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In vitro Evaluation of Thrombolytic and Antioxidant Scavenging Activity of *Eichhornia crassipes*

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Abstract

With the advancement of medical science, the importance of plants in search of new drug is gradually increasing day by day. We have evaluated the thrombolytic and antioxodent scavenging activity of organic extract of *Eichhornia crassipes* in this study. Thrombolytic activity of the plant extract was done with the help of human blood. The results found in thrombolytic test were as follows: methanol 23.37%, n-hexane 13.98%, carbon tetra chloride 19.01% whereas blank and standard were 4.63% and 71.25% respectively. It has been observed that the clot lysis effect of Eichornia *crassipes* was remarkable as compared to the positive control and blank. The antioxidant activity of the plant was carried out spectrophotometrically with the help of Tert-butyl-1-hydroxytolune (BHT) as standard. It has been estimated that Methanolic, n-haxane and carbon tetra chloride soluble partition of the leaf have got their free radical scavenging activity with IC_{50} value 0.018, 0.387, and 1.03µg/ml respectively. The study determined that *E. crassipes* extract have antioxidant activity. However, it possesses less thrombolytic activity. Therefore, it may be assumed that the plant may have potential bioactive compounds having antioxidant activity which may be used in pharmaceutical purpose.

I. Introduction

Since the early days of human civilization, the study of plants and its components for various activities and treatment of disease have been continuing. It has been declared by Norman R. Farnsworth of the University of Illinois that for every disease that affects mankind possesses its treatment of a cure is available on the earth. With the advancement of medical sciences, the importance of plants in search of new drug is rising gradually. A range of chemical compounds have been found in plant sources. A good number of these compounds having a wide range of biological activities are highly difficult to synthesize in laboratory. Pharmacy, the most important disciple in science and practice of medicine and its primary source has been playing an important role in discovering the new molecule of drug through chemical synthesis as well as from its natural source (ANM Alamgir, 2017).

At the early days of human civilization, people used to reduce their sufferings from injury or disease by utilizing the plant growing in the nature. Therefore, the history of drug from natural source is very resourceful and well established (D. A. Dias, 2012). It has been found form the literatures that the healing powers of some plants were established by accidents. We have found in the history that Babylonians had used a large number of medicinal plants for treating their disease. Many of their discovered plants are still in use almost in the similar ways. So, it has been safely assumed that the acquired knowledge of one generation is transmitted to another generation and additional information was incorporated to it by the next generation from their experience and observation (ANM Alamgir, 2017).

An antioxidant is an important molecule that possesses the property of slowing down or inhibiting the oxidation of other molecules. Oxidation is a chemical reaction which appears to transfer electrons from a substance to an oxidizing agent (MN Islam *et al*., 2011). An oxidation reaction has the capacity of producing free radicals leading to chain reactions causing cell damage. With the removal of free radical intermediates, antioxidants slow down these chain reactions. By being oxidized themselves, it also prevents other oxidation reactions (V. Lobo, 2010). Therefore, antioxidants are most of the cases found as reducing agents such as thiols, ascorbic acid or

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polyphenols (DM Kasote *et al*., 2015 and E. B. Kurutas, 2016).

Water hyacinth (Eichhornia crassipes belonging to Family Pontederiaceae) is one of the most productive plants on earth. It has been considered among one of the top ten world's worst weeds (AM Aboul-Enein *et al*., 2011). It is found to have a significant role in altering water clarity and decrease phytoplankton production. Dissolved oxygen, nitrogen, phosphorous, heavy metals and concentrations of other contaminants are also affected by this plant (AM Villamagna *et al*., 2010). It is extensively distributed to tropical and subtropical regions and now available in about 62 countries in Africa, Asia and North America, between 40 degrees North and 45 degrees South (AM Aboul-Enein *et al*., 2011).

Water hyacinth has been reported to be a rich source of natural bioactive compounds with antimicrobial, antitumoral, antiviral, anticancer and antioxidant activities (SMM Shanab *et al*., 2012). A good quantity of alkaloids, flavonoids, phenols, sterols, terpenoids, anthoquinones and protein is available in this plant (TP Lalitha *et al*., 2012). Highest percentage of tannins and alkaloids and moderate presence of glycosides and saponins and flavonoids have been found in this weed which leads to antimicrobial and chemotherapeutic activities (EN Ogamba *et al*., 2015). This aquatic weed has been reported to possess biologically active phytochemicals compounds that may be used for the treatment infectious diseases caused by bacterial and fungal pathogens (TP Lalitha *et al*., 2012; P Jayanthi *et al*., 2013).

As far as our knowledge goes, no thrombolytic activities on the plant have been done yet on this plant. Therefore, in this study we tried to evaluate the thrombolytic activity of *Eichhornia crassipes* as well as would like to validate its free radical scavenging activity.

II. Material and Methods

Collection and preparation of plant materials

During the month of January to February, 2017 the fresh plant *Eichhornia crassipes* was collected from Gazipur. The taxonomical identification of the plant *Eichhornia crassipes* was carried out by Bangladesh National Herbarium. To remove adhering dirt, the plant was first washed with water and then it was cut into small pieces. Furthermore, it had undergone to sun-dried for 4 days. When the drying was complete, with the help of grinding machine the entire portions were pulverized into a coarse powder. It was then stored in an airtight container for further use.

Extraction of the plant material

The extraction of the plant powder materials (600gm) was done by cold extraction process with methanol (1300ml) in a flat bottom glass container. Occasional shaking was done and it had been stirred for 7 days. Filtration of the extract was done through filter paper and by using a water bath at 40˚C; the filtrates were concentrated to afford solid masses. Solvent-solvent partitioning was carried out with the help of separating funnel. The concentrated methanol extract of whole plant were converted to slurry with water and it was then transferred to 100ml separating funnels. In order to get the n-hexane soluble fraction, 30 ml of n- hexane was added to the separating funnels. The n-hexane layers (upper layer) of the soluble extract were collected separately. Repetition of the process was done for three times. All the extract found each time was combined. It was then concentrated into solid masses using a water bath at 40˚C. The final mass of n-hexane extract of the plant was obtained as 1.37gm. Similar extraction process was done to get chloroform ($CHCl_3$) and carbon tetrachloride (CCL_4) soluble fractions that lead to 1.09gm and 0.93g extract respectively.

Thrombolytic Effect

Plant sample preparation

In 10 ml of distilled water 100mg extract was suspended. It was then taken in a vortex mixer for shaking and kept overnight. The decantation was done for soluble supernatant and then it was filtered. With 100µl of this aqueous preparation each micro centrifuge tubes were filled.

Blood specimen preparation

Five micro centrifuge tubes were sterilized and weighed. 5ml blood from volunteers was added to each of the pre weighed (w_1) micro centrifuge tubes (1 ml per each tube). The specimen of the blood was centrifuged at 2500 rpm for 5 minutes. It was then incubated for 45

minutes at 37^0C. With the help of decantation and capillary absorption, the serum was completely removed after clot formation. Without disrupting the clot, the serum was removed from inner surface of tube carefully with the help of cotton bar. After first removal of serum, the tubes were allowed to keep at lying position on a tray for 6 minutes. The liquids of the tube were removed with the help of the cotton rod and weight (w_2) of each tube was taken again. Weight of clot was obtained by subtracting the weight of tube alone (w_1) from the weight of clot containing tube (w_2). One hundred (100) µl of aqueous extract of plant (*Eichhornia crassipes)* was added to each of the micro centrifuge tube containing pre weighed clot. 100 µl of streptokinase as positive control was added to clot of one of the tubes which were used as standard. 100 µl water as negative control was added to clot of another tube that was used as blank.

Clot lysis was observed after incubating the samples at 37°C for 90 minutes. The released fluid was completely removed by decantation after the incubation period (90 minutes). With the help of cotton bud the clot containing liquid from the inner surface of the tube was removed carefully without disrupting the clot. Weight of the tubes (w_3) was measured again.

Evaluation of antioxidant effect

The antioxidant activity of the plant was evaluated spectrophotometrically with the help of the procedure described by Benzie and Strain (IF Benzie *et al*., 1996). According to this method, 2.0 ml of different concentration from 3.37 µg/ml to 100µg/ml of methanolic extract were mixed with 3.0 ml of a DPPH methanol solution with the concentration of 20 µg/ml. Reaction period at room temperature in dark place was set at 30 minutes. The absorbance was measured at 517 nm by UV spectrophotometer where methanol and BHT were used as blank and positive control respectively. By using the following formula free radical inhibition of DPPH was calculated in percent (I%)-

$$(I\%)=(1-A_{sample}/A_{blank})\times 100$$

,where A_{blank} is denoted as absorbance of the control reaction 50% inhibition (IC_{50}) provided by concentration of the extract was determined from the graph. Tests were carried out for three times and average value was determined.

III. Result and Discussions

Thrombolytic activity

The thrombolytic activity of *Eichornia crassipes* and comparative effects of its different extracts had been evaluated in this study. 100µl of three different extracts such as methanol, n-hexane and carbon tetrachloride of *Eichornia crassipes* as well as standard and distilled water were added to 20 different blood clots which were kept in incubation at 37°c for 90 minutes. After the incubation period it had been observed that the maximum clot lysis 71.25% was done by the positive control (standard) whereas very negligible clot lysis was carried out by negative control. The clot lysis of different materials was observed as follows: methanol 23.37%, n-hexane 13.98%, carbon tetra chloride 19.01%, blank 4.63% and standard 71.25%. It had been appeared that the clot lysis effect of *Eichornia crassipes* was not remarkable compared to the controls.

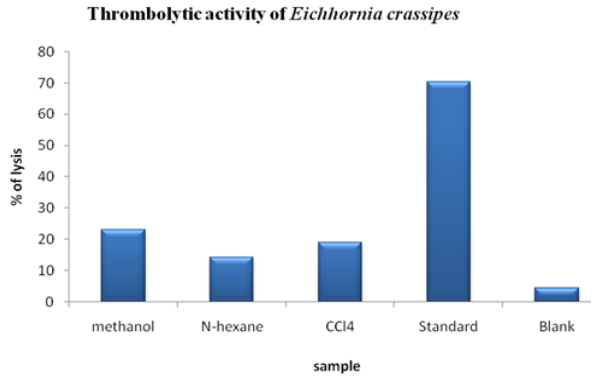

Figure 1: Thrombolytic activity of different extract of *Eichornia crassipes*

Antioxidant activity

Investigation of free radical scavenging activity of different fractions of methanol extract of ***Eichhornia crassipes*** were carried out in this study. Tert-butyl-1-hydroxytolune (BHT) was used as reference standard in this work. Free radical scavenging activity with IC_{50} value 0.018, 0.387, and 1.03 µg/ml had been observed for methanol, n-haxane and carbon tetra chloride soluble fraction of the leaf respectively. Free radicals as well as other reactive oxygen species

are available in all living organisms that have different biological roles. It has been reported in many scientific articles that signal transduction, sensing of oxygen tension and regulation of functions controlled by oxygen concentration (H Zhu *et al*., 1999; N Sommer *et al*., 2016 and RR Bartz *et al*., 2010). Synthesis of energy and essential molecules are also dependent on oxygen (B Alberts, 2002). In addition to this, in boosting of immune system oxygen plays a great role (X Chen *et al*., 2016).

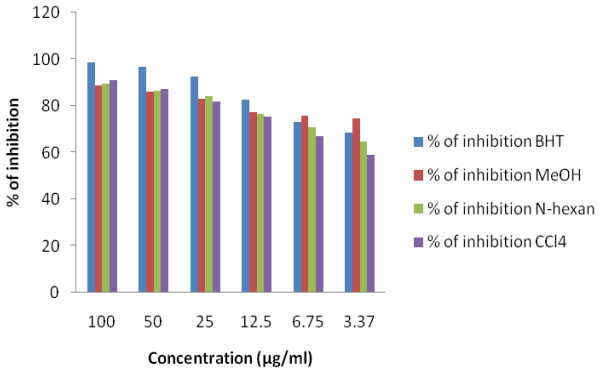

Figure 2: percentage of inhibition of different extract of *Eichornia crassipes*

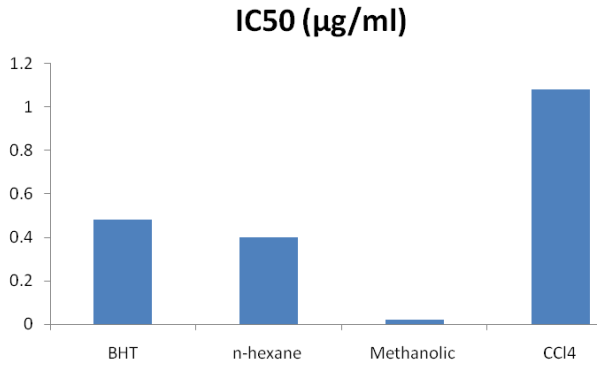

Figure 3: IC_{50} value of different extract of *Eichornia crassipes*

Due to fast electron transfer process, phenolic compounds are normally very active in scavenging DPPH free radicals (MC Foti *et al*., 2004). The extract containing high levels of total phenolic content (methanol extract of *Eichornia crassipes* leaves) was found to have potent DPPH radical scavenging activity. Therefore, it may be assumed that the phenolic compounds may be the main constituents responsible for antioxidant activity of the plant. It has been observed that the results of the present study have the consistency with the findings of some of the previous studies (A Surendraraj *et al*., 2013; P Thamaraiselvi *et al*., 2012 and P Sunitha *et al*., 2018).

Conclusion

It has been demonstrated through a large variety of studies for a long period of time that plant extracts are found to have a diverse range of bioactive compounds possessing phytochemical, antioxidant, antibacterial and cytotoxicity and many other biological activities. Our present study suggests that the *E. crassipes* extract may have bioactive compounds having antioxidant properties. However, it has been found to have few thrombolytic activities. Therefore, it is recommended that the plant may be subjected to further advanced research in search of the potential bioactive compounds having antioxidant properties as well as for human use through pharmaceutical processes.

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