



PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING OF *Enhydra fluctuans* Lour.

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Abstract: The study was aimed to evaluate pharmacological activities of the leaves of *Enhydra fluctuans* Lour. (Family-Asteraceae). The interesting reported pharmacological activities, coupled with the use of the plant in traditional medicine qualify *E. fluctuans* Lour. for possible analgesic, antidiarrhoeal and antioxidant activities. These activities were investigated on animal model like Swiss albino mice. Phytochemical analysis using standard screening methods indicated the presence of alkaloids, tannins, steroids, gums, glycosides, saponins and flavonoids in the ethanolic plant extract. The ethanolic extract obtained by cold extraction method, showed anti oxidant activity of $IC_{50} = 197 \mu\text{g}/\text{ml}$ in DPPH assay while that of the standard ascorbic acid was observed to be $IC_{50} = 18 \mu\text{g}/\text{ml}$. In Folin-Ciocalteu assay total phenolic content was found to be 157 mg GAE/100 gm of dry powder. This extract when applied at 250 and 500 mg/kg exhibits significant inhibition of writhing reflex 25.43% ($p < 0.02$) and 40.67 % ($p < 0.05$) respectively while with that of the standard drug diclofenac sodium inhibition was observed to be 68.82% ($p < 0.001$) at a dose of 25 mg/kg body weight. In castor oil induced method the extract produced 23.81 and 47.62 % ($p < 0.02$ and $p < 0.01$) inhibition of diarrhoea at the doses of 250 mg/kg and 500 mg/kg body weight, respectively while standard loperamide showed 85.72 % ($p < 0.001$) inhibition at a dose of 3 mg/kg body weight.

Keywords: *E. fluctuans*; antioxidant activity, DPPH, total phenolics, analgesic activity, antidiarrhoeal activity

Introduction

Enhydra fluctuans Lour. is an edible herbaceous vegetable with serrate leaves. The leaves are slightly bitter which have been reported to cure inflammation, skin diseases, small pox (Kirtikar and Basu, 2002), diseases for nervous system (Nadkarni and Nadkarni, 2002) and torpidity of liver (Chopra *et al.*, 2000). The plant has nutritional value and its methanolic extract has been reported to have antidiarrhoeal activity (Uddin *et al.*, 2005). The leaves have been reported hypotensive (Joshi and Kamat, 1972).

E. fluctuans (known as *Helencha shak*) grows all over in wetlands of Bangladesh and is accepted favorably as leafy vegetable. To reveal antioxidant and analgesic role of the plant and to promote its medicinal usage, the phytochemical constituents of the plant need to be

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identified. Therefore, the objective of present study was to identify the chemical constituents of the plant and to determine its antioxidant, analgesic and antidiarrhoeal activities.

Materials and methods

E. fluctuans were collected during February 2011 and shade dried. The plants were grounded and the powder was extracted following cold extraction method. About 100gm powder was soaked in 300 ml of 96% ethanol in a glass container for sixteen days with regular shaking and stirring. The extract was separated and the filtrate was evaporated by rotary evaporator. The concentrated extract was then dried at room temperature. The deep green paste designated as crude ethanolic extract of *E. fluctuans* was considered for further analysis.

Diclofenac sodium from Beximco Pharmaceutical Ltd. Dhaka, Bangladesh was collected as control drug. The chemicals used in this study were 1,1-diphenyl-2-picryl hydrazyl (DPPH) and gallic acid (Merck Germany); tween-80 (Loba Chemie Pvt Ltd., India); dimethyl sulfoxide (DMSO; Gaylord Chemical Company, USA) and acetic acid (Merck Germany).

Five percent (w/v) solution of plant extract in ethanol was made for major phytochemicals identification (unless otherwise mentioned) (Trease and Evans, 1989; Sofowara, 1982).

Young Swiss-albino mice aged 4-5 weeks having average weight of 28 to 35 gm purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR) were used for the experiment. The mice were kept in standard environmental condition for a week for adaptation when they were provided with standard laboratory food and tap water and were maintained at natural day-night cycle. The experiments were conducted on an isolated and noiseless condition.

Antioxidant activity screening: 1,1-diphenyl-2-picrylhydrazyl (DPPH) is used to evaluate antioxidant activity. The qualitative evaluation of antioxidant test was performed by spotting a suitably diluted stock solution on pre-coated silica gel TLC plates. The plates were developed in three solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extract. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. (Sadhu *et al.*, 2003).

DPPH free radical scavenging method was used in quantitative antioxidative analysis. The aliquots of concentrations 1.56 to 800 µg /ml of the extract were added to 3 ml 0.004% w/v solution of DPPH. Absorbance at 517 nm was determined after 30 min and IC₅₀ was estimated (Gupta *et al.*, 1971).

The percent inhibition has been estimated by following relationship as

$$\% \text{ inhibition} = \{(\text{Blank absorbance} - \text{Sample absorbance}) / \text{Blank absorbance}\} \times 100$$

Total phenolic content: In the present study phenolic content was measured using the Folin-Ciocalteu assay (Karim *et al.*, 2011) and gallic acid was used to set up a calibration curve. Dried and grounded plant material was extracted first with ethanol and centrifuged at 3000 rpm. Then the supernatant liquid was taken for the assay. An aliquote of extract or standard solutions of gallic acid and 0.1 ml (0.5N) FC reagent were mixed. The mixture was incubated at room temperature for 15 minutes. Then 2.5 ml saturated sodium carbonate

solution was added and further incubated for 30 min at room temperature. The absorbance was read at 760 nm against blank. Therefore, the total phenolic content was expressed in gallic acid equivalents (GAE) milligrams per 100 gram of dry powder (mg GAE/100 gm of dry powder).

Analgesic activity screening: Evaluation of analgesic activity was performed by acetic acid induced writhing method with slight modification (Podder *et al.*, 2011). Experimental mice were randomly selected and divided into four groups consisting of 5 in each group. Each group received a particular treatment i.e. negative control, positive control and the two doses of the extract (250 and 500 mg/kg body wt.). At 30 minutes interval the writhing inducing acetic acid solution (0.6%) was administered intraperitoneally to each of the animals of a group. At intervals of 5 minutes number of writhing was counted for 15 minutes.

Antidiarrhoeal activity screening: The antidiarrhoeal activity using castor oil-induced method was followed as that of Chatterjee (1993) with minor modification. Mice were divided into four groups consisting of five mice in each. Each group received a particular treatment i.e. negative control, positive control (loperamide at 3 mg/kg of body weight) and the two doses of the extract (250 and 500 mg/kg body wt.). After 30 min of treatment all mice were administered with castor oil and then these were kept in individual cages, having adsorbent paper underneath; diarrhoea episodes were counted every hour for the duration of the 4-hour study. Stool numbers and watery stools were counted on each adsorbent paper for the whole experiment time along with the latent period of each mouse.

Results

Phytochemical screening on *E. fluctuans* extract revealed the presence of chemical constituents such as alkaloids, tannins, steroids, gums, glycosides, saponins and flavonoids (Table 1).

Table 1: Result of chemical group test of ethanolic extract of *E. fluctuans*

Constituents	Result
Reducing sugar	-
Combined reducing sugar	-
Tannins	+
Flavonoids	+
Saponin	+
Gums	+
Steroids	+
Alkaloids	+
Glycoside	+
Proteins	-
Terpenoids	-
Acidic compounds	-

+ = Presence; - =Absence

In the TLC-based qualitative antioxidant assay using DPPH, *E. fluctuans* extract showed the free radical scavenging properties indicated by the presence of yellow spot on a purple background of the TLC plate. In the quantitative assay, the extract showed a free radical scavenging activity in the DPPH assay ($IC_{50} = 197 \mu\text{g/ml}$) which is comparable to that of ascorbic acid ($IC_{50} = 18 \mu\text{g/ml}$)(Fig. 1).

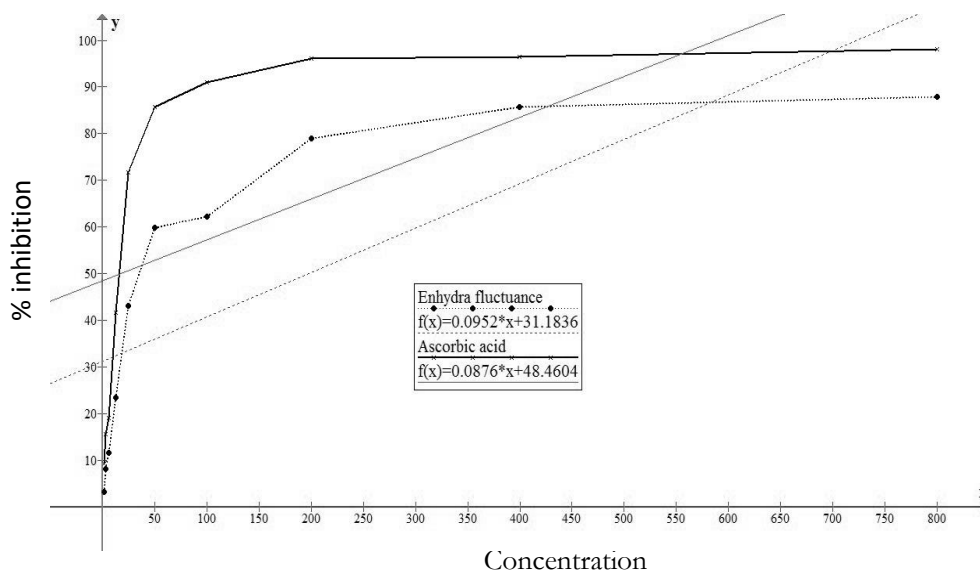


Fig. 1: % inhibition *vs* concentration of ascorbic acid and *E. fluctuans*

In Folin-Ciocalteu assay total phenolic content was found to be 157 mg GAE/100 gm of dry powder (Fig.2).

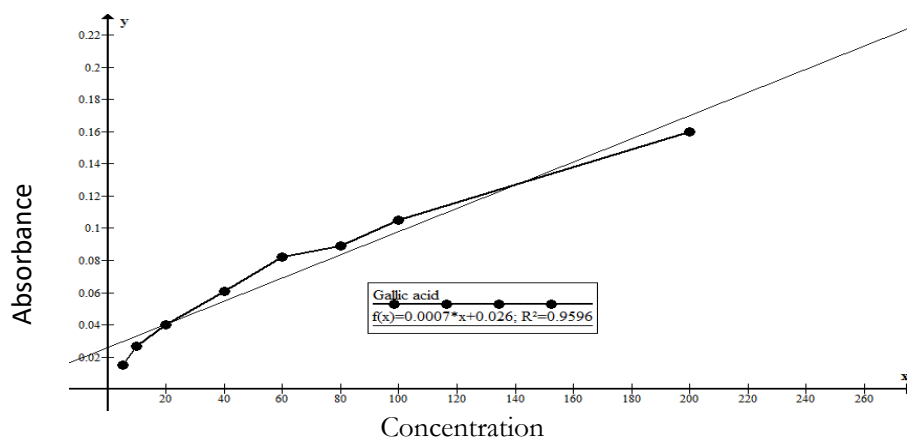


Fig 2: Absorbance *vs* concentration graph of gallic acid

The extract produced 40.67 % and 25.43 % ($p < 0.05$ and $p < 0.02$) writhing inhibition at the doses of 500 mg/kg and 250 mg/kg body weight, respectively while writhing

inhibition for Diclofenac Na inhibition was observed 68.82% ($p < 0.001$) at a dose of 25 mg/kg body weight (Table 2).

Table 2: Effects of *E. fluctuans* extract on acetic acid induced writhing of mice

Animal group	Mean writhing	% Writhing	% Writhing inhibition
Negative control	29.5± 2.6	100	-
Positive control (Diclofenac Na 25mg/kg)	9.2±1.5**	31.18	68.82
Extract (500 mg/kg)	17.5±0.9**	59.33	40.67
Extract (250 mg/kg)	22±2.8*	74.57	25.43

Values are mean± S E M, n = 5, * and ** represent $p < 0.05$ and $p < 0.001$ significant level respectively as compared to control

In castor oil induced antidiarrhoeal activity test the extract produced 47.62% and 23.81% ($p < 0.02$ and $p < 0.01$) inhibition at the doses of 500 mg/kg and 250 mg/kg body weight, respectively while standard loperamide showed 85.72 % ($p < 0.001$) inhibition at a dose of 3 mg/kg body weight (Table 3).

Table 3: Effects of *E. fluctuans* extract on castor oil induced diarrhea of mice

Animal group	Latent period (hr)	Average no of stool	% Inhibition
Negative control	0.35±0.2	4.2±1.2	-
Positive control (Loperamide 3mg/kg)	2.24± 1.3	0.6± 0.3	85.72
Extract (500 mg/kg)	0.65± 0.5	2.2±1.6	47.62
Extract (250 mg/kg)	1.23± 0.8	3.2±3.0	23.81

Values are mean± S E M, n = 5, * and ** represent $p < 0.05$ and $p < 0.001$ significant level as compared to control

Discussion

In the present study, ethanolic extract of *E. fluctuans* shows the presence of some important plant secondary metabolites. Vital and Rivera (2011) also found some phytochemicals like alkaloids, tannins, steroids, gums, glycosides, saponins and flavonoids in their study using methanolic extract of *E. fluctuans*. The biological activities of this plant may be due to the presence of these phytochemicals.

Antioxidants are molecules, which inhibit the oxidation of organic molecules. They are very important not only for food preservation but also for the defense of living systems against oxidative stress (Masuda *et al.*, 2003). Antioxidants have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals such as cancer, cardiovascular disease, neurodegenerative disease, diabetes etc (Soares *et al.*, 1997). On the other hand, oxidative degradation of lipids and microbial spoilage in foods leads to deterioration of taste and nutritional quality and could have harmful effect on consumers' health. Many studies have confirmed that food phenolics are a promising way to overcome these phenomena (Aruoma, 1994). *E. fluctuans* extract shows the free radical scavenging properties indicated by both qualitative and quantitative antioxidant activity test and flavonoids investigated by phytochemical screening are responsible for antioxidant properties (Beecher, 2003).

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plants (Randhirand Shetty, 2004). These compounds, one of the most widely occurring groups of phytochemicals, are of considerable physiological and morphological importance in plants. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Middleton *et al.*, 2000; Pimia *et al.*, 2001). In the present study the extract showed a good presence of phenolic components which could be a major determinant of antioxidant potentials and could therefore be a natural source of antioxidants.

The acetic acid-induced writhing model involves a localized inflammatory response, which triggered pain sensation. Acetic acid is used to induce algesia by releasing endogenous substances, which in turn excite nerve endings associated with pain sensation (Taesotikul *et al.*, 2003). This is a procedure used to test effectiveness of peripheral analgesic agents. The results obtained in this study showed the presence of analgesic activity of *E. fluctuans* extract which could play a potential role in developing a new drug molecule. Many studies show that the active principles responsible for the analgesic activity are alkaloids, gums, flavonoids and tannins (Ahmadiani *et al.*, 2000; Rajnarayana *et al.*, 2001 and Choi *et al.*, 2005). Therefore antinociceptive activity of the plant extract may be attributable to the existence of these secondary metabolites either in single form or in combination.

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract. In some diarrhoeas, the secretory component predominates, while other diarrhoeas are characterized by hypermotility. In castor oil induced diarrhea the autocoids and prostaglandins are involved these have been implicated in the causation of diarrhoeas in man (Horton *et al.*, 1968). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion (Pierce *et al.*, 1971). From the present study, it is evident that the plant extracts containing tannin, flavonoids, alkaloids, saponins and steroids possess anti-diarrheal activity (Shemsu *et al.*, 2013; Balaji *et al.*, 2012). Several studies have shown that castor oil induced method has been an excellent way to screen the antidiarrhoeal property of medicinal plants and for the isolation of a great variety of biologically active compounds (Izzo *et al.*, 1993)

Conclusion

Finally the overall results of this study act as a scientific basis to investigate the relationship between the phytochemicals of *E. fluctuans* extract and the biological activities. This may further help to explore the chemical constituents present in the plant responsible for bioactivities and isolation, purification, structure elucidation and activity determination of these phytochemicals.

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