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In vitro Assessment of Antioxidant, Membrane Stabilization and Thrombolytic Activities of Ethanolic Extract of *Lagenaria siceraria* Fruit

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Abstract

Lagenaria siceraria is a well-known vegetable fruit in Bangladesh. This study aimed to investigate the antioxidant, membrane stabilizing and thrombolytic activities of the ethanolic extract of *L. siceraria* fruits (EELSF). Total phenolic content and DPPH free radical scavenging activity tests were done to determine antioxidant properties. The anti-inflammatory effects were evaluated using heat- and hypotonic solution induced hemolysis. Human RBC from healthy volunteers were used to assess the thrombolytic activity of the extract. Ethanolic extract of *L. siceraria* fruits displayed noticeable antioxidant, membrane stabilizing and thrombolytic activities when compared to the standard drugs. Further studies are necessary to evaluate the pharmacological activities of different solvent fractions and plant parts of *L. siceraria*.

Key words: *Lagenaria siceraria*, antioxidant, thrombolytic, anti-inflammatory, membrane stabilizing activity.

Introduction

Natural products have long been recognized for their substantial contribution to the global treatment and prevention of various human diseases (Newman *et al.,* 2003; Paterson and Anderson, 2005). Natural medicines or drugs are derived from a diverse array of sources, encompassing terrestrial plants, microbes, marine organisms, as well as terrestrial vertebrates and invertebrates. The significance of natural products in contemporary medicine has been the subject of recent scholarly reviews and reports (Cragg and Newman, 2013; Croteau *et al.*, 2000; Demain and Fang, 2000; Harvey, 1999; Yuan *et al.*, 2016). Natural products have brought forth a broad range of chemical entities with diverse characteristics. These entities serve as templates for both semi-synthetic and total synthetic modifications, which can potentially be utilized in the development of treatments for various diseases (Ahire *et al.,* 2021). In this regard, herbal medicine is gaining significant ground in the management of numerous modern-day chronic diseases, surpassing conventional medicine (Ekor, 2014). Moreover, alternative medicine demonstrates superior efficacy compared to conventional medicine when addressing acute ailments such as coughs, viral respiratory tract infections, colds, cystitis and various other conditions that resolve on their own (Fintelmann *et al.*, 2012).

Lagenaria siceraria is a widespread vegetable fruit that is consumed all over the world. It is one of

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the best fruits for human beings that nature has ever created and given as a gift. The chemical composition of this plant includes all of the necessary components that are necessary for maintaining normal and good human health (Rahman, 2003). The fruits are commonly consumed as vegetables in Africa and Asia. Moreover, the shoots, tendrils and leaves are boiled and the seeds are extracted for oil extraction (Manandhar, 2002). Furthermore, seeds, tendrils and young leaves are utilized for medicinal reasons (Yetisir and Sari, 2003). *L. siceraria* is one of those vegetables that are available in both rich and poor people, so the pharmacological efficacy of *L. siceraria* may bring a new light to the disease treatment procedure in Bangladesh. Thus, this study aimed to assess the antioxidant, thrombolytic and membrane stabilization activities of ethanolic extract of *L. siceraria* fruits.

Materials and Methods

Collection and preparation of the plant material: Fresh *L. siceraria* or bottle gourd fruit was purchased from local market of Dhaka (Figure 1). Then the fruits were washed thoroughly for proper cleaning. After cleaning the fresh fruits, the seeds were separated from the fruit body and the remaining fruits were cut into very small pieces. Total weight was measured before they were sundried for 10 days from sunrise to sunset. During this period, temperature was (33 ± 5)^{o}C and relative humidity was (45 ± 5)%. After the fruits were fully dried, it was pulverized into a fine powder and weighed. Dried fruit powder was soaked in 70% ethanol for 15 days. The solution was filtered after every three days. The obtained extract was dried in a rotary evaporator at low temperature and pressure. Finally, the obtained residue was cooled and stored to conduct the required pharmacological tests.

Figure 1**.** Different plant parts of *Lagenaria siceraria***.**

Botanical authentication: According to the rules of Bangladesh National Herbarium, we deposited the sample of each part of our plant species presented in figure 2 and the herbarium authority authenticated the plant (Accession number: DACB 87071).

Chemicals and drugs: Sodium carbonate, Folin-Ciocalteu reagent, gallic acid and ascorbic acid were purchased from Merck Co., Germany. Acetylsalicylic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, USA. Streptokinase was obtained as a gift sample from Incepta Pharmaceuticals Ltd. Bangladesh.

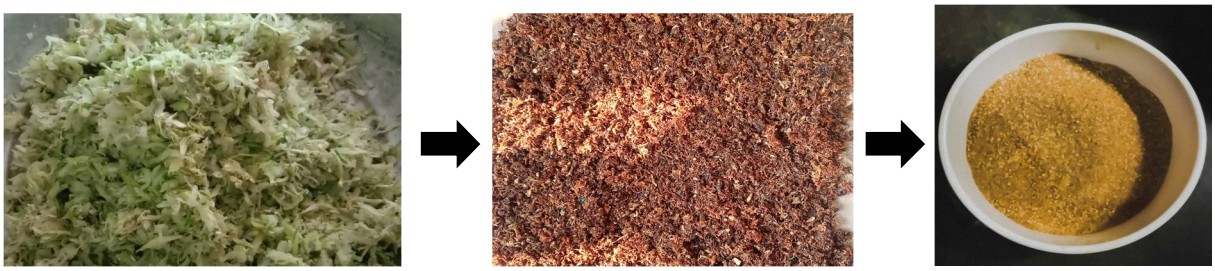

Figure 3. Preparation of fruit powder of *L. siceraria*.

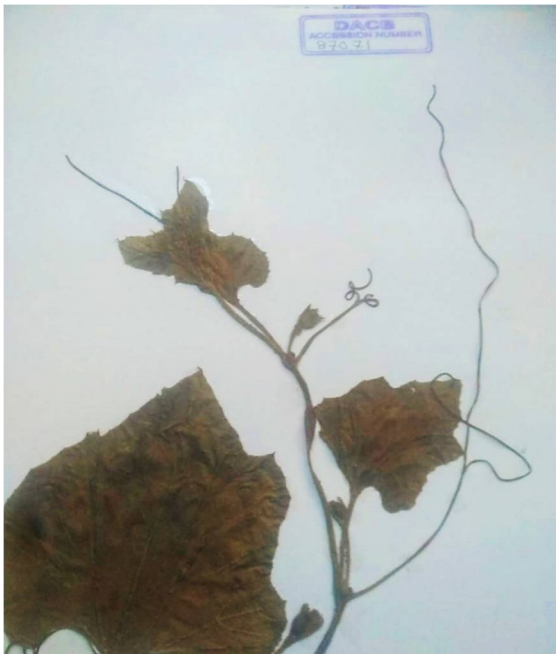

Figure 2. Accession number of *L. siceraria.*

*Determination of total phenolic content***:** The total phenolic content of ethanolic extract of *L. siceraria* fruits was determined using the Folin-Ciocalteu reagent as an oxidizing agent, as described by Skerget (Škerget *et al.*, 2005). Gallic acid was used to prepare standard solutions with their concentrations ranged from 0 to 100 µg/ml. 2.5 ml of the Folin-Ciocalteu reagent, which had been diluted ten times with water, 2.0 ml of a 7.5% w/v Na_2CO_3 solution, and 0.5 mL of the extract solution (concentration: 2 mg/ml) were mixed together to carry out the experiment. The mixture was then allowed to sit at

room temperature for 20 minutes. After the incubation period, the absorbance was measured at 760 nm using a UV spectrophotometer. To determine the sample's total phenolic content, a standard curve was constructed using different concentrations of gallic acid. Total phenolic content was expressed as mg of GAE (gallic acid equivalent)/g of extract.

DPPH free radical scavenging assay: The scavenging of stable radicals of 1,1-diphenyl-2-picrylhydrazyl (DPPH) of the plant fractions were estimated by the method described by Brand-Williams (Brand-Williams *et al.*, 1995). To measure an individual sample's antioxidant activity, 2.0 ml of various sample concentrations (0.97 g/ml to 500 g/ml) were mixed with 3.0 ml of a DPPH (20 g/ml) methanolic solution. The antioxidant efficiency of the standard ascorbic acid and samples was determined using a UV spectrophotometer absorbance at 517 nm after the purple DPPH radical in methanol solution faded to a yellow tint. The inhibition percentage (I%) of the DPPH radical was computed using the following equation:

$$\% \text{ inhibition} = [1-(A_{sample}/A_{blank})] \times 100$$

where A_{blank} is the absorbance of the solution that contain all reagents except test samples and A_{sample} absorbance of the solution that contain fractions or standards.

Standard curves were generated by graphing the percentage of inhibition vs the extract concentration for each sample and the standard. These curves were used to calculate the sample strength required to cause 50% inhibition (IC_{50}). The IC_{50} value is the concentration at which 50% of the DPPH molecules were neutralized. By comparing IC_{50} values, the antioxidant capacity of the samples may be calculated, with lower values indicating more antioxidant capability. This approach provides critical information on the samples' ability to battle oxidative stress and scavenge free radicals.

*Thrombolytic activity assay***:** The thrombolytic activity of the plant extract was evaluated using streptokinase (SK) as the standard drug by the method described by Prasad (Prasad *et al.*, 2007). 5 ml of aliquots of venous blood was collected and split among 10 pre-weighed sterilized Eppendorf tubes containing 0.5 ml each. The tubes were then incubated for 45 minutes at 37°C to induce clot formation. After carefully removing the serum without disrupting the formed clot, the Eppendorf tubes holding the clots were weighed again to estimate their weight. Then, 100 µl of different concentrations of test extract were added in separate volumes to the pre-weighed Eppendorf tubes holding the clots. As a positive control, 30000 I.U. of streptokinase (SK) stock solution was added to the control Eppendorf tubes and 100 µL of distilled water was used as a negative non-thrombolytic control. Clot lysis was visually examined in the Eppendorf tubes after 90 minutes of incubation at 37°C. The ratio of masses after and before clot lysis was represented as a percentage (%) of clot lysis using the following formula:

$$\text{Percentage (\%) of clot lysis} = (\text{Mass of clot after lysis}/\text{Mass of clot before lysis}) \times 100$$

*Membrane stabilizing activity assay***:** The plant extract's membrane stabilizing ability was evaluated using hypotonic solution-induced hemolysis and heat-induced hemolysis of human erythrocytes, as illustrated by Sikder (Sikder *et al.*, 2012). To create an erythrocyte suspension of red blood cells (RBCs), 5 ml of whole blood was taken from a human volunteer. The blood was taken in a sterile test container and stored at room temperature and relative humidity of 55±2%. To inhibit blood coagulation, dipotassium-EDTA (2.2 mg/ml of blood) was added to the test tube before blood collection. The mixture was centrifuged at 3000 rpm for 10 minutes. The blood cells were separated using an isotonic NaCl solution (154 mM, produced in 10 mM Na_3PO_4 at pH 7.4).

Hypotonic solution-induced hemolysis: 4.5 ml of hypotonic solution (50 mM NaCl, made in Na_3PO_4 buffer saline at pH 7.4) containing the plant extract (2 mg/mL) was mixed with 0.50 mL erythrocyte suspension. Control sample was prepared without using the plant extracts. The mixtures were left at room temperature for 10 minutes. To separate the supernatant, the mixture was then centrifuged at 3000

rpm for 10 minutes. The absorbance of supernatant was measured at 540 nm. The % inhibition of hemolysis was calculated using the optical density (OD) mentioned in the following equation:

$$\% \text{ Inhibition} = [(OD_{\text{control sample}} - OD_{\text{experimental sample}}) / OD_{\text{control sample}}] \times 100$$

`Heat-induced hemolysis: Aliquots (5 mL) of an isotonic solution of plant extract (2.0 mg/mL) or acetylsalicylic acid (0.10 mg/ml) were placed in centrifuge tubes. As a control, the same amount of isotonic solution was placed in another tube. Each solution underwent a gentle mix after the addition of 30µl of erythrocyte suspension. The centrifuge tubes were then incubated in a water bath for 20 minutes at 54°C with control samples. Another batch of prepared samples was incubated in an ice bath at 0-5°C. After incubation, the prepared mixtures were centrifuged for 3 minutes at 1300 rpm. At 540 nm, the absorbance of the collected supernatant was measured. Using the following equation, the percentage (%) of hemolysis inhibition was estimated from optical density (OD):

$$\% \text{ Inhibition} = [1 - (OD_{\text{heated experimental sample}} - OD_{\text{unheated experimental sample}}/OD_{\text{heated control}} - OD_{\text{unheated experimental sample}})] \times 100$$

Results and Discussion

Total phenolic content: The antioxidant properties of *L. siceraria* fruits extract may be due to its phenolic content. The antioxidant potential of an extract might be increased with phenolic content augmentation (Madaan *et al.*, 2011; Henríquez *et al.*, 2010). Total phenolic contents of the ethanolic extract of *L. siceraria* fruits were calculated using the regression equation, y = 0.0036x + 0.1339, R^2 = 0.9516, which was obtained from standard curve of gallic acid (x = absorbance and y = gallic acid equivalent in mg/g extract), illustrated in figure 1.

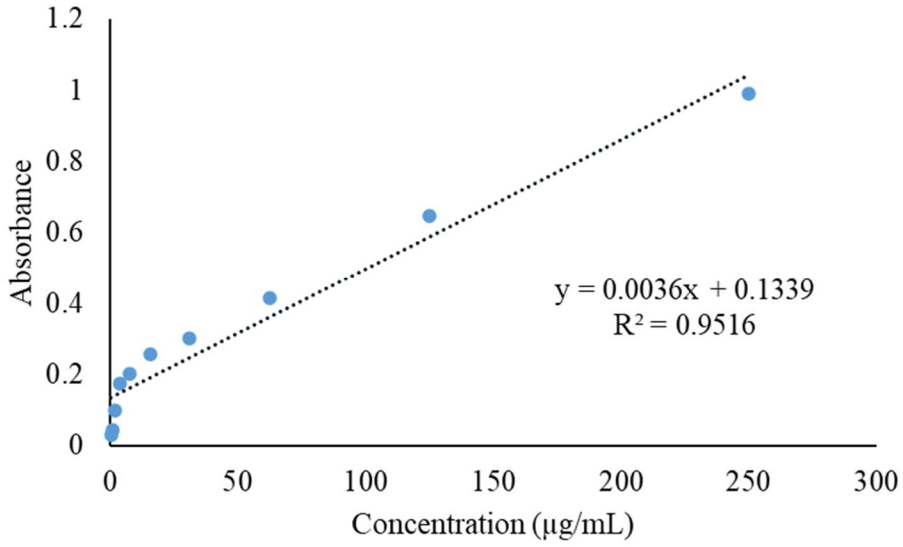

Figure 1. Standard curve of gallic acid for total phenolic content determination.

Absorbance for the ethanolic extract of *L. siceraria* fruits was obtained as 0.328. So, total phenolic content was obtained as 53.92µg/mL.

DPPH free radical scavenging assay: The outcome of the DPPH free radicals scavenging inhibition, was shown in figure 2. The inhibition potentialities increased with concentration of plant extract. It was evident that the ethanolic extract of *L. siceraria* had antioxidant properties with an IC_{50} of 185.83 µg/ml. Moreover, the effect was much weaker than that of the ascorbic acid (IC_{50} = 3.05 µg/ml).

Thrombolytic activity: The ethanolic extract of *L. siceraria* fruits showed lower thrombolytic efficacy than standard streptokinase, illustrated in figure 3.

Membrane stabilization activity: It is thought that a cell's membranes' integrity determines how healthy it is (Ferrali *et al.*, 1992). When RBC is exposed to hypnotic solution, the rupturing of the cell membrane is associated with the deposition of fluid inside the cell. This additional harm results in lipid peroxidation caused by free radicals, which in turn causes cellular harm (Augusto *et al.*, 1982; Halliwell *et al*., 1988). As a result, it is reasonable to assume that chemicals with membrane stabilizing capabilities provide a large amount of protection for the cell membrane against harmful substances (Shinde *et al.*, 1999).

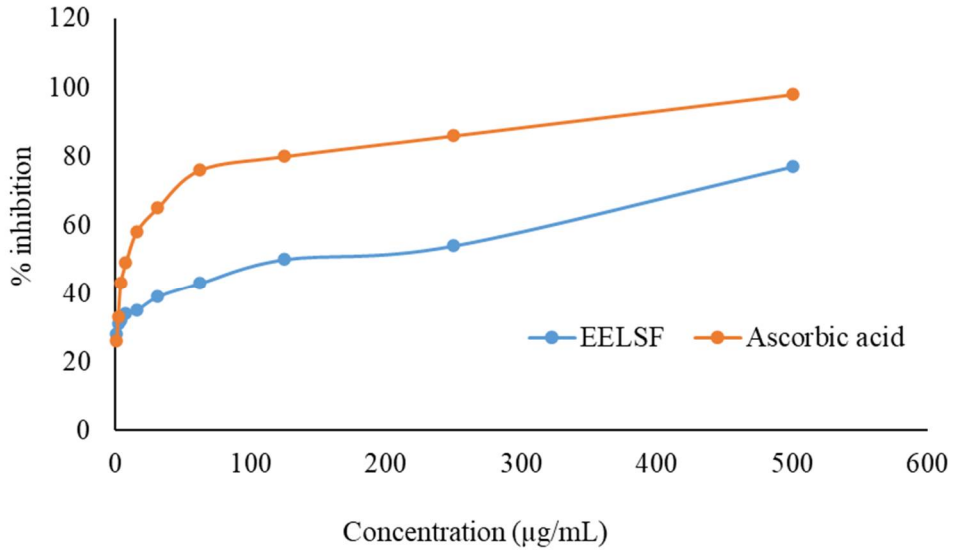

Figure 2. Comparison of the percentage (%) of DPPH inhibition exerted by the ascorbic acid and ethanolic extract of *L. siceraria* fruits (EELSF).

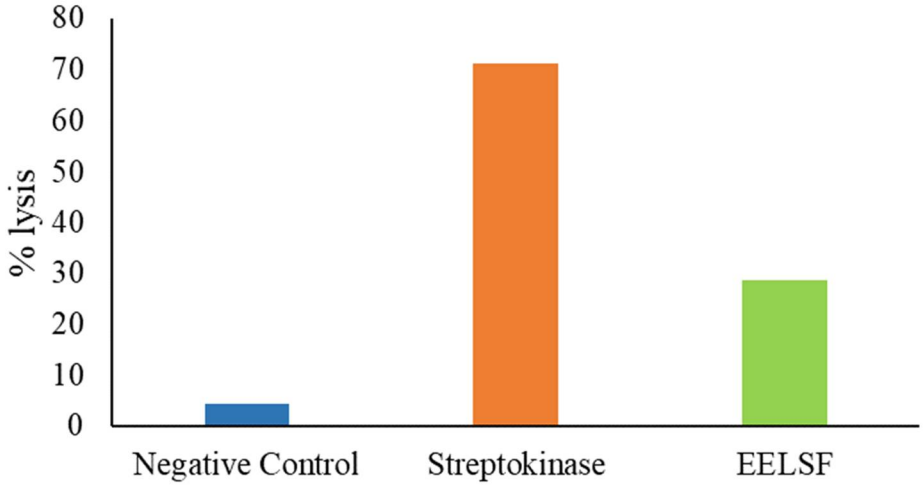

Figure 3. Percentage of clot lysis of streptokinase and the ethanolic extract of *L. siceraria* fruits (EELSF).

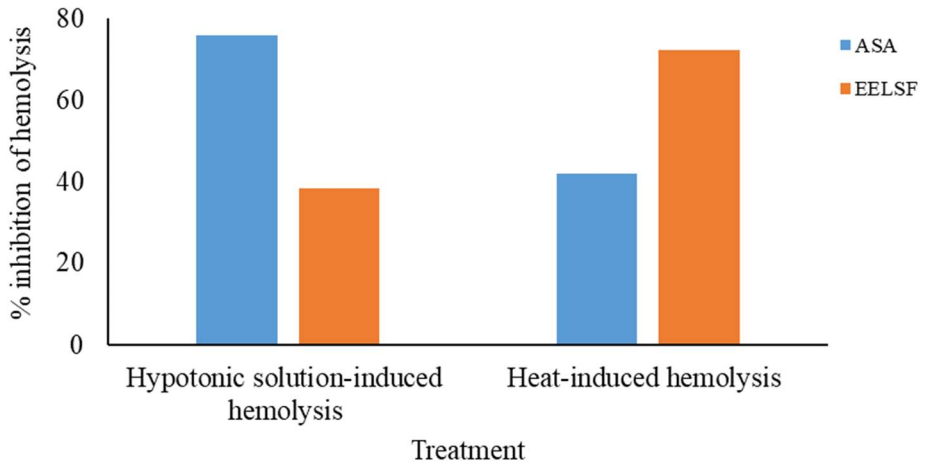

Figure 4. Membrane stabilizing effect of standard acetyl salicylic acid and ethanolic extract of *L. siceraria* fruits (EELSF).

Ethanolic extract of *L. siceraria* fruits exerted greater affinity compared to acetyl salicylic acid (ASA) in heat induced hemolysis, which is illustrated in figure 4. Opposite results were obtained in hypotonic solution-induced hemolysis. Although standard acetyl salicylic acid shown stronger effectiveness than test extract, the latter can still be a suitable source of an anti-inflammatory drug. Numerous earlier researches shown that plants containing flavonoids have the ability to stabilize membranes (Sikder *et al.*, 2012; Shinde *et al.*, 1999).

Conclusion

The current study found that the ethanolic extract of *L. siceraria* fruits has promising free radical scavenging, thrombolytic and membrane stabilization properties. The effects might be attributed to their high phenolic content to some extent. However, more research into *in vivo* effects, as well as separation of the plant's bioactive metabolites are required.

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Author's contribution

MSA has originated the concept. AS worked in the laboratory, AS and FA performed the literature study, JAC, AAC and SK critically reviewed the whole activities. MSA supervised the overall work.

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