



Research Article

Cytotoxic, phytochemical and antioxidant potential of marshy herb *Enhydra fluctuans* Lour.

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ABSTRACT

Enhydra fluctuans is a marshy herb usually eaten by folk people as vegetable and also used for anti diarrhoeal activity. In the present study solvent extracts of the leaf were screened for phytochemical, cytotoxic and antioxidant activity. All the extracts showed the presence of tannin, saponins and flavonoids. Except for chloroform extract, terpenoid was also present in all other extracts. Cytotoxic activity was conducted using brine shrimp mortality assay, Chloroform extract showed highly significant activity of 93.9%. Antioxidant activity was tested qualitatively as well as quantitatively and polar extracts showed better activity in comparison to non-polar extracts.

KEY WORDS: *Enhydra fluctuans*, tannin, saponins, flavonoids, cytotoxic, antioxidant

INTRODUCTION

E*nhydra fluctuans* is a marshy herb found in tropical & subtropical region. Plant is used in ascites, dropsy & anasarca. It is cooked with fish curry & taken to revive appetite after long weakness due to fever/typhoid. Whole plants are useful. Basically leaves and roots are used for medicinal purposes [1]. Traditionally folk people use it as a cooling agent, Leaves are made in to paste and applied cold over the head [2]. As the plant is eaten as edible so exploration of its nutritive value as an antioxidant and phytochemical analysis for the presence of medicinally important class of compounds seemed an interesting proposition.

MATERIALS AND METHODS

Collection & processing of plant materials:

Fresh leaves of *Enhydra fluctuans* were collected from medicinal germplasm garden of Regional Plant Resources Center (RPRC), Bhubaneswar. Leaves were dried in shade and were made in to fine powder in grinder (Lexus make). Solvent extraction was done using Soxhlet extraction

method. 15 gm of powdered leaf sample of plant *Enhydra fluctuans* was taken in a thimble & was subjected to serial extraction with different solvents like Hexane, Chloroform, Acetone, and Methanol on the basis of increasing polarity. 250ml of solvent was taken in around bottom flask & extraction was carried out soxhlet reflux was continued till solvent becomes colourless. Extracts obtained were concentrated in vacuum under pressure using Buchi (R-200) Rotavapour. Concentrated extract was stored in screw cap vials until further use.

Phytochemical tests

Phytochemical tests were conducted standard protocols of Harborne [3].

Test for alkaloid:

Mayer's Test: 100 µl extract was taken in a test tube to which 2 ml of dilute HCl was added and 1ml of Mayer's reagent was added in drop wise manner. Yellow buff or cream colour precipitate indicates the presence of alkaloid.

Wagner's test: To small amount of extract solution 2 ml dilute HCl and 1 ml of Wagner's reagent was added drop

wise. The reddish brown precipitate shows the presence of alkaloid.

Dragendroff's test: To 200 µl of extract, 2 ml of dilute HCl and 1ml of reagent was added in a test tube, the orange brown precipitate shows the presence of alkaloid.

Test for flavonoids: To 5 ml of extract, 1 ml of 10% NaOH solution was added, yellow colour turning to colourless is an indication of presence of flavonoids.

Test for anthraquinones: To 1ml of extract, 2 ml of 5% KOH was added. Then observed pink colour shows the presence of anthraquinone.

Test for saponins: About 2 ml of 1% NaHCO₃ was added to 1 ml of extract and shaken. Lather like formation persistent for sometime is indication of presence of saponins.

Test for tannin: 1gm of sample was added with 100ml of distilled water boiled and cooled. Then 1% of FeCl₃ was added drop wise to the aqueous solution. Green black precipitate shows the presence of tannins.

Test for terpenoids: 400 µl of chloroform was added to 1ml of extract. Then 2-3 drops of H₂SO₄ was added. Reddish brown color shows the presence of terpenoids.

Test for starch: 1gm of dried powder was taken and grinded thoroughly using mortar pestle with 10 ml of distilled water and filtrate alcoholic iodine solution was added to the filtrate. Blue colour indicates the presence of starch.

Test for phlobatannin: Fresh leaves powder of plant was grinded with distilled water to make aqueous solution. Then the mixture was filtrate and filtrate was taken as sample. 1ml of aqueous 1% HCl was added to the 1ml of sample followed by boiling. A red precipitate is indication of phlobatannin.

Test for cardiac glycoside (Keller killani test): About 5 ml of the extract was mixed with 2 ml of glacial acetic acid containing 1 drop of FeCl₃ solution. To this 1ml of concentrated H₂SO₄ was slowly added to the sample mixture. A positive test result was confirmed by the presence of a brown ring at the interface.

ANTI OXIDANT ACTIVITY

Qualitative antioxidant assay

TLC based antioxidant assay:

TLC is one of the most widely used and potent techniques to resolve mixture of plant compounds. The TLC sheets supplied by Merck, Germany (TLC Silica gel 60 F₂₅₄) was used to observe the separation of individual compounds as a single spot from the selected crude extract employing varying solvent polarities. The developed TLC plate after visualizing with a staining procedure (DPPH), serves as a reference to identify and confirm the compounds eluted through column chromatography. Three solvents were used for quantitative antioxidant assays, these were as follows:

- **BEA** - Benzene : Ethanol : Ammonium hydroxide(90:10:1) [Non polar/Basic]
- **EMW** - Ethyl acetate : Methanol : Water(40:5.4:4) [Polar/neutral]
- **CEF** - Chloroform : Ethyl acetate : Formic acid(5:4;1) [Acidic]

The precoated TLC sheets 60 F₂₅₄ (Merck Company) were activated at 100°C for 10 minutes. The samples were then spotted with the help of micro tips leaving 2 cm from the bottom of the sheet. Samples were run in all the above solvents. After drying of sheets DPPH solution was sprayed. Yellow bands in purple background represent the antioxidant bands of the extract [4] R_f values of all the antioxidants was calculated using the following formula.

Retardation factor (R_f) = Distance travelled by the compounds / Total distance travelled by the solvents.

Quantitative antioxidant assay

DPPH free radical scavenging assay: The radical scavenging activity of different extracts (hexane, chloroform, ethyl acetate and methanol) against DPPH was determined by the standard protocol[5].

Ferric reducing anti-oxidant power assay (frap assay)

Total anti-oxidant activity was measured by ferric reducing anti-oxidant power (FRAP) assay [6,7]

CYTOTOXIC ACTIVITY

Brine shrimp mortality assay: Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of the medicinal plant as per standard protocol[8]. Brine shrimp (*Artemia salina*) were hatched using brine shrimp eggs in a 250 ml beaker with artificial seawater, which was prepared using sea salt 1.8 gm/100ml distilled water. This experiment was conducted in triplicate vials. In each vial 20 numbers of brine shrimp larvae were taken and the experiment was divided in three groups such as negative control, positive control and experimental vials. Then brine shrimp larvae motility readings were taken at each hour difference up to four hours. After 24 hours vials were checked for live and dead larvae. Standard deviation was also calculated.

Percentage Inhibition = $\frac{\text{Control} - \text{experimental}}{\text{Control}} \times 100$

RESULTS AND DISCUSSIONS

Phytochemical analysis of medicinal plant *Enhydra fluctuans*

It is evident from the table that all the samples showed the presence of tannin and saponin. Terpenoids were present in fresh sample, hexane, chloroform and acetone extracts. Remaining class of compounds were absent in all the samples. As terpenoids is an important class of medicinal compounds so it can be assumed that plant does have some important molecules of medicinal importance. Some workers have reported flavonoids in *Enhydra fluctuans* [9, 10] but same was absent in the samples collected from the medicinal germplasm garden of RPRC. This could be due

to the changes in climatic or edaphic factors as they play a major role in the presence or absence of secondary metabolites in medicinal plants[11]

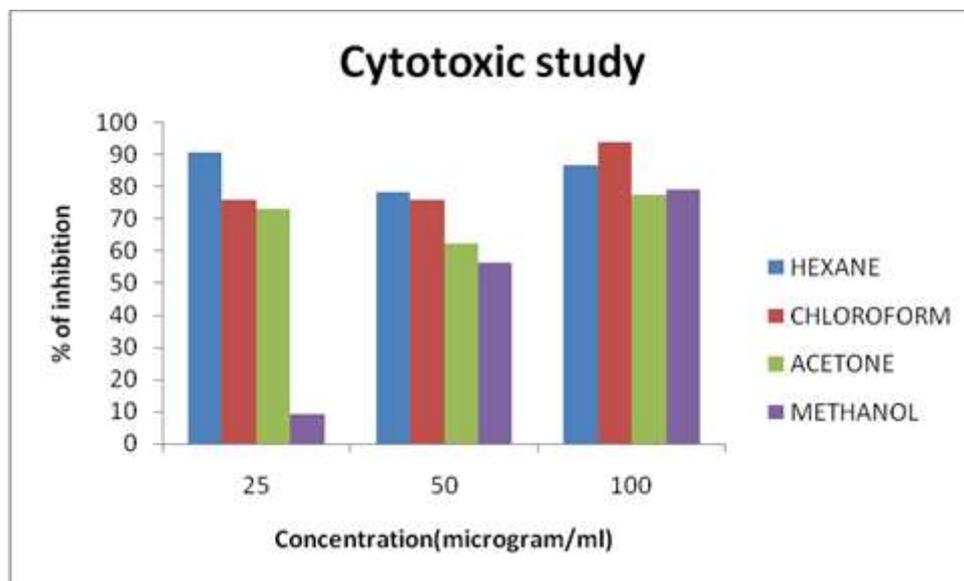
Table 1:Phytochemical analysis:

Class of compounds	Fresh samples	Hexane extract	Chloroform extract	Acetone extract	Methanol extract
Tannin	+ve	+ve	+ve	+ve	+ve
Anthraquinone	-ve	-ve	-ve	-ve	-ve
Saponin	+ve	+ve	+ve	+ve	+ve
Flavonoid	-ve	-ve	-ve	-ve	-ve
Terpenoids	+ve	+ve	+ve	+ve	-ve
Alkaloid	-ve	-ve	-ve	-ve	-ve
Glycoside	-ve	-ve	-ve	-ve	-ve

Cytotoxic activity using brine shrimp assay:

All the extracts were tested in three doses (25, 50 and 100µg/ml). Highest cytotoxic activity(93.99%) was found in chloroform extract at the dose 100 µg/ml. Remaining extracts also showed good cytotoxic activity(Figure 1). This study is in confirmation with another work where chloroform extract has shown significant hepatotoxic activity[12]] suggesting that chloroform extract of leaf does have some significant component and needs further exploration.

Figure 1:Cytotoxic activity of solvent extracts of Enhydra fluctuans

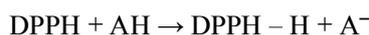


Antioxidant activity of extracts of Enhydra fluctuans

Antioxidant activity of *Enhydra fluctuans* was evaluated by one qualitative and two quantitative tests.

TLC based qualitative antioxidant assay

The presence of antioxidant compounds were detected by yellow spots against a purple background on the TLC sheet sprayed with 0.2% DPPH in methanol. Yellow bands are the result of radical scavenging activity of the molecules on the chromatographs.



(Purple color)

(Yellow color)

molecules like Combretastatin from Combretum species[4], Urosolic acid and Phrymarolin from Phryma leptostachya [13]] etc. Thus, a number of bands obtained in the solvent extracts is a positive indication of presence of more than one antioxidant molecules in *Enhydra fluctuans*.

On the basis of TLC, Maximum antioxidant bands were obtained in acetone extract in CEF solvent followed by methanol extract in the same solvent. TLC based antioxidant activity has lead to the isolation of a number of

Table 2:Qualitative Analysis of Antioxidant

EXTRACTS	SOLVENT	NO.OF BANDS	R.F Values
HEXANE	BEA	1	0.44
	CEF	0	0
	EMW	1	0.4
CHLOROFORM	BEA	2	0.13, 0.26
	CEF	1	0.55
	EMW	1	0.47
ACETONE	BEA	3	0.16, 0.26, 0.42
	CEF	5	0.1, 0.18, 0.25, 0.66, 0.84
	EMW	4	0.4, 0.54, 0.74, 0.86
METHANOL	BEA	0	0
	CEF	4	0.55, 0.34, 0.26, 0.71
	EMW	0	0

QUANTITATIVE ANTIOXIDANT ASSAY

DPPH radical scavenging assay;

As can be observed from the figure 2 below, Methanol extract showed maximum activity as compared to the other

extracts, at higher dose of 1250 microgram/ml it was even more active as compared to the standard ascorbic acid. This is in confirmation with earlier studies[14]

Figure 2:DPPH radical scavenging assay of leaf extracts of Enhydra fluctuans

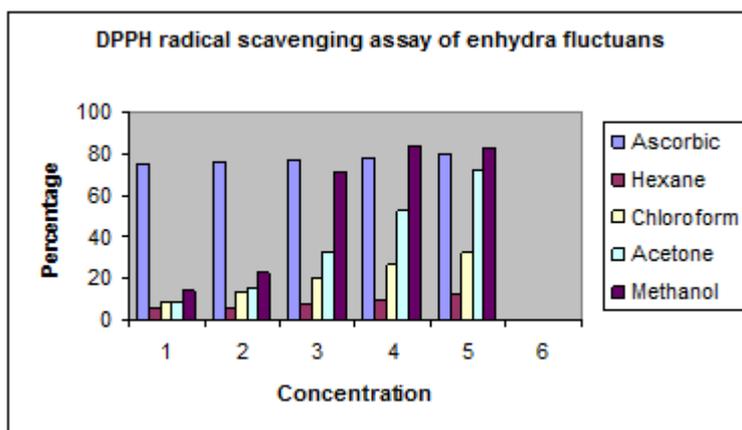
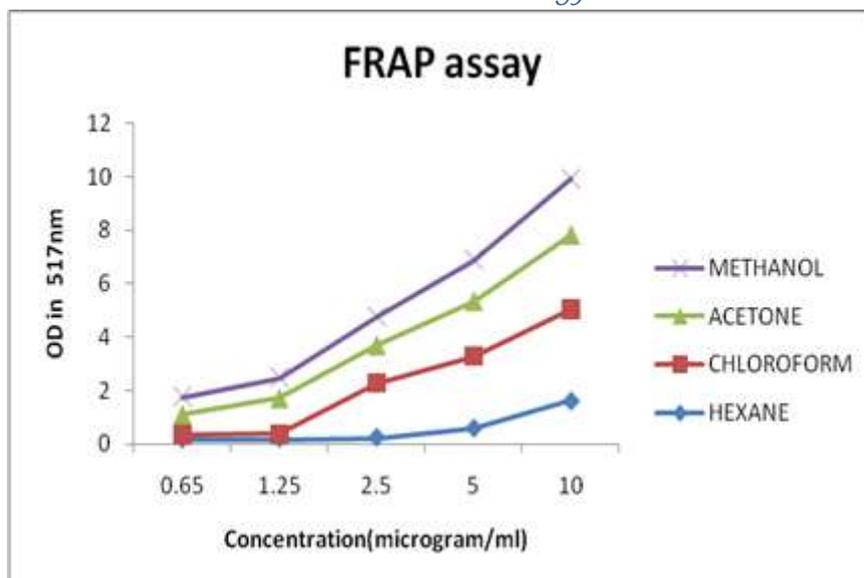


Figure 3:FRAP assay of solvent leaf extracts of Enhydra fluctuans.





Ferric oxide reducing power assay (FRAP assay)

At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) can be monitored by measuring the change in absorbance at 593nm. The reaction is nonspecific, i.e. any half reaction that has lower redox potential under reaction condition than that of ferric ferrous half reaction will drive the ferrous (Fe iii to Fe ii) ion formation. The change in absorbance is therefore directly related to the combined or 'total' reducing power of the electron donating antioxidants present in the reaction mixture. Standard antioxidant has a FRAP value of 2. Any sample having similar or higher value is considered significant antioxidant activity. As can be observed from Figure 3 except for hexane extract all the other three extract showed a FRAP value higher than the standard antioxidant value of 2, so all these extracts could be considered to possess significant antioxidant activity. Thus anti-oxidant potential of *Enhydra fluctuans* further makes the nutritional value of the leaves even more attractive. Overall it can be concluded that medicinal as well as nutritional potential of the herb is worthwhile.

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