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Antioxidant and thrombolytic activities of *Crinum asiaticum* L.

S. A. Rakhi[1], M. S. Islam[2], M. R. Kuddus[3], M. R. Sarker[4] and F. Ahmed[3]*

[1]*Department of Pharmacy, United International University, United City, Madani Avenue, Badda, Dhaka 1212, Bangladesh*
[2]*Pharmaceutical Sciences Research Division, BCSIR Dhaka Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh*
[3]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh*
[4]*Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh*

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Abstract

Crinum asiaticum L. is a toxic plant and can be grown as an ornamental plant in Bangladesh. The present study was designed to determine *in vitro* antioxidant and thrombolytic activity of n-hexane, dichloromethane dichloromethane, ethyl acetate and aqueous fractions of *C. asiaticum.* Antioxidant activity was determined by total phenolic content (TPC) analysis and DPPH radical scavenging assay method. The ethyl acetate fraction showed maximum antioxidant activity (IC_{50} = 35.44 μg/ml), which may be due to the presence of the highest amount of phenolic compounds (52.04 mg of GAE per gram of extractives) in this fraction. During the thrombolytic assay, dichloromethane fraction of *C. asiaticum* (Conc.= 10 mg/ml) showed the highest 32.59% clot lysis activity. The present study revealed that *C. asiaticum* possesses significant antioxidant and thrombolytic potential.

Keywords: *Crinum asiaticum*; Antioxidant; Total phenol content; DPPH; Thrombolytic

Introduction

Since ancient time, several medicinal plants have played a critical part in ensuring human welfare. These medicinal plants have a variety of therapeutic components that allow them to be used in medications or formulations to treat various human ailments. As per WHO estimation, the plant is used by around 80% of developing nation populations for basic health care and about 25% of synthetic medications are produced from different natural plant sources (Beyene, 2016; Rao *et al*. 2004). Many official drugs currently available on the markets are obtained from plants and these include cardiotonic drugs such as digitoxin, digoxin (Digitalis species), anticancer taxol (*Taxus brevifolia*), antimalarial for example Artemisinin (*Artemisia annua*), quinine (*Cinchona officinalis*), and anti-inflammatory drug aspirin from salicin (*Salix alba*) etc. (Veeresham, 2012).

Crinum asiaticum is a perennial bulbous herb in the Amaryllidaceae family that grows widely across the world. Because of its gorgeous blossoms, *C. asiaticum* has a high commercial value as an ornamental plant (Patel, 2017). The plant is well reported for anticancer, immune-stimulating, analgesic, antimicrobial and antimalarial properties. Crinums have been the focus of several phytochemical, pharmacological, and toxicological studies since the 1950s, owing to their abundance of pharmacologically active chemicals (Refaat *et al*. 2012 and 2013). The plant is rich in alkaloids but qualitative analysis of its essential oils revealed the presence of alcohols, phenols, flavonoids, terpenoids and terpenes. These compounds showed potent antioxidant, cytotoxic and thrombolytic activity (Patel, 2017). In our previous studies, alkaloids such as crinamine, lycorine and 6-hydroxycrinamine

*Corresponding author's e-mail: firoj72@du.ac.bd

isolated from this plant demonstrated strong cytotoxic activity against human pancreatic and prostate cancer cells (Arai *et al*. 2015). In our recent study (Rakhi *et al*. 2024), cycloneolitsol, hippeastrine and *β*-sitosterol were isolated by the chromatographic separation of *C. asiaticum* leaves. Here, both cycloneolitsol and hippeastrine showed mild to moderate cytotoxicity against various cancer cell lines whereas hippeastrine showed strong TRAIL-resistance abrogating activity against AGS cells (Rakhi *et al*. 2024). Due to its significant biological importance, in this study *C. asiaticum* was investigated for its antioxidant and thrombolytic potential by *in vitro* procedures.

Materials and methods

Sample collection and preparation

C. asiaticum leaves were harvested from the Botanical Garden, Mirpur and were authenticated (Accession number 56819) in Bangladesh National Herbarium, Dhaka. After collection, leaves were air-dried for two weeks and then turned into fine powder using a grinding device.

Extraction and fractionation of plant material

About 400 g of the powdered materials of *C. asiaticum* was soaked into methanol and kept for several days with occasional shaking and stirring. After cold extraction, the mixtures were filtered through a fresh cotton plug in a large funnel and then through Whatman No.1 filter paper. The obtained filtrate was then concentrated using a Buchi rotary evaporator (Heidolph, UK) and kept for complete drying. This process was done multiple times over a period to get enough amount of extract. Finally, the dried crude extract (45 g) was fractionated following the modified Kupchan partitioning protocol (VanWagenen *et al*. 1993) to obtain *n*-hexane, dichloromethane ethyl acetate and aqueous soluble fraction.

Total phenolic content (TPC) analysis

The total phenolic content of different fractions of *C. asiaticum* was calculated using Folin-Ciocalteu reagent as an oxidizing agent and gallic acid as a reference (Skerget *et al*. 2005). About 2.0 ml Na_2CO_3 (7.5% w/v) solution and 2.5 ml Folin reagent were added into 0.5 ml extract solution (conc. 2 mg/ml) and incubated for 20 min. After incubation, the absorbance was measured with a UV-spectrophotometer (at 760 nm) and the total phenolic content of the sample was calculated using a quality curve made from gallic acid solutions of various concentrations.

Antioxidant activity

The antioxidant activity by DPPH scavenging assay (Islam *et al*. 2019) was expressed as the percentage of inhibition (I%) that was measured against blank:

$$(\mathrm{I}\%) = \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \times 100\%$$

Where, A = Absorbance for each group.

Thrombolytic activity

The thrombolytic activity of all extractives was assessed using streptokinase (SK) as a standard drug (Prasad *et al*. 2006). In separate vials, 10 mg of different partitions of methanolic extracts of *C. asiaticum* were mixed with 1.0 ml of distilled water. Healthy volunteers provided aliquots (5 ml) of venous blood, which were divided into five pre-weighed sterile vials (1.0 ml/tube) and incubated at 37°C for 45 min. 100 µl of aqueous solutions of different fractions were added separately to each vial containing pre-weighed clot. After that, all of the vials including positive (streptokinase, 100 µl) and negative (distilled water, 100 µl) were incubated at 37°C. The released fluid after incubation was withdrawn carefully and the vials were weighed again. The percentage difference was calculated using weight obtained before and after clot lysis as shown below:

$$(\%\ \text{clot lysis}) = \frac{\text{Weight of the lysis clot}}{\text{Weight of clot before lysis}} \times 100\%$$

Results and discussion

Plant-derived secondary metabolites such as phenolics serve as antioxidants through various mechanisms. The hydroxyl group of these compounds can scavenge free radicals which are well reported to exacerbate oxidative stress in biological systems (Karim *et al*. 2020). The amount of total phenolic content ranged from 26.95 to 52.04 mg of gallic acid equivalent (GAE) per gram of extractives (Table I). Here, the maximum phenolic content was observed in the ethyl acetate fraction (52.04 mg of GAE per gram of extractives) and the lowest phenolic content was found in the aqueous fraction (26.95 mg of GAE per gram of extractives). The total phenolic content in the plant extractives signifies mild to moderate antioxidant properties of *C. asiaticum*.

Table I. Total phenolic content and DPPH scavenging activity of different fractions of C. *asiaticum*

Sample	TPC (mg of GAE/g of dry extract)	DPPH scavenging activity (IC_{50} µg/ml)
HXF	30.51	45.78
DCMF	39.52	71.53
EAF	52.04	35.44
AQF	26.95	49.33
AA	--	4.31

Here HXF= Hexane fraction, DCMF= Dichloromethane fraction, EAF= Ethyl acetate fraction, AQF= Aqueous fraction of *Crinum asiaticum*, AA= Ascorbic acid.

In DPPH assay, among all, the ethyl acetate soluble fraction possessed the strong DPPH radical quenching activity with IC_{50} of 35.44 µg/ml (Table I) as compared with the standard ascorbic acid (IC_{50} of 4.31µg/ml). Antioxidant potential was in the following order: ethyl acetate fraction (IC_{50} = 35.44 μg/ml) > hexane fraction (IC_{50} = 45.78 μg/ml) > aqueous fraction (IC_{50} = 49.33 μg/ml). The free radical neutralizing property of the ethyl acetate fraction of *C. asiaticum* might be due to the presence of more phenolic compounds in this fraction (Table I). This finding proved a positive connection between the amount of phenolics and antioxidant activity via DPPH radical scavenging capacity (Anjum *et al*. 2021). Phytochemicals such as phenolics and flavonoids have antioxidant activities as they are reported to scavenge reactive oxygen species and free radicals in biological systems.

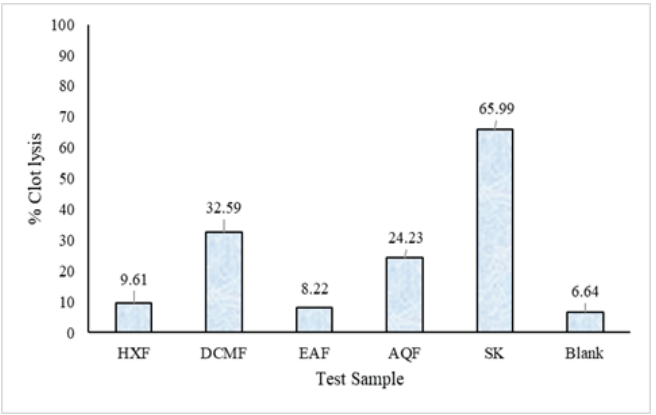

Fig. 1. Percentage of clot lysis activity of different fractions of *C. asiaticum*

Blood clotting agents are important in the treatment of cardiovascular diseases. Thrombolytic agents of plant origin are safer than synthetic drugs that have noteworthy limitations such as limited fibrin specificity, and bleeding tendency (Hussain *et al*. 2014). Therefore, the exploration of cardiovascular agents from natural sources has become a promising field of medical science. In the search for new cardiovascular drugs from plant sources (Rahman *et al*. 2013) the extracts obtained from *C. asiaticum* were measured for thrombolytic activity and the results are presented in Figure 1. Addition of 100 µl streptokinase (30,000 I.U.), to the clots, exhibited 68.22% lysis of the clot. In this study, dichloromethane fraction of *C. asiaticum* at conc. of 10 mg/ml demonstrated highest 32.59% thrombolytic activity while the ethyl acetate fraction demonstrated the lowest thrombolytic activity of 8.22 %. The current experiment showed that the test samples of *C. asiaticum* have moderate thrombolytic potential because it can reduce the weight of blood clots in our test conditions. These antithrombotic properties could be ascribed to the phytochemicals present in *C. asiaticum*.

Conclusion

The different fractions of *Crinum asiaticum* were investigated for antioxidant and thrombolytic activities. Based on the study, it may be concluded that the plant *C. asiaticum* can be considered as a vital source of bioactive compounds with antioxidant and thrombolytic potential. Supplementary comprehensive investigations are suggested to find out the lead from this plant accountable for the exerted pharmacological actions.

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References

Anjum A, Kuddus MR, Sultan MZ, Al-Mansur A, Hasan CM and Rashid MA (2021), Phytochemical and Biological

Investigation of *Bridelia tomentosa* Blume Growing in Bangladesh, *Dhaka Univ J Pharm Sci*. **20**(2): 213-218. DOI: 10.3329/dujps.v20i2.57171

Arai MA, Akamine R, Sadhu SK, Ahmed F and Ishibashi M (2015), Hedgehog/GLI-mediated transcriptional activity inhibitors from *Crinum asiaticum*, *J Nat Med*. **69**(4): 538-542. DOI: 10.1007/s11418-015-0922-8

Beyene B (2016), Review on application and management of medicinal plants for the livelihood of the local community, *J Res Dev Manag.* **22**: 33-39.

Hussain F, Islam A, Bulbul L, Moghal MR and Hossain MS (2014), In vitro thrombolytic potential of root extracts of four medicinal plants available in Bangladesh, *Anc Sci Life*. **33**(3): 162-164. DOI: 10.4103/0257-7941.144620

Islam M, Jannat T, Kuddus MR, Rashid MA and Haque MR (2019), In vitro and in vivo evaluation of pharmacological potentials of *Campsis radicans* L, *Clin Phytosci*. **5**: 1-9. DOI: 10.1186/s40816-019-0144-9

Karim MA, Islam MA, Islam MM, Rahman MS, Sultana S, Biswas S, Hosen MJ, Mazumder K, Rahman MM and Hasan MN (2020), Evaluation of antioxidant, anti-hemolytic, cytotoxic effects and anti-bacterial activity of selected mangrove plants (*Bruguiera gymnorrhiza* and *Heritiera littoralis*) in Bangladesh, *Clin Phytosci*. **6**: 8. DOI: 10.1186/s40816-020-0152-9

Patel D (2017), *Crinum asiaticum* Linn: A Medicinal Herb as Well as Ornamental Plant in Central India, *Int J Environ Sci Nat Resour*. **6**(1): 1-7. DOI: 10.19080/IJESNR.2017.06.555678

Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM and Daginawala HF (2006), Development of an *In vitro* model to study clot lysis activity of thrombolytic drugs, *Thromb J*. **4**: 14. DOI: 10.1186/1477-9560-4-14

Rahman MA, Sultana R, Bin Emran T, Islam MS, Rahman MA, Chakma JS, Rashid HU and Hasan CM (2013), Effects of organic extracts of six Bangladeshi plants on *in vitro* thrombolysis and cytotoxicity, *BMC Complement Altern Med*. **13**: 25. DOI: 10.1186/1472-6882-13-25

Rakhi SA, Hara Y, Islam MS, Manome T, Alam S, Emon NU, Al-Mansur MA, Kuddus MR, Sarkar MR, Ishibashi M and Ahmed F (2024), Isolation of bioactive phytochemicals from *Crinum asiaticum* L. along with their cytotoxic and TRAIL-resistance abrogating prospect assessment, *Heliyon*. **10**(3): e25049. DOI: 10.1016/j.heliyon.2024.e25049

Rao MR, Palada MC and Becker BN (2004), Medicinal and aromatic plants in agroforestry systems, *Agrofor Syst.* **61**: 107-122. DOI: 10.1007/978-94-017-2424-1_8

Refaat J, Kamel MS, Ramadan MA and Ali AA (2012), Crinum; An endless source of bioactive principles: a review. Part I- Crinum alkaloids: Lycorine type alkaloids, *Int J Pharm Sci Res*. **3**(7): 1883-1890

Refaat J, Kamel MS, Ramadan MA and Ali AA (2013), Crinum; An endless source of bioactive principles: A review. Part IV: Nonalkaloidal constituents, *Int J Pharm Sci Res*. **4**(3): 941-948.

Skerget M, Kotnik P, Hadolin M, Hras A, Simonic M and Knez Z (2005), Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities, *Food Chem*. **89**: 191-198. DOI: 10.1016/j.foodchem.2004.02.025

VanWagenen BC, Larsen R, Cardellina JH, Randazzo D, Lidert ZC and Swithenbank C (1993), Ulosantoin, a potent insecticide from the sponge, *Ulosa ruetzleri*, *J Org Chem*. **58**: 335-337. DOI: 10.1021/jo00054a013

Veeresham C (2012), Natural products derived from plants as a source of drugs, *J Adv Pharm Technol Res*. **3**(4): 200-201. DOI: 10.4103/2231-4040.104709