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Phytochemical and *in vitro* biological investigations of methanolic extracts of *Enhydra fluctuans* Lour.Sourov Kuri¹, Md. Mustahsan Billah^{1*}, S. M. Masud Rana¹, Zannatul Naim², Md. Mahmudul Islam¹, Md. Hasanuzzaman¹, Md. Ramjan Ali¹, Rana Banik¹¹Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh²Department of Pharmacy, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh

PEER REVIEW

ABSTRACT

Peer reviewer

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Comments

This is a valuable research work in which authors have demonstrated three important activities of *E. fluctuans*, antioxidant, anthelmintic and thrombolytic activities. In general, people eat this herb as vegetables. Therefore, the findings of this study will encourage people to use this as medicinal plants in future.

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Objective: To study the phytochemical and biological properties (antioxidant, anthelmintic and thrombolytic) of methanolic extracts of *Enhydra fluctuans* Lour., a plant belonging to the Asteraceae family.

Methods: The phytochemical evaluation was carried out by qualitative analysis. *In vitro* antioxidant activity of extract was studied using free radical scavenging assay, ability of reduction, total phenol and total flavonoid contents determination assays. The anthelmintic activity was determined using paralysis and death time of *Pheretima posthuma* (earthworm) and thrombolytic activity by clot disruption assay.

Results: The phytochemical evaluation showed significant presence of flavonoids, triterpenes, carbohydrate, reducing sugars, saponins, phenols, diterpenes, protein and tannin. The antioxidant activity was found significant [IC₅₀=(135.20±0.56) µg/mL] as compared to ascorbic acid [(130.00±0.76) µg/mL]. The reducing power was increased with concentration. Total phenol and total flavonoid contents were (153.08±0.38) mg/mL and (172.04±0.56) mg/mL respectively. The paralysis and death time of earthworms for different concentrations of extract were determined and compared with albendazole. The results showed that 10 mg/mL of the crude extract had similar effect with albendazole. Additionally, the crude extract showed a concentration depended relationship with its anthelmintic property. The clot lysis activity of crude extract was compared to the standard streptokinase's clot lysis (40.13%) activity and found significant (31%).

Conclusions: The study proves that the crude methanolic extract of *Enhydra fluctuans* Lour. has significant antioxidant, anthelmintic and thrombolytic activity containing wide range of phytochemicals.

KEYWORDS

Enhydra fluctuans Lour., Phytochemicals, Antioxidant, DPPH radical scavenging, *Pheretima posthuma*, Streptokinase

1. Introduction

Nature has been a supply of medicative agents for thousands of years and a formidable variety of recent medications were isolated from natural sources, several of that supported their use in ancient medication. In

sight of this, our attention has been targeted significantly to *Enhydra fluctuans* Lour. (*E. fluctuans*) (Family: Asteraceae), native name (helencha), edible semi aquatic nonwoody vegetable plant with separate leaves. It is native to India, Bangladesh, Burma, Sri Lanka and a number of other places in South East Asia[1]. This plant may be a

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prostate, spreading, annual herb. The stems are somewhat fleshy, thirty centimeters or a lot of long, branched development at the lower nodes, and somewhat bushy. The leaves are sessile, linear–oblong, three to five centimeters long, pointed or blunt at the tip, sometimes truncate at the bottom[1]. The leaves are slightly bitter, cure inflammation, skin diseases and small pox. It possesses biological value and its fuel extract has been reportable to own analgesic, cytotoxic, antimicrobial, hepatoprotective, hypotensive, CNS depressant, antidiarrheal activity[2–7].

The role of free radical reactions in disease pathology is well established. The free radicals are responsible for not only in support of aging but also many age–related diseases[8]. Free radical damage within cells has been connected to a range of disorders including cancer, arthritis, atherosclerosis, Alzheimer's disease, and diabetes[9–11]. There has been some proof to suggest that free radicals and some reactive nitrogen species trigger and increase cell death mechanisms such as apoptosis and in extreme cases necrosis[12,13]. Scientists recommend that antioxidant can reduce the activity of free radicals including their so called side effects and thus increase the cell survival times effectively[14].

Thrombosis is the fundamental patho–physiological process that underlies the acute coronary disorders which are the main causes of morbidity and mortality in developed countries. Portal vein thrombosis frequently caused by thrombus formation in vein leads to the constricting of portal vein followed by portal hypertension. Cerebral epithelial duct occlusion may be a common disorder that in the midst of vital morbidity and mortality. Clot buster medication like tissue proteinase, alteplase (Activase), reteplase (Retavase), tenecteplase (TNKase), streptokinase, urokinase *etc.* play an important role in the management of patients with cerebral epithelial duct occlusion[11].

Helminthiasis, a macro–parasitic disease, is observed in humans and animals which reflects serious social and economic problems throughout the world, especially in the third world countries. In this disease, a part of the body is infested with parasitic worms like roundworms (nematodes), tapeworms (cestodes) or flukes (trematodes)[15]. In the medical field, helminthes have been a matter of concern for centuries and they still cause considerable problems to human and other animals. World Health Organization estimates that about two billion of people throughout the world are affected by parasitic worm infection and the reason for it is associated with poor management practices and inadequate control measures[16]. Although numerous advances were made in understanding the mode of transmission and the treatment of the helminthes during the last few decades, there is still no potential product which can control specific helminthes[17].

However, indiscriminate use of several anthelmintic, antioxidant, clot buster medication has emerged problems,

leading to the development of resistance as well as chemical residue and toxicity problems[18]. For these reasons, phytochemical screening of medicinal plants for their antioxidant, anthelmintic and clot lysis activity has become a matter of great scientific interest though synthetic chemicals are extensively used in modern clinical practices worldwide[19]. On the contrary, our traditional system of medicine and folklore are using the whole medicinal plant or a part of it for the treatment of all types of disease successfully including antibacterial, anthelmintic, anti–inflammatory *etc.* since the time immemorial[20]. This is because the traditional medicines act as an easily available and effective source of medicines to people with a broad spectrum of action like high percentage of cure with single therapeutic dose, cost effective and free from toxicity[21].

Thus the native use of *E. fluctuans* as medicament prompted us to research the phytochemical analysis, antioxidant, anthelmintic and clot buster activity of *E. fluctuans* that has not been explored so far. The methanolic extracts of *E. fluctuans* were evaluated for phytoconstituents, total phenolic content, total flavonoid content, the 1, 1–diphenyl–2–picrylhydrazyl (DPPH) scavenging activity, ability of reduction, the anthelmintic and clot lysis activity in the present study.

2. Materials and methods

2.1. Chemicals

Lyophilized *S–Kinase*TM (streptokinase) vial (1 500 000 IU) was purchased from Popular Pharmaceuticals Ltd., Bangladesh; Batch No: VEH 09. DPPH (1, 1–diphenyl 2–picryl hydrazyl), trichloroacetic acid, gallic acid, ferric chloride and quercetin were obtained from Sigma Chemical Co. Ltd, (St. Louis, MO, USA). Ascorbic acid was obtained from SD Fine Chem. Ltd., India. All other chemicals and reagents were of analytical grade.

2.2. Plant materials

Whole plant of *E. fluctuans* was collected from Lakshmipur district, Bangladesh in July 2012. After collection whole plant were thoroughly washed with water. The plant was identified and authenticated by taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (accession number–37925). The collected plant parts were separated carefully. The separated samples were then dried at room temperature in the shade and away from direct sunlight for 5 d and finally kept in hot air oven for 3 d.

2.3. Preparation of crude extract

After drying, the total plants were coarsely fine–grained (120 g) and extracted by dissolving with methanol (500 mL)

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