

Phytochemical Screening, Cytotoxic and Thrombolytic Activity of Extract of *Brassica oleracea* Flower (Cauliflower)

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Abstract: The study was aimed for phytochemical screening and evaluate the cytotoxic and thrombolytic activity of methanol extract of the flower of *Brassica oleracea*. The cytotoxic activity of crude extract was determined using brine shrimp lethality bioassay and LC_{50} values of the sample was $62.087 \pm 1.22 \mu\text{g/ml}$ whereas for standard vincristine sulphate was $8.50 \pm 0.56 \mu\text{g/ml}$ as a positive control. The plant's extract showed (42.75 ± 3.72 %) clot lytic as compared to standard streptokinase's (67.32 ± 5.25 %) clot lytic activity in case of thrombolysis assay.

Key words: *Brassica oleracea* • Cytotoxic • Thrombolytic Activity • Methanol Extract

INTRODUCTION

Plant-based foods contain significant amounts of bioactive compounds, which provide desirable health benefits beyond basic nutrition. Epidemiological evidence suggests that consumption of a diet rich in vegetables and fruits has positive implications for human health. The World Health Organization reported that 80 % of the world populations rely chiefly on indigenous medicine and that the majority of traditional therapies involve the use of plant extracts or of their active constituents [1] and over 25% of modern medicines that are commonly used worldwide contains compounds extracted from medicinal plants [2]. The active principles differ from plants to plants due to their biodiversity and produce a definite physiological action on the human body that develops interest on their medicinal properties [3]. In recent years, there has been a revival in the use of traditional medicinal plants and therefore, pharmaceutical companies are investigating a lot of money in developing natural products extracted from plants [4]. In Bangladesh thousands of plant species are known to have medicinal value [5] and ninety percent of the medicinal plants are wild sourced [6,7].

During recent decades, there has been an increasing demand for finding newer and safer chemotherapeutic agents. Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries [8]. It is the third leading cause of death worldwide, only preceded by cardiovascular disease, infectious and parasitic disease [9]. Extracts of medicinal plants are believed to contain a wide spectrum of polyphenolic, flavonoids, alkaloids, terpenoids and saponin compounds, which might have therapeutic properties and hinder cancer formation [10]. Over 60% of current cytotoxic agents have been derived from natural sources including plants, marine organisms and microorganisms, either directly or by chemical synthesis based on natural lead compounds [11, 12]. Therefore, natural products have a wide application in cancer chemotherapy [12].

Cardiovascular disease caused by blood clot (thrombus) formation is one among the most severe diseases which are increasing at an alarming rate in the recent years [13]. Homeostasis maintains the integrity of circulatory system after damaging of the vascular channel [14]. Thrombus development is a critical event in the arterial diseases associated with myocardial infarction, anoxia, hypertension [15], stroke, reduction of the blood

supply to the liver [16] and venous thromboembolic disorders that account for considerable number of deaths worldwide [17]. Remarkable efforts have been made towards the discovery and development of natural constituents from various plant and animal sources which have antiplatelet [18, 19], anticoagulant [20, 21], antithrombotic [22] and thrombolytic activity [23-25].

Thrombolytic agents are used to dissolve clot and in the management of thrombosis in patients [26]. Thrombolytic agents such as tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) [27] etc, are used all over the world for the treatment [28] but their use is associated with hyper risk of haemorrhage [29], anaphylactic reaction and lacks specificity [14, 29]. Because of the shortcomings in the existing thrombolytic agents, a number of researches are underway to improve the variants of these drugs for their better effective nature [30].

Cruciferous vegetables are one of the dominant food crops which have high vitamin C, soluble fibre and contain multiple nutrients and phytochemicals with potential anticancer properties. *Brassica oleracea* (Cauliflower) belongs to the family *Brassicaceae* is an annual plant that reproduces by seed. The plant have leaves which are more divided and petiolate. The main head consists of clusters of fully differentiated flower buds which are less densely arranged with longer peduncles. It is an annual herb reaching 400 mm during vegetative stage and 1-2 m at the end of flowering [31]. Cauliflower is low in fat, but high in dietary fibre, potassium, folate, water and vitamin and possesses a high nutritional density. Cauliflower contains several phytochemicals which are beneficial to human health [32]. It has antimicrobial [33, 34] and antioxidant [35] activities. The present study was undertaken to investigate the cytotoxic and thrombolytic activity of flower extract of this plant.

MATERIAL AND METHODS

Chemicals: Lyophilised streptokinase vial (1 500 000 IU) was purchased from Square Pharmaceuticals Ltd, Bangladesh. Methanol was purchased from Merck, Germany. Normal saline solution was purchased from Beximco Infusion Ltd. All chemicals used were of analytical reagent grade.

Plant Materials: Fresh flower of *B. oleracea* for this study were collected from the local market of Chittagong, Bangladesh and were authenticated by Dr. Sheikh

Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Preparation of Crude Extract: The collected flowers were dried for a period of 2 weeks under shade and ground. The ground flower(750 g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring. The sediments were filtered and the filtrates were dried at 40°C in a water bath. The solvent was completely removed by filtering with Whatman number-1 filter paper. The solvent was evaporated under reduced pressure at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use [36].

Phytochemical Screening: Phytochemical screening of the crude methanol extract of the four plants was carried out using standard phytochemical methods described by Muanda, 2010 [37].

Thrombolytic Test: This test was performed according to the method described by Prasad *et al.* [38]. In the commercially available lyophilised streptokinase vial (1 500 000 IU) 5 mL sterile distilled water was added and mixed properly. This suspension was used as a stock solution from which appropriate dilution was made. Five milliliter of venous blood was drawn from the healthy volunteers (n=10) without the history of oral contraceptive or anticoagulant therapy and was distributed (0.5 mL/tube) to each ten previously weighed sterile micro centrifuge tube and incubated at 37°C for 45 min to form the clot. After the clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight. A volume of 100 µL of methanol extract (10 mg/mL) was added to each micro centrifuge tube containing pre weighed clot. As a positive control, 100 µL of streptokinase and as a negative control 100 µL of distilled water were separately added to the control tube numbered. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis [39].

Brine Shrimp Lethality Assay: The assay was carried out according to the principle and protocol previously described by many authors [40-42], with slight

modifications. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. Dried cysts of *Artemia salina* were collected from an aquarium shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. After hatching, active naupli free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. The test sample (extract) were prepared by dissolving them in DMSO (not more than 50 μ L in 5 mL solution) plus sea water (3.8% NaCl in water) to attain concentrations of 10, 25, 50, 100, 200, 300, 500 and 800 μ g/mL. A vial containing 50 μ L DMSO diluted to 5 mL was used as a control. Vincristine sulphate [43] was used as positive control. After 24 hours the number of survival of nauplii was counted and percentage of mortality was determined using the equation:

$$\% \text{ mortality} = (\text{no. of dead nauplii} / \text{initial no. of live nauplii}) \times 100.$$

Statistical method of probit analysis (Finney's table) [44] was used to calculate LC_{50} . Criterion of toxicity for fractions was established according to Déciga-Campos *et al.* [45]. LC_{50} values $> 1000 \mu\text{g/mL}$ (non-toxic), $\geq 500 \leq 1000 \mu\text{g/mL}$ (weak toxicity) and $< 500 \mu\text{g/mL}$ (toxic).

Statistical Analysis: All the results obtained by *in vitro* experiment were expressed as mean \pm SEM of three measurements followed by Dunnet's test where $P < 0.01$ was considered as statistically significant.

RESULTS

The phytochemical screening of the crude extract of *B. oleracea* flower indicates qualitative presence of alkaloid, saponins, steroids, flavonoids, tannins and reducing sugar (Table 1). The lethality of the crude extract to brine shrimp was determined on *Artemia salina* after 24 h of exposure the samples, the negative control DMSO and sea water and vincristine sulphate used as standard. This technique was applied for the determination of general toxic property of the plant extract. The LC_{50} value (Figure 1) of the extract was $62.087 \pm 1.22 \mu\text{g/mL}$ and that for standard vincristine sulphate was $8.50 \pm 0.56 \mu\text{g/mL}$. No mortality was found in the control group, using DMSO and sea water. The plant extract showed moderate clot lysis activity ($42.75 \pm 3.72\%$) as compared to standard streptokinase's clot lysis ($67.32 \pm 5.25\%$) activity (Figure 2).

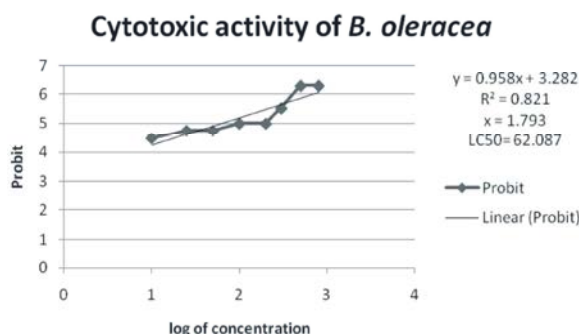


Fig. 1: Toxicity assay of *B. oleracea* on brine shrimp. The results are expressed as mean \pm SEM of three measurements

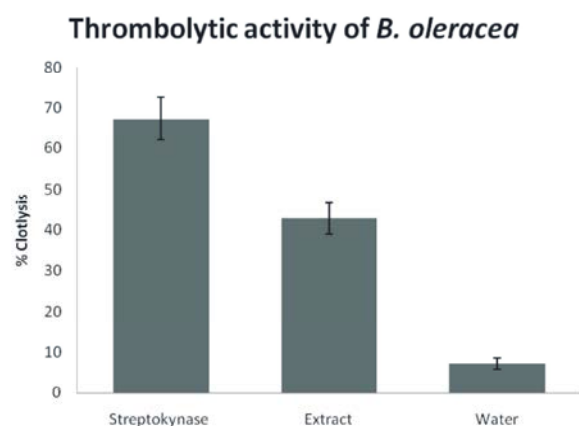


Fig. 2: The clot lysis activity of *B. oleracea* extract and streptokinase. All results are mean \pm SEM of three consecutive experiments

Table 1: Phytochemical constituents identified in the plant extracts of *Brassica oleracea*.

Phytochemical constituents	Results	Phytochemical constituents	Results
Alkaloids	+	Flavonoids	+
Terpenoids	-	Tannins	+
Glycosides	-	Resin	-
Saponins	+	Anthraquinones	-
Steroids	+	Reducing sugar	+

+: indicate presence; -: indicate absence.

DISCUSSION

Most thrombolytic agents work by activating the enzyme plasminogen, which clears the cross-linked fibrin mesh. This makes the clot soluble and subject to further proteolysis by other enzymes and restores blood flow over occluded blood vessels. Thus thrombolytic agents are useful for the treatment of myocardial infarction, thromboembolic strokes, deep vein thrombosis and PE to clear a blocked artery and avoid permanent damage to the perfused tissue (e.g. myocardium, brain and leg).

The cytotoxic and antitumor activity of plant derived product is either through induction of apoptosis or inhibition of neovascularization [46]. Ideally, any agent useful in the treatment of cancer should not be toxic to normal cell. However, in reality, anticancer agents are often toxic to normal cells, particularly towards rapidly growing cells [47]. It is necessary to test this extract in low concentration to evaluate its potency and also against various cancer cell lines as well as normal cell line so justify the potential to further investigate this plant for anticancer activity.

CONCLUSION

This study concludes that the plant might have potential to be developed useful economic and safe cytotoxic and thrombolytic alternative, but it demands more thorough study to find out the exact chemical responsible for those activity of plant so as to isolate and extract it separately so as to improve the potency.

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