

## Cytotoxic, Anthelmintic and Thrombolytic Activities of the Methanol Extract of *Holdina Cordifolia* Bark

Md. Imran Hasan, Md. Jakaria, Mohammad Parvez,  
Rashaduz Zaman, Arifujjaman and Md. Rabiul Islam

Department of Pharmacy, International Islamic University Chittagong (IIUC),  
Chittagong 4203, Bangladesh

**Abstract:** The present study was designed to investigate *in vitro* cytotoxic, anthelmintic and thrombolytic activities of crude methanol extract of *Holdina cordifolia* bark. Evaluation of cytotoxic activity was done using the brine shrimp lethality bioassay, anthelmintic activity was done by counting of paralyzed time and death time on the aquarium worm *Tubifex tubifex*. The clot lysis activity was done to evaluate thrombolytic activity. The crude methanol extract of *Holdina cordifolia* bark showed significant cytotoxic potential (LC 50 value = 236.68 $\mu$ g/ml) comparing with that of standard vincristine sulphate (0.825 $\mu$ g/ml). It also produced a significant anthelmintic activity in dose dependent manner compared with standard drug levamisole. At the higher dose of crude extract 20mg, the time taken for paralysed was 14 minutes and 17 seconds but 18 minutes and 06 seconds were taking for death. In the case of standard drug levamisole, at higher dose 1mg where paralysed time was 3 minutes and 30 seconds but death time was 6 minutes and 50 seconds. It has significant thrombolytic activity (51.57%) compared to standard streptokinase (80.51%). We concluded that *H. cordifolia* bark has got the potential as a candidate for future antitumour, anthelmintic and thrombolytic agent.

**Key words:** *In vitro* • Cytotoxic • Anthelmintic • Thrombolytic • *Holdina Cordifolia* Bark

### INTRODUCTION

Medicinal plants are an important therapeutic aid for various ailments. Most of the people in rural and urban areas of the world depended on the medicinal plants for the treatment of infectious diseases [1]. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicines for some aspect of primary health care. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis and quinine [2].

Cancer is a class of diseases in which a cell or a group of cells display uncontrolled growth, invasion and sometimes metastasis. It is largest non-communicable disease and it has a sizable contribution in the total number of deaths. The world cancer report documents that cancer rates are set to increase at an alarming rate globally. Cancer rates could increase by 50% new cases for the year 2020 [3]. Natural products extracts,

fractions or pure compounds can be tested for their bioactivity by this method [4].

The major phyla of helminthes are nematodes (Round worms) which are soil transmitted helminthes that mostly cause the intestinal infection, filarial worms cause the onchocerciasis and lymphatic filariasis, but plathelminths (Flatworms) also known as trematodes like schistosomes and cestodes causes cyticercosis. Health Organization estimated 2 billion people infected with helminthes and it was also estimated that 100% of all age groups of school children are at risk of morbidity [5]. Recent estimation suggest that over half of the world population is infected with intestinal helminthes, such as *Ascaris*, hookworms, *Trichuris*, *Enterobius*, *Strongyloides* and tapeworms and that most of these infected people live in remote rural areas in the developing countries [6, 7]. Morbidity from nematodes is common with diabetes and lung cancer. The helminthes is a type of parasite mainly subsists in human body in intestinal tract [8]. It is important to look for alternative strategies (Other than synthetic agents)

against gastrointestinal nematodes, which have led to the proposal of screening medicinal plants for their anthelmintic activity.

Atherothrombosis may be defined as the hardening and narrowing (Medically known as ‘Stenosis’) of the body’s arteries. It is caused by a slow and progressive build-up of plaque under the lining of the arterial wall which may gradually narrow the artery and restrict blood flow to the target organ [9].

It was realized from the various reports that *Haldina cordifolia* (Roxb.), Syn. *Adina cordifolia* (Roxb.) belongs to the family Rubiaceae, have displayed plethoras of potential biological activities [2, 10, 11]. It is found throughout central and south India to Srilanka. Different studies on this plant revealed that it has several biological activities isolated from various parts such as anticancer [12], antiulcer [13], hepatoprotective [14], anti-inflammatory [15], anti-fertility [16], anti-diabetic [17], anti-amoebic [18], anti-nociceptive [19] etc. Traditionally, this plant has also used in curing various ailments such as rheumatism, stomachache, headache, cold/cough, toothache, fever, pain and swelling, bacterial infection, urinary problems, conjunctivitis, miscarriage etc. [2].

In this study, we have attempted to evaluate the cytotoxic, anthelmintic and thrombolytic activities of the methanol extract of *H. cordifolia* bark by using *in vitro* methods.

## MATERIALS AND METHODS

**Plant Materials:** The bark of *H. cordifolia* were collected from Vatiari area of Chittagong district and then authenticated by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor, Department of Botany, University of Chittagong, Chittagong 4331, Bangladesh.

**Extraction of Plant Materials:** The fresh barks of *H. cordifolia* were cut, washed and air dried at room temperature (24±2°C) for about 10 days. Dried leaves were macerated into coarse powder. Dried powder (250 gm) was then emerged using methanol. Then methanol extract was shaken by rotary shaking apparatus for 7 days. The extract was collected using Buckner funnel. The methanol was evaporated at a temperature below 45°C and concentrated extract was weighed 25gm, stored at 4°C temperature.

**In vitro Cytotoxic Activity Evaluation:** Brine shrimp lethality bioassay: Brine shrimp lethality bioassay was used to evaluate cytotoxic activity. The dried cyst of the

brine shrimp (*Artemia salina*) were collected from an aquarium shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) with aeration for 48 hours day/dark cycles to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii according to protocol of Mayer *et al.* [20]. The test samples (Extract) were prepared by dissolving them in DMSO (Not more than 50µL in 5mL solution) plus sea water (3.8% NaCl in water) to attain concentrations of 50, 100, 150, 200, 300, 500, 800 and 1000µg/ml. A vial containing 50µL DMSO diluted to 5mL was used as a control. Standard vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental and control vials. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial were counted. From this data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ Mortality} = (N_0 - N_t) \times 100$$

where,

$N_t$  = Number of alive nauplii after 24 hrs of incubation,

$N_0$  = Number of total nauplii transferred i.e 10.

The  $LC_{50}$  (Median lethal concentration) was then calculated by using Microsoft Excel 2003.

**In vitro Anthelmintic Activity Evaluation:** The anthelmintic activity of methanol extract of bark of *H. cordifolia* was carried out as per the protocol of Kamal *et al.* [5]. The aquarium worm *Tubifex tubifex* were used in the present study. The worm was collected from the local market of Chittagong and average size of worms 2-2.5cm. were selected for study. The standard drug levamisole and three different concentrations of methanol extract (5, 10 and 20mg/ml) in double distilled water were prepared freshly and used for the study of anthelmintic activity [21, 22]. In the case of negative control group distil water was added in the place of extract and standard drug. The anthelmintic activity was determined at two different stages ‘Time of paralysis’ and ‘Time of death’ of the worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors [23]. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased [24].

**In vitro Thrombolytic Activity:** The thrombolytic activity of this extract was evaluated according to protocol of Sweta *et al.* [25] where streptokinase used as a positive standard. The dry crude extract (10mg) was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered. Aliquots (5ml) of venous blood were drawn from healthy volunteers which were distributed in five different pre weighed sterile micro centrifuge tube (1ml/tube) and incubated at 37° C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube–weight of tube alone). To each microcentrifuge tube containing preweighed clot, 100µl aqueous solutions of different partitionates along with the crude extract was added separately. As a positive control, 100µl of streptokinase (SK) and as a negative non thrombolytic control, 100µl of distilled water were separately added to the control tubes. All the tubes were then incubated at 37° C for 90 minutes and observed for clot lysis. After incubation, the released of fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. The differences in weights taken before and after clot lysis were expressed as percentage of clot lysis as shown below:

$$\% \text{ of clot lysis} = (\text{wt of released clot /clot wt}) \times 100$$

**Statistical Analysis:** Statistical analysis was performed using Microsoft Office Excel 2003 and online calculator [www.endmemo.com](http://www.endmemo.com) (<http://www.endmemo.com/math/sd.php>).

## RESULTS

**Cytotoxic Activity:** The crude methanol extract of *H. Cordifolia* bark produced cytotoxic effect. The LC<sub>50</sub> value was 236.68µg/ml (Table 1 and Figure 1). This LC<sub>50</sub> value of this extract compared with standard vincristine sulphate LC<sub>50</sub> = 0.831µg/ml.

**Anthelmintic Activity:** The crude methanol extracts of *H. cordifolia* bark produced a significant anthelmintic activity in dose dependent manner at 5mg, 10mg and 20mg and the activity of crude extract was comparable with that of standard drugs levamisole doses at 0.5mg, 0.8mg and 1mg. At the highest dose of crude extract 20mg, the time taken for paralysed 14 minutes 17 seconds and 18 minutes 06 seconds taking for death. In terms of, the standard drug levamisole, at highest dose 1mg where paralysed time was 3 minutes and 30 seconds but death time was 6 minutes and 50 seconds (Table 2).

Table 1: Cytotoxic activity of *H. Cordifolia* bark

Conc. (µg/ml)	Log C	Total Nauplii	No. of Nauplii Dead	No. of Naupli Live	% of Mortality	LC <sub>50</sub>
50	1.69	10	0	10	0%	
75	1.87	10	1	9	10%	
100	2	10	3	7	30%	
150	2.17	10	4	6	40%	236.68 µg/ml
250	2.39	10	5	5	50%	
500	2.69	10	7	3	70%	
800	2.9	10	8	2	80%	
1000	3	10	10	0	100%	

Table 2: Anthelmintic activity of methanol extract of *H. Cordifolia* Bark

Test Sample	Conc. (mg/ml)	Time taken for paralysis (min:sec)	Time taken for death (min:sec)
Extract	20	14.170 ± 0.553	18.067 ± 1.810
	10	28.610 ± 0.196	37.797 ± 1.472
	5	58.274 ± 1.119	58.237 ± 1.201
	1	3.30 ± 0.645	6.50 ± 0.314
Standard (Levamisole)	0.8	6.260 ± 0.261	12.210 ± 0.512
	0.5	14.410 ± 0.643	51.320 ± 0.825
Control (water)	--	--	--

All results are mean ± SEM of three consecutive experiments

Table 3: Thrombolytic Activity of methanol extract of *H. Cordifolia* Bark

Volunteer	Empty wt. of tube (A) gm	Wt. of clot with tube (B) gm	Wt. of Clot (C)=B-A	Clot weight after lysis (D) gm	Wt. of lysis, (E)=B-D	% of clot lysis	Average % of clot lysis
1	0.874	1.379	0.505	1.047	0.332	65.742	51.57%
2	0.809	1.246	0.437	0.995	0.251	57.437	
3	0.815	1.277	0.462	1.011	0.266	57.575	
4	0.836	1.303	0.467	1.056	0.247	52.890	
5	0.836	1.385	0.549	1.051	0.334	60.837	
6	0.826	1.294	0.468	1.063	0.231	49.358	
7	0.842	1.265	0.423	1.064	0.201	47.517	
8	0.821	1.28	0.459	1.068	0.212	46.187	
9	0.848	1.312	0.464	1.044	0.268	57.758	
10	0.84	1.32	0.48	1.222	0.098	20.416	

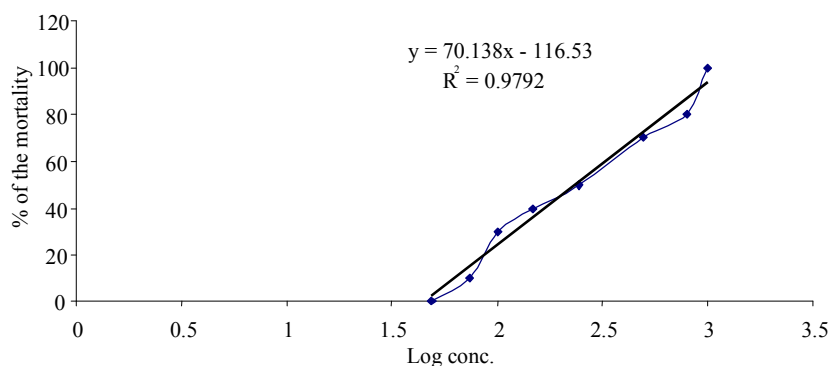


Fig. 1: Cytotoxic activity of *H. Cordifolia* Bark

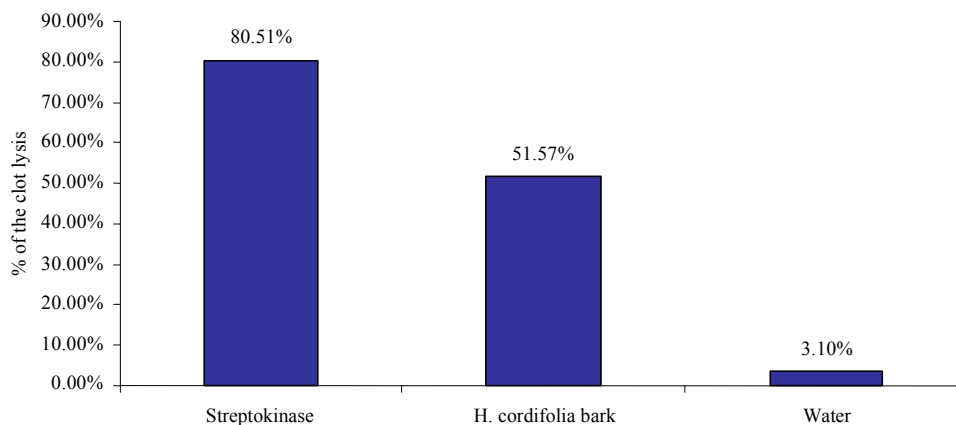


Fig. 2: Clot lysis activity of *H. Cordifolia* Bark with standard (Streptokinase) and negative control (Water).

**Thrombolytic Activity:** The *in vitro* thrombolytic activity study revealed that *H. cordifolia* showed 51.57% of the clot lysis (Table 3 and Figure 2). Addition of 100µl SK (Streptokinase), a positive control to the clots along with 90 min of incubation at 37°C, showed 80.51% clot lysis. Clots when treated with 100µl water (Negative control) showed only negligible clot lysis (3.098%).

## DISCUSSION

According to results, the crude extract has good cytotoxic potency. Some studies tend to suggest that flavonoids and tannin which may contain significant cytotoxic and antitumor potency [26]. It is confirmed that this extract contains a higher concentration of these bioactive compounds were responsible for cytotoxic

activity. Cytotoxic and anthelmintic activities of a plant are inter-related because the confirmation of cytotoxicity also confirmed that plant is capable to produce toxic effect on parasites. There are several phyto-constituents such as alkaloids, tannins, phenols etc. may be responsible for anthelmintic activity. It was also reported that, tannins may interfere with energy generation of worms by uncoupling oxidative phosphorylation and lead to death and alkaloids were also reported to cause paralysis of the worms by acting on its central nervous system. In addition to the major effect of anthelmintic drug is to cause a flaccid paralysis of the worm [26, 27]. Therefore, in our study, it is clear that this plant showed similar effects like anthelmintic drugs against *Tubifex tubifex*.

Thrombolytic activity also known as thrombolysis, as the breakdown (*Lysis*) of blood clots by pharmacological means. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator (tPA), the protein that normally activates plasmin. A number of plant sources especially several fruits and vegetables have been studied for their supplements having anti-coagulant, anti-platelet and fibrinolytic activity and there is evidence that consuming such food leads to prevention of coronary events and stroke [28-30]. In our present *in vitro* preliminary thrombolytic test confirmed that, this extract of *H. cordifolia* bark showed the thrombolytic activity. The maximum clot lysis activity was mostly observed in methanol extract that means methanol soluble compounds are mainly responsible for the thrombolytic activity.

### CONCLUSION

Numbers of pharmaceuticals approved by the Food and Drug Administration (FDA) currently have origins to plant sources. The result of evaluation revealed in near future this plant will be a good source of pharmaceuticals as an agent for the improvement of patients suffering from different pathological ailments such as cancer, helminth infection and atherothrombotic diseases. Further studies are needed to isolate, characterize the compounds responsible for these pharmacological activities. This study may be helpful for further related research works on this plant.

### ACKNOWLEDGEMENT

The authors acknowledge to the respective authority of the Department of Pharmacy, International Islamic

University Chittagong for laboratory facilities during the period of laboratory work. Authors also acknowledge to the faculty members of Department of Pharmacy, International Islamic University Chittagong (IIUC).

### REFERENCES

1. Fakruddin, M., K.S.B. Mannan, R.M. Mazumdar and H. Afroz, 2012. Antibacterial, antifungal and antioxidant activities of the ethanol extract of the stem bark of *Clausena heptaphylla*. BMC Complementary and Alternative Medicine, 12: 232.
2. Dash, P.P., A.K. Chaturvedi, A. Mishra and D. Chaturvedi, 2014. Search for an herbal medicine: Antibacterial activity of methanolic extract of *Haldina cordifolia*. International Journal of Natural Products Research, 4(4): 95-99.
3. Marimuthu, P., 2008. Projection of cancer incidence in five cities and cancer mortality in India. Ind. J. Cancer, 45(1): 1-7.
4. Hasanat, A., M. Jakaria, M.I. Tarek, T.A. Chowdhury, Arifujjaman and M.S. Ali, 2015. Bioassay of brine shrimp lethality and thrombolytic activity of methanolic extract of *Macaranga denticulata* leaves. Journal of Pharmacognosy and Phytochemistry, 3(6): 43-46.
5. Kamal, A.T.M.M., K.A.A. Chowdhury, M.M.H. Chy, L.K. Shill, S. Chowdhury, M.A.H. Chy and M.Z. Habib, 2015. Evaluation of anthelmintic activity of seeds of *Sesamum indicum* L. and fruits of *Capsicum frutescens* L. Journal of Pharmacognosy and Phytochemistry, 3(6): 256-259.
6. De Silva, N.R., S. Brooker, P.J. Hotez, A. Montresor, D. Engels and L. Savioli, 2003. Soil transmitted helminth infections: updating the global picture. Trends Parasitol, 19: 547-551.
7. Horton, J., 2003. Human gastrointestinal helminth infections: Are they now neglected diseases?. Trends Parasitol, 19: 527-531.
8. Krogstad, D.J., 1993. Charting a course for the future: a society at the interface. American Journal of Tropical Medicine and Hygiene, 48(3): 303-312.
9. Zaman, R., M. Parvez, M. Jakaria, M.A. Sayeed and M. Islam, 2015. In vitro Clot Lysis Activity of Different Extracts of *Mangifera sylvatica* Roxb. Leaves. Research Journal of Medicinal Plant, 9(3): 135-140.
10. Iqbal, P.F., A.R. Bhat and A. Azam, 2009. Antiamoebic coumarins from the root bark of *Adina cordifolia* and their new thiosemicarbazone derivatives. Eur. J. Med. Chem., 44: 2252-2259.

11. Sharma, A., B. Sangameswaran, V. Jain and M.S. Saluja, 2012. Hepatoprotective activity of *Adina cordifolia* against ethanol induce hepatotoxicity in rats. International Current Pharmaceutical Journal, 1(9): 279-284.
12. Sangameswaran, B. and M.S. Saluja, 2012. Anticancer activity of *Adina cordifolia* against ehrlich ascites carcinoma (EAC) in mice. Cont. J. Pharmacol. Toxicol. Res., 5: 7-16.
13. Kasinadhuni, V.V.R., G. Rajashekhar, R. Rajagopalan, V.M. Sharma, C.V. Krishna, P. Sairam, G.S. Prasad, S. Sadhukhan and G.G. Rao, 1999. Anti-ulcer potential of *Haldinia cordifolia*. Fitoterapia, 70: 93-95.
14. Agarwal, M., V.K. Srivastava, K.K. Saxena and A. Kumar, 2006. Hepatoprotective activity of beta-Vulgaris against carbon tetrachloride induced hepatic injury in rats. Fitoterapia, 77: 91-93.
15. Kausik, V., L. Khosa and V. Parcha, 2009. Evaluation of anti-inflammatory potential of *Haldinia cordifolia* bark extracts. Int. J. Pharmbit., 2: 126-130.
16. Sabir, M. and M.K. Razdan, 1970. Antifertility study with leaf extract of *Adina cordifolia* (Karam ki Gaach). Ind. J. Physiol. Pharmacol., 14: 209-210.
17. Chaudhary, P., B. Goel and A.K. Ghosh, 2012. Antidiabetic activity of *Adina cordifolia* (Roxb) leaves in alloxan induced diabetic rats. Asian Pacific J. Trop. Biomed., pp: S1630-S1632.
18. Iqbal, P.F., A.R. Bhat and A. Azam, 2009. Antiamoebic coumarins from the root bark of *Adina cordifolia* and their new thiosemicarbazone derivatives. Eur. J. Med. Chem., 44: 2252-2259.
19. Jain, A.P., R.S. Pawar and A. Singhai, 2006. Anti-inflammatory and anti-nociptive activity of *Adina cordifolia* bark. Nig. J. Nat. Prod. Med., 10: 90-93.
20. Mayer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. Mclaughlin, 1982. Brine shrimp: a convenient bioassay for active plant constituents. Planta Med., 45: 31-34.
21. Satish, B.K. and A. Ravindra, 2009. Fursule investigation of in vitro anthelmintic activity of *Thespesia lampas* (cav). Asian Journal of Pharmaceutical and Clinical Research, 2: 69-70.
22. Iqbal, Z., Q.K. Nadeem, M.N. Khan, M.S. Akhtar and F.N. Waraich, 2001. In vitro Anthelmintic Activity of *Allium sativum*, *Zingiber officinale*, *Cucurbita mexicana* and *Ficus religiosa*. International Journal of Agriculture and Biology, 3(4): 454-457.
23. Grime, A.S., R.D. Bhalke, P.B. Ghogare, V.D. Tambe, R.S. Jadhav and S.A. Nirmal, 2005. Comparative *in vitro* anthelmintic activity of *Mentha piperita* and *Lantana camara* from Western India. Dhaka Univ. J. Pharm. Sci., 5(1-2): 5-7.
24. Temjenmongla and A.K. Yadav, 2005. Anticestodal efficacy of folklore medicinal plants of Naga tribes in Northeast India. Afr J Trad CAM, 2(2): 129-133.
25. Sweta, P., S.K. Rajpal, Y.D. Jayant, J.P. Hemant, M.T. Girdhar and F.D. Hatim, 2007. Effect of *Fagonia Arabica* (Dhamasa) on *in vitro* thrombolysis. BMC Complementary and Alternative Medicine, 7: 36.
26. Majumder, M.S., M.N. Amin, M.M.R. Moghal, S. Banik, A. Kar and M.M. Hossain, 2014. Anthelmintic and Cytotoxic Activities of Two Medicinal Plants: *Polygonum viscosum* and *Aphanamixis polystachya*. Growing in Bangladesh J. Sci. Res., 6(2): 339-345.
27. Aziz, A., G.S. Raju, A. Das, J. Ahmed and M.M.R. Moghal, 2014. Evaluation of In vitro Anthelmintic Activity, Total Phenolic Content and Cytotoxic Activity of *Crinum latifolium* L. (Family: Amaryllidaceae). Adv. Pharm. Bull., 4(1): 15-19.
28. Emran, T.B., M.A. Rahman, M.M.N. Uddin, M.M.R. Rahman, M.Z. Uddin, R. Dash and C. Layzu, 2015. Effects of organic extracts and their different fractions of five Bangladeshi plants on *in vitro* thrombolysis. BMC Complementary and Alternative Medicine, 15: 128.
29. Ali, M.R., M. Hossain, J.F. Runa, M. Hasanuzzaman and M.M. Islam, 2014. Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds. Avicenna Journal of Phytomedicine, 4(6): 430-436.
30. Zaman, R., M. Parvez, M. Jakaria and M.A. Sayeed, 2015. *In-vitro* Cytotoxic and Thrombolytic Potential of Methanolic Extract of *Podocarpus neriifolius* D. Don leaves, International Journal of Pharma Sciences and Research, 6(2): 273-277.