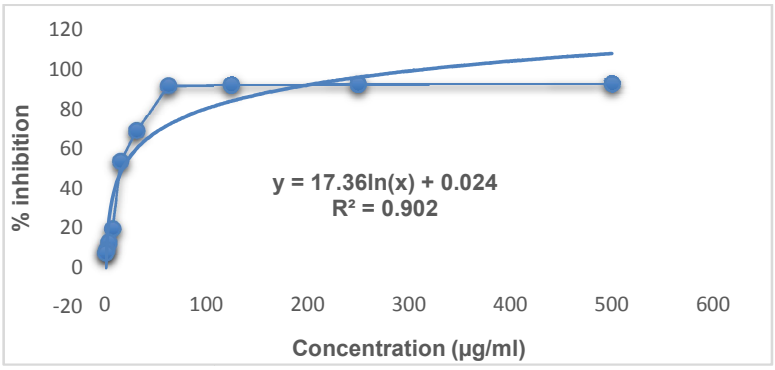

Fig. 1. IC_{50} value of ascorbic acid (ASA)

Table 4. IC_{50} value of methanolic extract of *E. fluctuans* lour

Absorbance of control	Concentration (microgram/ml)	Absorbance of sample	Inhibition (%)	IC_{50}
	500	0.275	58.96	
	250	0.340	49.25	
	125	0.420	37.31	
	62.5	0.428	36.12	
0.670	31.25	0.456	31.94	619.55
	15.625	0.463	30.90	
	7.813	0.464	30.75	
	3.906	0.466	30.45	
	1.953	0.478	28.65	
	0.977	0.480	28.35	

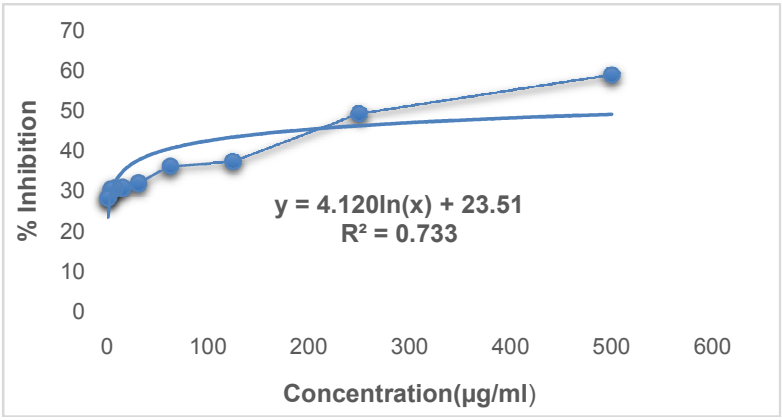

Fig. 2. IC_{50} value of methanolic extract of *E. fluctuans* lour

3.3 Total Flavonoids Content

Total flavonoid content of crude methanolic extract, crude ethanolic extract were determined and The results were expressed as mg of quercetin equivalent per gram of dried sample, where quercetin was used as a standard and the summery expressed in following table. Total flavonoid content was determined for 500 µg/ml and 250 µg/ml concentration of both ethanolic and methanolic extract of *E. fluctuans* lour. The methanolic extract of 500 µg/ml and 250 µg/ml concentration was showed highest total flavonoid content. The total flavonoid content of methanolic extract for 500 µg/ml and 250 µg/ml concentration were 67 and 33 mg QE/gm dry extract and for same concentration total flavonoid content of ethanolic extract were 21,16.67 mg QE/gm dry extract.

Table 5. IC_{50} value of ethanolic extract of *E. fluctuans* lour

Absorbance of control	Concentration (microgram/ml)	Absorbance of sample	Inhibition (%)	IC_{50}
0.670	500	0.185	72.38	115.67
	250	0.255	61.94	
	125	0.305	54.48	
	62.5	0.425	36.57	
	31.25	0.485	27.61	
	15.625	0.521	22.24	
	7.813	0.551	17.76	
	3.906	0.611	8.81	
	1.953	0.643	4.03	
	0.977	0.656	2.09	

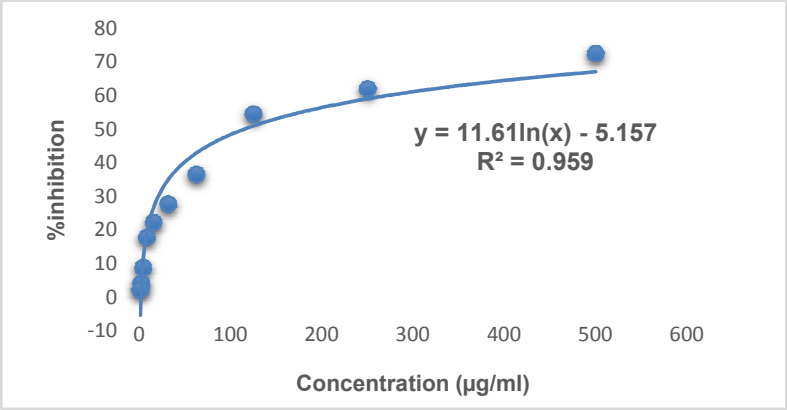

Fig. 3. IC_{50} value of ethanolic extract of *Enhydra fluctuans* lour

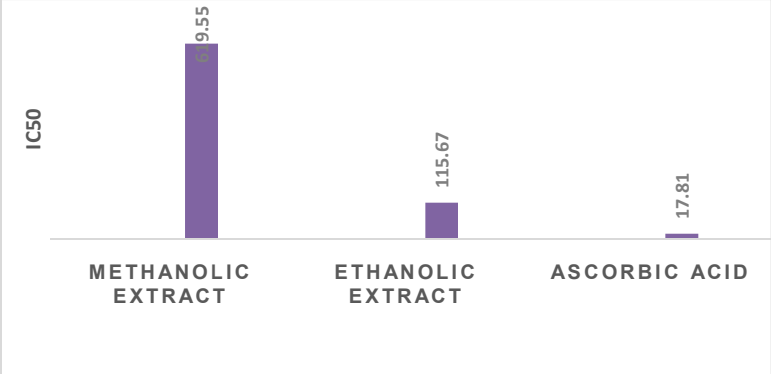

Fig. 4. Comparison of IC_{50} value between extract and standard

Table 6. Result Total flavonoid content of two extracts of *E. fluctuans* lour

Sample	Concentration (µg/ml)	Total flavonoid content (mg QE/gm dry extract)
Crude methanolic extract	500	67
	250	33
Crude ethanolic extract	500	21
	250	16.67

Table 7. Absorbance of ethanolic quercetin (standard) at different concentrations for quantitative determination of total flavonoids

Concentration of the standard (µg/ml)	Absorbance	Regression line	R^2
500	0.178		
250	0.176		
125	0.175		
62.5	0.174		
31.25	0.165	y = 0.003x + 0.142	R² = 0.955
15.625	0.164		
7.813	0.157		
3.906	0.152		
1.953	0.150		
0.977	0.147		

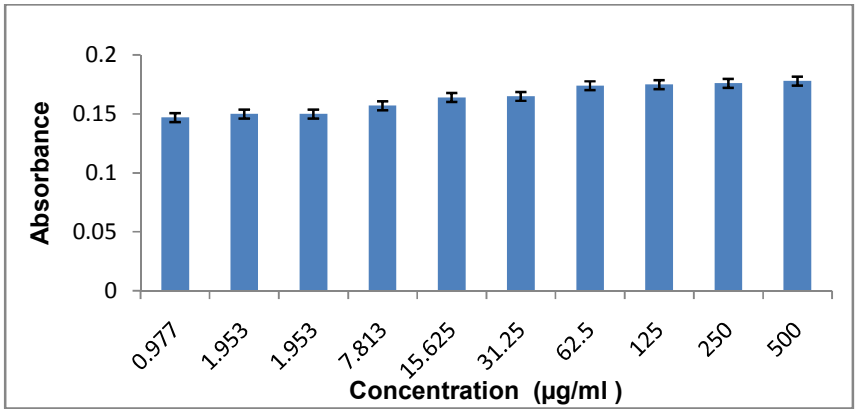

Fig. 5. Standard curve for ethanolic quercetin for the determination of total flavonoid content

Table 8. Data for determination of total flavonoid content of ethanolic crude extract of *E. fluctuans* lour

Concentration of extract	Absorbance at 510 nm	Regression line	Total flavonoid content (mg QE/gm dry extract)
500 µg/ml	0.205	y = 0.003x + 0.142	21
250 µg/ml	0.192		16.67

Table 9. Absorbance of methanolic quercetin (standard) at different concentrations for quantitative determination of total flavonoids

Concentration of the standard (µg/ml)	Absorbance	Regression line	R^2
500	0.115		
250	0.114		
125	0.112		
62.5	0.111	y=0.001x+ 0.097	R² = 0.982
31.25	0.109		
15.625	0.106		
7.815	0.105		
3.906	0.104		
1.953	0.100		
0.977	0.098		

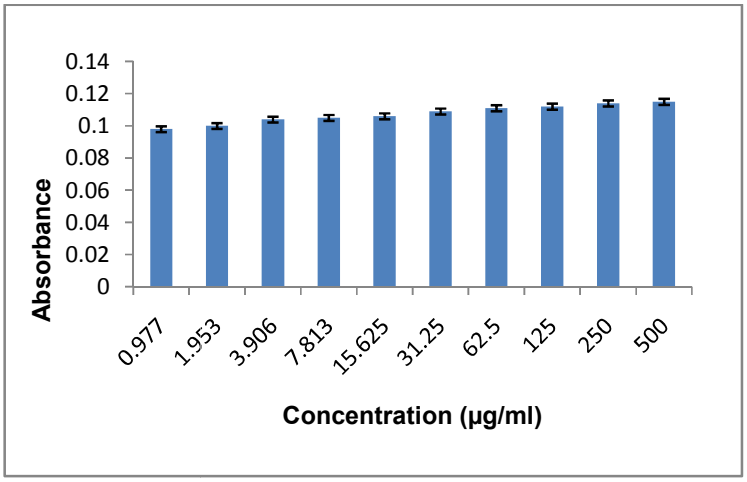

Fig. 6. Standard curve for methanolic quercetin standard for the determination of total flavonoid content

Table 10. Data for determination of total flavonoid content of methanolic crude extract of *E. fluctuans* lour

Concentration of extract	Absorbance at 510 nm	Regression line	Total flavonoid content (mg QE/gm dry extract)
500 µg/ml	0.164	y = 0.001x + 0.097	67
250 µg/ml	0.130		33

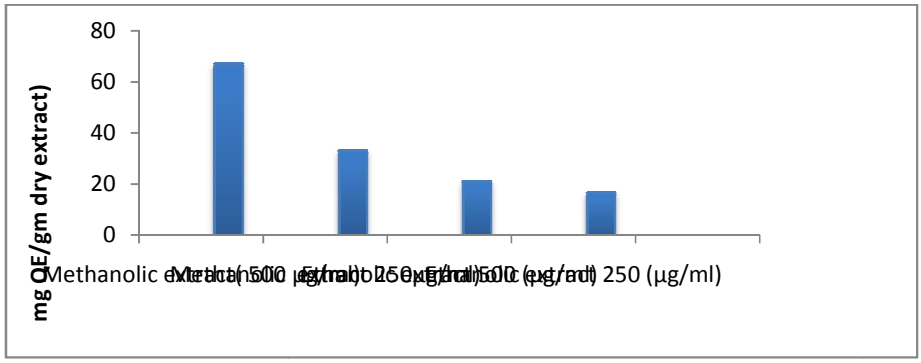

Fig. 7. Comparison of total flavonoid content between methanolic and ethanolic sample

3.4 Antimicrobial Screening

The ethanolic extract of *E. fluctuans* lour was subjected to antimicrobial screening with a concentration of 50,100,150, 200, 250, 300, 400 µg/disc in every case. The results are given in the following table (Table 11). The ethanolic extract exhibited the highest inhibition against microbial growth. The maximum zone of inhibition produced by ethanolic extract was found to be 12 mm against *E. coli.* The zone of inhibition was not found against *Salmonella enteritidis, Pseudomonas aeruginosa, Staphylococcus aureus.* Those test organisms became resistance by concentration 50 to 400 µg/disc. Ciprofloxacin was used as a standard.

3.5 Cytotoxicity Test

Methanolic extract of *E. fluctuans* was tested and brine shrimp lethality bioassay using the following the procedure of Meyer [24]. This method was applied for the determination of toxic property of the extract. The LC_{50} values for standard vincristine sulphate, methanolic and ethanolic extract were found to be 0.632, 3.29 and 27.446 µg/ml, respectively, which indicate that the plant has potent cytotoxic effect.

Table 11. Antimicrobial activity of *E. fluctuans* lour

Type of bacterial strains	Bacterial strains	Diameter of the zone of inhibition (mm)							
		Crude ethanolic extract							Ciprofloxacin
		50	100	150	200	250	300	400	30
Gram negative	*E. coli*	R	R	R	6 mm	8 mm	10 mm	12 mm	44 mm
	Salmonella enteritidis	R	R	R	R	R	R	R	42 mm
	Pseudomonas aeruginosa	R	R	R	R	R	R	R	40 mm
Gram positive	*Staphylococcus aureus*	R	R	R	R	R	R	R	42 mm

R= Resistance

Table 12. Effect of Vincristine Sulphate (positive control) on Brine Shrimp Nauplii

Concentration (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
40	1.6020	100	0.632
20	1.301	90	
10	1.000	90	
5	0.699	80	
2.5	0.398	70	
1.25	0.097	70	
0.625	-0.204	60	
0.312	-0.506	50	
0.156	-0.807	20	
0.078	-1.108	10	

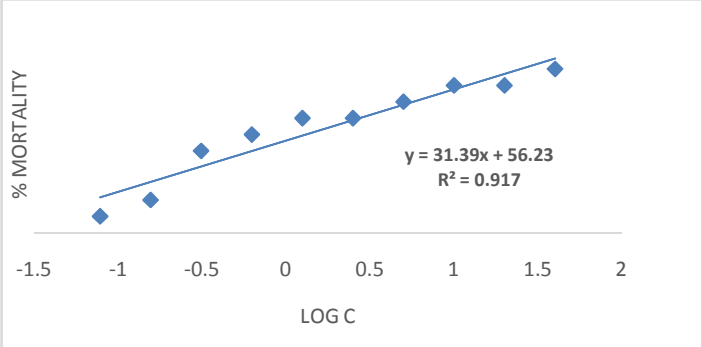

Fig. 8. Effect of Vincristine Sulphate on Brine Shrimp Nauplii

Table 13. Effect of crude ethanolic extract of *E. fluctuans* lour on brine shrimp nauplii

Concentration (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
400	2.60205	90	
200	2.30102	70	
100	2	50	
50	1.69897	50	
25	1.39794	40	27.446
12.5	1.09691	40	
6.25	0.79588	40	
3.12	0.49485	30	
1.56	0.19382	30	
0.78	-0.1072	20	

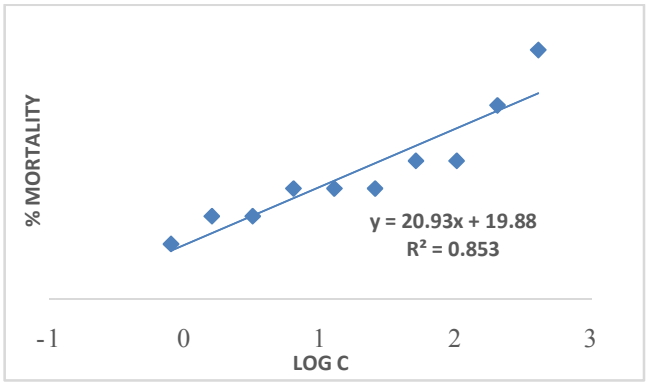

Fig. 9. Effect of crude ethanolic extract of *E. fluctuans* lour on Brine Shrimp Nauplii

Table 14. Effect of crude methanolic extract of *E. fluctuans* lour on Brine Shrimp Nauplii

Concentration (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
400	2.60205	100	
200	2.30102	100	
100	2	100	
50	1.69897	80	
25	1.39794	70	
12.5	1.09691	70	3.29
6.25	0.79588	60	
3.12	0.49485	50	
1.56	0.19382	40	
0.78	-0.1072	30	

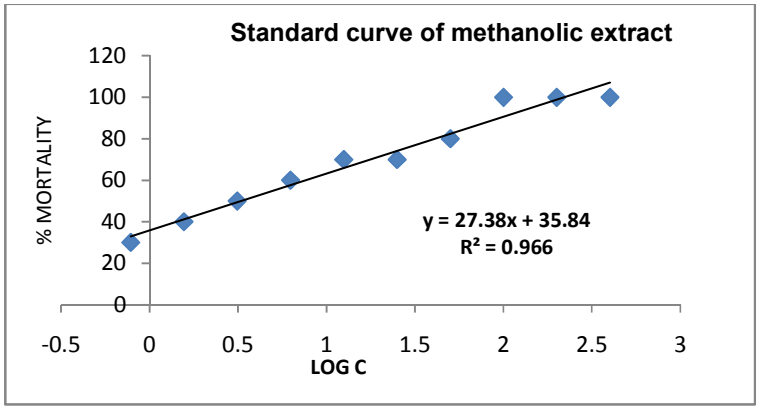

Fig. 10. Effect of crude methanolic extract of *E. fluctuans* lour on Brine Shrimp Nauplii

3.6 Discussion

Plants are the primary sources of different potentially useful bioactive compounds for the beneficial role of different healing of diseases and malady agents [26]. The plant was subjected to the preliminary phytochemical screening to determine the nature of compounds present in their methanolic and ethanolic extract. The result revealed the presence of different compounds such as flavonoids, steroid, saponins, carbohydrate, alkaloid, diterpenes, amino acids, steroids, saponins, tannins, glycosides, carbohydrates, alkaloids, flavonoids, diterpenes, amino acids. Presence of flavonoids was observed in case of the plant, recently different activities of the flavonoids have been demonstrated by several authors. Protective effect of flavonoids to animal liver injury and liver fibrosis has been proved by some authors

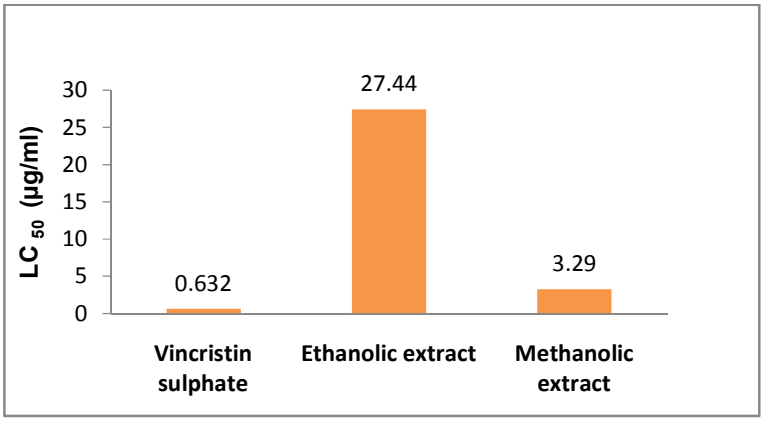

Fig. 11. LC_{50} (µg/ml) values of different extractives of *E. fluctuans* lour on Brine Shrimp mortality

[27-29]. Quantification of bioactive compounds revealed that *E. fluctuans* contain significant amount of flavonoids. Flavonoids, including flavones, flavanols and condensed tannins, are secondary plant metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity in vitro and also act as antioxidants in vivo. [30,31]

4. CONCLUSION

The methanolic and ethanolic extract of *Enhydra fluctuans* lour were subjected to different biological investigations such as phytochemical screening, total flavonoid content, free radical scavenging assay, brine shrimp lethality bioassay, antimicrobial screening. The pharmacological activities were evaluated. The plant extract showed mild and moderate activities. Further studies of this plant species should be directed to carry out in vivo studies of its active medicinal components to prepare natural pharmaceutical products of high value.

ETHICAL APPROVAL

The protocol of the experiment was approved by the animal ethics committee of the Department of Pharmacy, Daffodil International University, Dhaka, Bangladesh. The animals care and health were maintained according to the guidelines of National Institutes of Health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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