

# Phytochemical Analysis of *Enhydra fluctuans* (Fam. – Asteraceae) Extracts to Detect their Chemical Components with Antioxidant Property

Alisha Nasreen\* Kamal Choudhury\*\*

\* Department of Zoology, Research Scholar, Gauhati University, Guwahati- 14, Assam, India

\*\* Department of Zoology, Associate Professor, B. Borooah College, Guwahati-07, Assam, India

## Abstract :

Antioxidants are the substances that inhibit oxidation and have the ability to remove the potentially damaging oxidizing agents in a living organism. Phytochemicals like flavonoids and phenols present in plants are able to reduce or prevent the oxidative damage to the human cells. *Enhydra fluctuans* Lour. (Fam. Asteraceae) is a marshy herb used by many tribal communities of North East India as well as in traditional medicine for treatment of various ailments. In the present study, ethanolic and aqueous extract of this plant were tested for Phytochemical and antioxidant activities. Phytochemical screening of the extracts done by routine analytical procedures revealed the presence of saponin, terpenoids, phenol and proteins in both the extracts. However, alkaloids, and tannins which were found to be present in the ethanolic extract were found to be absent in the aqueous extract of *E. fluctuans*. Quantitative determination of total phenolics and *in-vitro* DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging activity of both the extracts were carried out using spectrophotometric analysis. The total Phenolic content was found to be  $60.67 \pm 0.083 \mu\text{g/ml}$  GAE and  $39.83 \pm 0.083 \mu\text{g/ml}$  GAE for ethanolic and aqueous extract, respectively. They exhibited strong antioxidant DPPH radical scavenging activity with IC<sub>50</sub> value of  $1.38 \mu\text{g/ml}$ ,  $43.65 \mu\text{g/ml}$  and  $158.49 \mu\text{g/ml}$  for ascorbic acid, aqueous extract and ethanolic extract respectively. The result of present comprehensive analysis demonstrated that *E. fluctuans* possesses potential antioxidant activity, and could be used as a viable source of natural antioxidants and might be exploited for pharmaceutical applications.

**Keywords:** Antioxidants, DPPH, *Enhydra Fluctuans*, Free radicals, Phytochemical screening, total phenol content.

## 1. INTRODUCTION

Since ancient times, people have been using plants as an origin of drugs to treat various diseases. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Sandhya *et al.*, 2011). The plant kingdom harbours enormous reservoir of biologically active compounds called phytochemicals with various chemical structures and protective / disease preventing properties. Scientists estimate that there may be as many as 10,000 different phytochemicals with the potential to affect disease such as cancer, stroke or metabolic syndrome.

Therefore, phytochemical screening is very important in identifying new sources of therapeutically and industrially important secondary metabolites like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc. (Akindele *et al.*, 2007).

Reactive oxygen species (ROS) exert oxidative damaging effects to cell components and molecules (Bansal *et al.*, 2011). Damage to DNA, proteins and other macromolecules due to oxidation has been implicated in the pathogenesis of a wide variety of degenerative diseases, most notably cancer and heart disease (Halliwell *et al.*, 1994). Antioxidants can help prevent oxidative damage induced by free radicals. However commercially available synthetic antioxidant agents like butylated hydroxyanisole (BHT) have been reported to be toxic to animals including human beings which have stimulated the interest of many researchers to search natural antioxidants (Vinay *et al.*, 2010). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of diseases (Shekhar *et al.*, 2011). It has been reported that antioxidant activity of plants might be due to their flavonoids and phenolic compounds (Cook and Samman, 1996; Samantha *et al.*, 2012). Plant phenolics are widely distributed in the tissues of plants and play a very important role in free radical scavenging due to their hydroxyl groups.

*Enhydra fluctuans*, commonly known as water crest or Marsh herb, belonging to the family Asteraceae, is a hydrophytic plant. In India, this plant is predominantly found in the North-Eastern region and mostly in Assam (Chakraborty *et al.*, 2012). *E. fluctuans* is used by the tribal communities for treatment of various diseases. It has many beneficial effects such as anti-inflammatory activity (Sannigrahi *et al.*, 2010); antioxidant activity (Uddin *et al.*, 2005); anti-cancer activity (Kumar *et al.*, 2012); anti-diarrhoeal activity (Kumar and Khanum, 2012); hepatoprotective activity (Patil KS *et al.*, 2008); analgesic activity; neuroprotective potential (Alebiosu *et al.*, 2015); Phagocytic and cytotoxic activity (Hassan *et al.*, 2015).

This study has been undertaken to determine the phytochemicals present, total Phenolic content and the antioxidant property of ethanolic and aqueous extract of *Enhydra fluctuans* by DPPH free radical scavenging method.

## 2. MATERIALS AND METHODS

### 2.1. COLLECTION OF PLANT MATERIALS

Fresh leaves and stems of *Enhydra fluctuans* were collected in August from local area of Guwahati. The plant material collected was authenticated (Ref. no. Herb./Bot./GU/2016/144) in the Department of Botany, Gauhati University, Guwahati.

### 2.2. PREPARATION OF CRUDE ETHANOLIC EXTRACT

The leaves and stems were washed carefully with water and shade dried at room temperature and then ground into a coarse powder with the help of a suitable grinder. The powders were stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

About 100g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 500ml of 95% ethanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrates so obtained were allowed to evaporate under normal temperature. Gummy concentrate of greenish and brownish colour was obtained which was designated as ethanol extract of *E. fluctuans* (EEEF), dried in vacuum evaporator and stored under 4°C until further investigation (Islam *et. al.*, 2011).

### 2.3. PREPARATION OF AQUEOUS EXTRACT

About 27g powder was macerated with distilled water and kept for a period of 3 days with occasional stirring. The mixture was filtered using whatman filter paper and the filtrate was lyophilized. A brownish colored aqueous extract of *E. fluctuans* (AEEF) was obtained that was stored under 4°C until further investigation (Nabi *et. al.*, 2013; Semwal *et. al.*, 2012).

### 2.4. QUALITATIVE ANALYSIS (TESTS FOR CHEMICAL GROUP)

EEEF and AEEF were subjected to preliminary quantitative phytochemical investigation for the detection of some important secondary metabolites (Ncube *et. al.*, 2008; Sandhya *et. al.*, 2011).

#### 2.4.1. Test for alkaloids

0.2g of the extracts were heated on a boiling water bath with 2N HCl (5ml). After cooling, the mixtures were filtered.

- I. **Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of yellow coloured precipitate indicated the presence of alkaloids.
- II. **Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloids.

#### 2.4.2. Test for glycosides

1.2g of the extracts were hydrolyzed by 10ml of 1% HCl solution and neutralized with 10% of NaOH solution. A few drops of Fehling's solution A and B were added. The formation of red precipitate indicated the presence of glycosides.

#### 2.4.3. Test for tannins (Ferric Chloride Test)

0.2g of each extracts were mixed with 10ml of distilled water and heated. The mixtures were filtered and to each filtrate 5% (w/v) solution of ferric chloride were added and the formation of dark green solution indicated the presence of tannins.

#### 2.4.4. Test for flavonoids (Alkaline reagent Test)

0.2g of each extracts were taken and dissolved in diluted NaOH and 1M of HCl was (5ml each) added. A yellow solution that turns to colorless indicated the presence of flavonoids.

#### 2.4.5. Test for saponins (Froth Test)

0.2g of each extracts was diluted each with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicated the presence of saponins.

#### 2.4.6. Test for terpenoids

0.2g of each extracts were mixed separately with 2ml of chloroform and concentrated sulphuric acid (3ml) and then added carefully, to form a layer. A formation of reddish brown coloration of the solution at inert face indicated the presence of terpenes.

**2.4.7. Test for phenols (Ferric Chloride Test)** About 0.2g of each extracts was dissolved in a mixture of water and ethanol, and a few drops (3-4) ferric chloride solution was added. Red, blue, green or purple coloration indicated presence of phenols.

#### 2.5. Quantitative analysis of total phenol content using the Folin-Ciocalteu reagent assay adapted by Singloten and Rossi, 1965.

10mg of gallic acid was dissolved in 100ml of 50% ethanol (100µg/ml). Further diluted to 20, 40, 60 and 80µg/ml. 1ml of each dilution was taken in a test tube. Concentration of 1mg/ml of plant extract was prepared in ethanol. 1ml of each sample were taken in test tubes. 1.5ml Folin-Ciocalteu reagent was added to each test tubes. Incubated at room temperature for 8 mins for reactions to take place. 2ml of 20% Na<sub>2</sub>CO<sub>3</sub> was added to each test tubes. Mixed well. Test tubes allowed to stand for 1 hour at room temperature. Absorbance of the standard measured at 760nm. Blank : 1ml 50% methanol, 1.5ml FCR and 2ml Na<sub>2</sub>CO<sub>3</sub> solution (Singleton and Rossi, 1965).

#### 2.6. Evaluation of antioxidant activity by DPPH radical scavenging method

The principle of DPPH scavenging assay is based on the reduction of purple colored ethanolic DPPH solution in the presence of hydrogen donating antioxidants by the formation of yellow colored diphenyl-picryl hydrazine. Lower absorbance of the reaction mixture indicated higher free radical activity. This reaction has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate the anti-oxidative activity of foods and plant extracts (Zhou *et. al.*, 2004; Panovska *et. al.*, 2005).

0.1mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml. of EEEF and AEEF extracts in ethanol at different concentration (1, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then, absorbance was measured at 517 nm by using spectrophotometer (UV-VIS Shimadzu) (Ahmed *et. al.*, 2013). Absolute alcohol was used as blank. Reference standard compound being used was ascorbic acid and experiment was done in triplicate (Patel *et. al.*, 2011). The IC<sub>50</sub> value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity (Koleva *et. al.*, 2002). The percent DPPH scavenging effect was calculated by using equation (1):

$$\text{DPPH scavenging effect (\%)} \text{ or Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100. \quad (1)$$

Where A<sub>0</sub> = the Absorbance of control reaction

And A<sub>1</sub> = the Absorbance in presence of test or standard sample (Achola *et al.*, 1998).

### 3. RESULTS

#### 3.1. Percentage yield of plant extract.

The percentage yield of EEEF and AEEF is shown below (Table 1)

**Table 1 : Percentage yield of plant extract**

Sl. No.	Extracts	Color of the extracts	Yield of extracts (g)	Yield of extracts (%)
1	EEEF	Dark green	5.46	3.64
2	AEEF	Dark brown	2.32	8.59



Photoplate 1. Extracts of *E. fluctuans* –  
 A. Ethanolic extract B. Aqueous extract

**3.2. Phytochemical Screening of plant extracts**

The Phytochemical screening of EEEF revealed the presence of alkaloids, tannins, saponins, terpenes, phenols and proteins while AEEF revealed the presence of saponins, terpenes, carbohydrates, phenols and proteins. Flavonoids and glycosides were found to be absent in both the extracts (Table 2).

**Table2. Phytochemical screening of EEEF and AEEF.**

Chemical components	EEEF	AEEF
Alkaloids	+	-
Glycosides	-	-
Tannins	+	-
Flavonoids	-	-
Saponins	+	+
Terpenes	+	+
Carbohydrates	-	+
Phenols	+	+
Proteins	+	+

Present (+) ; Absent (-)

**3.3. QUANTITATIVE ANALYSIS OF TOTAL PHENOLIC CONTENT (TPC) OF EEEF and AEEF**

Using the calibration curve of Gallic acid (Fig.1), TPC in the EEEF was found to be 60.67µg/ml GAE and in AEEF 39.8367µg/ml GAE, respectively (Table. 3). TPC was found to be higher in EEEF than in AEEF.

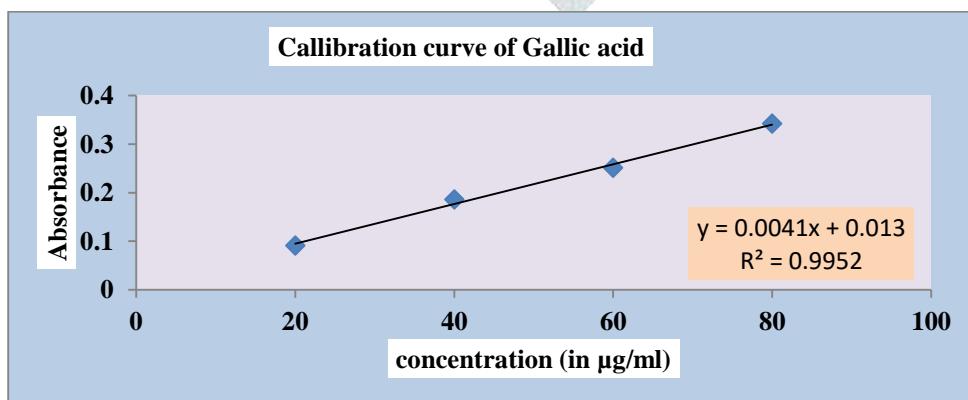


Fig 1. Calibration curve of Gallic acid for TPC determination.

Table 3. Total Phenolic content (TPC) as Gallic acid Equivalent

Sl. No.	Extract	TPC as Gallic acid equivalent (in $\mu\text{g/ml}$ )
1.	AEEF	$39.83 \pm 0.083$
2.	EEEEF	$60.67 \pm 0.083$

### 3.4. DPPH Free Radical Scavenging activity of EEEF and AEEF

As shown in table 4, DPPH decolorization was increased by the *E. fluctuans* extracts in a concentration dependent manner. Both the extracts are good scavengers of DPPH free radical. AEEF at a concentration of  $100\mu\text{g/ml}$  was able to scavenge  $54.83 \pm 0.07\%$  of DPPH free radical while EEEF was able to scavenge  $42.32 \pm 0.009\%$  of DPPH free radical. Thus, AEEF showed a better free radical scavenging activity. Also,  $\text{IC}_{50}$  value of AEEF ( $43.65\mu\text{g/ml}$ ) is lower than that of the EEEF ( $158.49\mu\text{g/ml}$ ) that indicates towards higher activity of AEEF (Table 4)

Table 4 : DPPH Free Radical Scavenging potential of EEEF, AEEF and Ascorbic acid. Values are represented as mean  $\pm$  SEM

Concentration (in $\mu\text{g/ml}$ )	% inhibition		
	EEEEF	AEEF	Ascorbic acid
10	$26.73 \pm 0.03$	$40.07 \pm 0.03$	$79.1 \pm 0$
20	$27.43 \pm 0.01$	$41.6 \pm 0$	$80.6 \pm 0$
30	$32.53 \pm 0.03$	$48.97 \pm 0.03$	$88 \pm 0.058$
40	$34.63 \pm 0.01$	$51.93 \pm 0.03$	$90.9 \pm 0.033$
50	$34.70 \pm 0.01$	$52.2 \pm 0.15$	$91.2 \pm 0.145$
60	$37.96 \pm 0.003$	$52.4 \pm 0.12$	$91.4 \pm 0.115$
70	$38.63 \pm 0.03$	$52.2 \pm 0.17$	$91.7 \pm 0.173$
80	$40.6 \pm 0.006$	$53.67 \pm 0.17$	$92.6 \pm 0.167$
90	$41.27 \pm 0.006$	$53.97 \pm 0.07$	$92.9 \pm 0.067$
100	$42.32 \pm 0.009$	$54.83 \pm 0.07$	$93.5 \pm 0.067$
IC50	<b><math>158.49\mu\text{g/ml}</math></b>	<b><math>43.65 \mu\text{g/ml}</math></b>	<b><math>1.38 \mu\text{g/ml}</math></b>

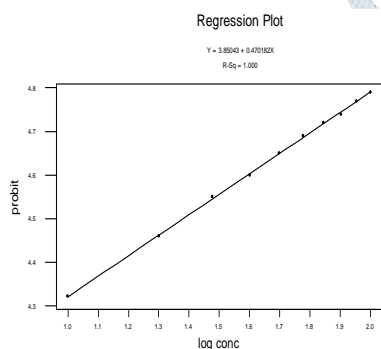


Fig 2. Regression graph for EEEF

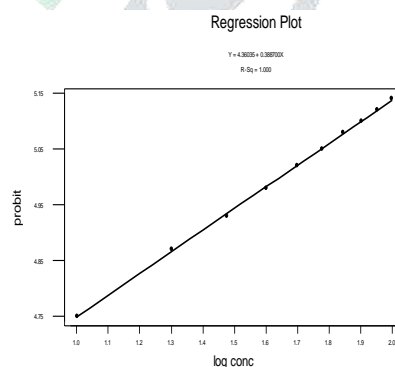


Fig 3. Regression graph for AEEF

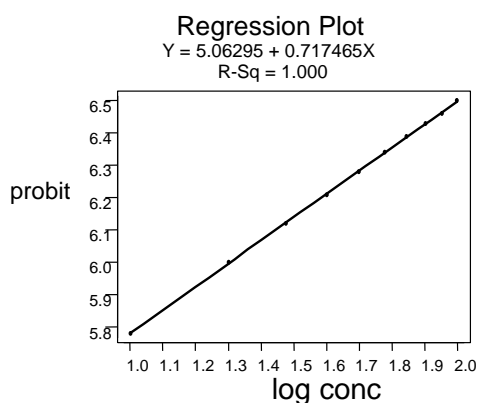


Fig.4 Regression graph for Ascorbic acid

#### 4. DISCUSSION

Result from the present investigation shows that *E. fluctuans* is rich in Phytochemicals. Phytochemical screening of Ethanolic extract of *Enhydra fluctuans* (EEEEF) revealed the presence of alkaloids, tannins, saponins, terpenoids, and phenols. Till now, there has been no report on the phytochemical analysis of ethanolic extract of *E. fluctuans*. However, methanolic extract of *E. fluctuans* has been screened for Phytochemical constituents and workers reported the presence of tannin, saponin and absence of terpenoids, flavonoids, alkaloids, glycoside (Panigrahi et al., 2015) which is in agreement to our findings. Some other workers (Kuri et al., 2014) reported the presence of flavonoids, triterpenes, carbohydrates, reducing sugar, saponins, phenols, diterpenes, proteins and tannins and absence of alkaloid, cardiac glycoside and phytosterols. On the other hand, the AEEF showed the presence of saponins, terpenes, carbohydrates and phenols. However, Phytochemical analysis of AEEF by Dua et al., 2016 revealed the presence of flavonoids, phenols, carbohydrates, glycosides, alkaloids, tannins and amino acids (Dua et al., 2016). The difference in results may be due to differences in area of plant collection (Ghasemzadeh et al., 2018), choice of solvent and method of extraction (Simon et al., 2015).

Plant phenolics are widely distributed in the tissues of plants and play a very important role in free radical scavenging due to their hydroxyl groups. The total Phenolic content in the AEEF and EEEF were found to be  $39.83 \pm 0.083 \mu\text{g/mlGAE}$  and  $60.67 \pm 0.083 \mu\text{g/mlGAE}$ , respectively. EEEF has shown higher amount of TPC which is similar to the findings of Pramod et al., 2012, where the EEEF showed higher content of phenol compared to pet-ether and chloroform extracts, and Dua et al., 2016, wherein TPC of AEEF was found to be  $\sim 21.3 \text{mg/g}^{\text{DW}}$  pyrocatechol equivalent. (Dua et al., 2016; Swain et al., 2012)

In the present study,  $\text{IC}_{50}$  of EEEF, AEEF and Ascorbic acid were found to be  $158.49 \mu\text{g/ml}$ ,  $43.65 \mu\text{g/ml}$  and  $1.38 \mu\text{g/ml}$ , respectively at a concentration of  $100 \mu\text{g/ml}$ . Both the extracts have shown potent antioxidant activity as compared to ascorbic acid which is in agreement with the works done by Swain et al., 2012 and Dua et al., 2016. However, there has been no report on the DPPH free radical scavenging activity of AEEF, but, AEEF ( $400 \mu\text{g/ml}$ ) significantly returned the  $\text{NaAsO}_2$ -induced alteration of lipid peroxidation, protein carbonylation, and antioxidant markers to close to untreated levels (Dua et al., 2016). Our results suggests that AEEF showed higher free radical scavenging activity in a concentration dependent manner. In spite of having a lower TPC than EEEF, AEEF exhibited a higher free radical scavenging activity. This may be due to the antioxidant activity of other active phytoconstituents present in AEEF. This fact can be validated by the findings of Ghosh et al., 2013, wherein biochemical analysis of a water extracted carbohydrate polymer of *E. fluctuans* containing esterified Phenolic acids, revealed similarity in antioxidant potential when compared with known standard antioxidant. Thus, our findings represent an interesting approach in phytotherapeutic treatments that might include the use of the aqueous extract of *E. fluctuans* as a possible new source of natural antioxidant to combat diseases like cancer, diabetes, etc. caused by oxidative stress.

#### 5. CONCLUSION

From this study, we can conclude that the AEEF was found to exhibit higher free radical scavenging activity and could be investigated as a possible new source of natural antioxidants in the food and pharmaceutical industry. This may also lead to the development of a new generation of drugs that possess both chemotherapeutic and chemo protective properties which can result in ways of combating the serious problems of diseases including diabetes.

## 6. Acknowledgements

We are deeply indebted to Department of Zoology, B. Borooah College for their kind help and support. Authors are also thankful to Biotech HUB, B. Borooah College, for providing the laboratory facility to carry out the research work.

## REFERENCES

1. A. Dewanji, S. Matai, L. SI, S. Barik & A. Nag, *Plant foods for Human Nutrition*, 1993, 44; 11-16
2. Achola, KJ, et al, Bronchodilating and uterine activities of *Ageratum conyzoides* extract, *Pharmaceutical Biology*, 36(2), 1998, 93-96.
3. Ahmed, M, Saeed, F, et al, Evaluation of Insecticidal and Antioxidant activity of selected Medicinal plants, *Journal of Pharmacognosy & Phytochemistry*, 2013, 2(3), 153-158
4. Alebiosu C. O., Yusuf A. J. (2015), Phytochemical Screening , Thin-layer Chromatographic Studies and UV Analysis of Extracts of *Citrullus lanatus*, *Journal of Pharmaceutical, Chemical and Biological Sciences*; 3(2): 214-220.
5. A J Akindele, O O Adeyemi, *Fitoterapia*, 2007, 78, 25-28.
6. Assanga SB, Lujan LML, Espinoza CL, Salido AAG, Angulo DF, Pino JLR, Haines DD. 'Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*'. *BMC Research Notes* (2015)8.
7. Bansal, A.K., Bilaspuri, G.S., 2011, Impacts of oxidative stress and antioxidants on semen functions (review article), *Vet. Med. Int.*
8. Chakraborty R, De R, Devanna N, Sen S., North East India an ethnic Storehouse of Unexplored Medicinal Plants, *J Nat Prod Plant Resour* 2012; 2(1):143-152.
9. Cook, N.C., and Samman, S. 1996, Flavonoids chemistry, metabolism, cardioprotective effects, and dietary sources, *J. Nutr. Biochem.*, 7:66 - 76.
10. Dua TK, Dewanjee S, Khanra R, Prophylactic role of *Enhydra fluctuans* against arsenic-induced hepatotoxicity via anti-apoptotic and antioxidant mechanisms, *Redox report*, 2016, Vol.21, no.4.
11. Ghosh D., Ray S., Ghosh K., Micard V., Chatterjee U.R., Ghosal P.K., Ray B., Antioxidative Carbohydrate Polymer from *Enhydra fluctuans* and its interaction with Bovine Serum Albumin, *Biomacromolecules*, Vol.14:, Issue. 6, : Pages. 1761-1768. May 1, 2013.
12. Ghasemzadeh A, Jaafar HZE, Bukhori MFM, Rahmat H, Rahmat A. 'Assessment and comparison of phytochemical constituents and biological activities of bitter bean (*Parkia speciosa* Hassk.) collected from different locations in Malaysia', 2018. *Chem. Cent. J*;12:12.
13. Hossain A., Uddin N., Salim A., Haque R (2014), Phytochemical and Pharmaceutical screening of *Coccinia grandis* Linn., *Journal of Scientific and Innovative Research*, 3(1): 65-71.
14. Halliwell, B., 1994, Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 344, 721–724.
15. Hassan I.A., Nasiru I.A., Malut A.M., Abdulkadir I., Ali A.S. (2015), Phytochemical studies and thin layer chromatography of leaves and flower extracts of *Senna siamea lam* for possible biomedical applications, *Journal of Pharmacognosy and Phytotherapy*, Vol. 7(3), pp. 18-26.
16. Islam A., Khan R.I., Hossain S., Alam A. H. M. K., Wahed M. I. I, Rahman B. M., Anisuzzaman A. S. M., Shaheen S. M., Ahmed M., Antidiabetic and Hypolipidemic effects of different fractions of *Coccinia cordifolia* L. on normal and streptozotocin-induced diabetic rats, *Pak. J. Pharm. Sci.*, Vol.24, No.3, July 2011, pp. 331-338.
17. Kumar SP, Jagannath PV, Chandra DS, Prasan ND (2012), Hepatoprotective activity of *Enhydra fluctuans* Lour. aerial parts against CCl4-induced hepatotoxicity in rats, *International Journal of Research in Ayurveda and Pharmacy* ; 3(6)893-896.
18. Kumar GP, Khanum F (2012), Neuroprotective potentials of Phytochemicals, *Pharmacognosy Review*; 6(12): 81-90.
19. Koleva I.I., van Beek T.A., Linssen J.P.H., Groot A., Evstatieva L.N., Screening of Plant Extracts for Antioxidant Activity: a Comparative Study on Three Testing Methods, *Phytochem. Anal.* 13, 8-17(2002).
20. Kuri S, Billah MM, Rana SM, Naim Z, Islam MM, Hasanuzzaman M, Ali MR, Banik R., Phytochemical and *in-vitro* biological investigations of methanolic extracts of *Enhydra fluctuans* Lour., *Asian Pac J Trop Biomed*, 2014 Apr;4(4):299-305.
21. Nabi S.A., Kasetti R.B., Sirasanagandla S., Tilak T.K., Venkateshwarulu M., Kumar J. and Rao C.A., Antidiabetic and antihyperlipidemic activity of *Piper longum* root aqueous extract in STZ induced diabetic rats, *BMC Complementary and Alternative Medicine* 2013, 13:37.
22. Ncube NS, Afolayan AJ, Okoh AI (2008), Assessment techniques of antimicrobial properties of natural compounds of plant origin, current methods and future trends, *African Journal of Biotechnology*; 7(12): 1797-1806.
23. Pandey AK, Bora HR., Edible plants of Shan Tribe of Assam, *Ancient Science of Life* 1997; XVI(4): 258-276

24. Panigrahi R, Bhatnagar S, Cytotoxic, Phytochemical and antioxidant potential of marshy herb *Enhydra. fluctuans* Lour., *International Journal of Ethnobiology & Ethnomedicine*, 2015, Vol.1, Issue 1.
25. Patil KS, Majumder P, Wadekar RR, Effect of EF Lour leaf extract on phagocytosis by human neutrophils, *Journal of Natural Remedies* 2008; 8(1):76-81
26. Patel, R.M, Patel , N.J, *In vitro* antioxidant activity of coumarin compounds by DPPH, superoxide and nitric oxide free radical scavenging methods, *Journal of advanced American Journal of Ethnomedicine* ISSN: 2348-9502 Page 244-249
27. Panovska TK, Kulenova S, Stefova M (2005), In-vitro antioxidant activity of some *Teucrium* species (Lamiaceae), *Acta Pharm.* 55:207-214.
28. Sannigrahi S, Majumdar UK, Mondal A, Pal D, Mishra SL, Roy S (2010), Flavonoids of *Enhydra fluctuans* exhibit anticancer activity against Ehrlich's ascites carcinoma in mice, *Nat Product Commun*; 5(8):1239-42.
29. Sandhya B, Thomas S, Isabel W, Shenbagarathai R (2011), *Complementary and alternative medicines*, 3, 101-114.
30. Shekhar HU, Goto M, Watanabe J, Konishide-Mikami I, Bari ML, Takanoishikawa Y., Multi food functionalities of Kalmi Shak (*Ipomoea aquatica*) grown in Bangladesh, *Agric Food Anal Bacteriol.*, 2011;1(1):24-32
31. Samantha T, Acharya RS, Srinivas P, Ramaswamy N, Quantification of total Phenolic and total flavonoids contents in extracts of *Oroxylum indicum* L. Kurz., *Asian J Pharm Clin Res.* 2012;5(4):177-179
32. Sandhya B, Thomas S, Isabel W, Shenbagarathai R (2011), *Complementary and alternative medicines*, 3, 101-114.
33. Semwal A, Singh F.M., *In-vitro* antihelminthic activity of *Diplazium esculantum* (Retz.) Swiss rhizome extract, *Journal of Pharmacognosy and phytochemistry* 2012;1(4):84-87.
34. Singleton V.L., Rossi J.A., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *American Journal of Enology and Viticulture*, 16(3), 144-158, 1965.
35. Swain PK, Dinda SC, Nayak DP, Kar B, Patro VJ, Antioxidant activity of *Enhydra fluctuans* Lour. aerial parts, *Journal of Phytotherapy and Pharmacology*, (2012)-4, Vol.-1(2), pp:23-34
36. Uddin SJ, Ferdous MS, Rouf R, Alam MS, Sarker MAM, Shilpi JA (2005), Evaluation of Anti-diarrhoeal activity of *Enhydra fluctuans*, *J Med Sci*; 5(4):324-327.
37. Vinay RP, Prakash RP, Sushil SK, Antioxidant activity of some selected medicinal plants in western region of India', *Adv Biol Res.* 2010;4(1):23-26.
38. Vaidyaratnam, Varier, PS, *Indian Medicinal Plants- A Compendium of 500 species*, I, Orient longman publishing house, Kottakkal-India, 2002, 146.
39. Zhou K, Su L, Yu L (2004), Phytochemicals and antioxidants in wheat Bran. *J. Agric. Food Chem*, 52:6108-6114.