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**Tanvir Ahmad Chowdhury**
Department of Pharmacy, International Islamic University Chittagong, (IIUC), Chawkbazar, Chittagong-4203, Bangladesh.

**A.T.M Mostofa Kamal**
Department of Pharmacy, International Islamic University Chittagong, (IIUC), Chawkbazar, Chittagong-4203, Bangladesh.

**Kazi Ashfak Ahmed chy**
Department of Pharmacy, International Islamic University Chittagong, (IIUC), Chawkbazar, Chittagong-4203, Bangladesh.

**Afrina Jahan**
Department of Pharmacy, BGC Trust University Bangladesh, Chittagong 4000, Bangladesh.

**Md. Shakhawat Hossain**
Department of Pharmacy, International Islamic University Chittagong, (IIUC), Chawkbazar, Chittagong-4203, Bangladesh.

**Arafatul Mamur**
Department of Pharmacy, International Islamic University Chittagong, (IIUC), Chawkbazar, Chittagong-4203, Bangladesh.

**Mahmudul Hasan**
Department of Pharmacy, International Islamic University Chittagong, (IIUC), Chawkbazar, Chittagong-4203, Bangladesh.

**Javed Hossain**
Department of Pharmacy, International Islamic University Chittagong, (IIUC), Chawkbazar, Chittagong-4203, Bangladesh.

**Correspondence:**
**Tanvir Ahmad Chowdhury**
Department of Pharmacy
International Islamic University Chittagong, (IIUC) Chawkbazar, Chittagong-4203, Bangladesh.

# Cytotoxic & thrombolytic activity of methanolic extract of Macaranga denticulata Bark

**Tanvir Ahmad Chowdhury, A.T.M.Mostafa Kamal, Kazi Ashfak Ahmed Chowdhury, Afrina Jahan, Md. Shakhawat Hossain, Arafatul Mamur, Mahmudul Hasan, Javed Hossain**

**Abstract**
Various forms of cancer are rising all over the world, requiring newer therapy. The quest of anticancer drugs both from natural and synthetic sources is the demand of time. The present study was to investigate the cytotoxicity & thrombolytic activity of methanolic extracts Macaranga denticulata bark. An in vitro thrombolytic model was used to evaluate the clot lysis effect of different extracts of M. denticulata along with Streptokinase as a positive control and distilled water as a negative control. Plant was evaluated for cytotoxicity by brine shrimp lethality bioassay comparing with standard cytotoxic drug vincristine sulphate. The cytotoxic activity of methanolic extracts of M. denticulata bark was evaluated by Brine shrimp lethality bioassay. The brine shrimp lethality bioassay result was (LC50=57.25 µg/ml) compared with standard vincristine sulphate (LC50=0.512 µg/ml). It has significant thrombolytic activity (31.59%) compared to standard streptokinase (70%).

**Keywords:** Brine shrimp, Thrombolytic, Cytotoxic, Streptokinase, Vincristine sulphate

## 1. Introduction
**Thrombolysis** is the breakdown (*lysis*) of blood clots by pharmacological means, and commonly called *clot busting*. It works by stimulating secondary fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator (tPA), the protein that normally activates plasmin. Thrombolysis mainly involves the use of thrombolytic drugs, which dissolve blood clots. These drugs are either derived from *Streptococcus* species, or, more recently, using recombinant biotechnology whereby tPA is manufactured by bacteria, resulting in a recombinant tissue plasminogen activator or rtPA.
Some commonly used thrombolytics are: Streptokinase, Urokinase. Formation of blood clots lies at the basis of a number of serious diseases (see below). By breaking down the clot, the disease process can be arrested, or the complications reduced. While other anticoagulants (such as heparin) prevent the "growth" of a clot, thrombolytic agents actively reduce the size of the clot [1].
Most thrombolytic agents work by activating the enzyme plasminogen, which clears the cross-linked fibrin mesh (the backbone of a clot). This makes the clot soluble and subject to further proteolysis by other enzymes, and restores blood flow over occluded blood vessels.
Brine shrimp lethality bioassay is a simple, high throughput cytotoxicity test of bioactive chemicals. It is based on the killing ability of test compounds on a simple zoological organism-brine shrimp (*Artemia salina*) [2]. This assay was first proposed by Michael *et al*. [3], and further developed by several groups. The brine shrimp lethality bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines especially natural plant extracts and etc. It's a preliminary toxicity screen for further experiments on mammalian animal models.
Macaranga denticulata Muell. Arg. (Euphorbiaceae) is a small to medium-sized, evergreen tree and is a common pioneer species in moist open areas and secondary forests [4]. In the mountains of Northern Thailand, M. denticulata is used as a fallow enriching species by Karen hill tribe farmers [5]. In folk medicine, traditional healers use fresh or dried leaves of some Macaranga species to treat swellings, cuts, sores, boils and bruises [6]. A phytochemical review of literatures indicates the genus Macaranga to be a rich source of the isoprenylated, geranylated and farnesylated flavonoids and stilbenes. Furthermore, more classes of secondary metabolites like terpenes, tannins, coumarins and other types of compounds are known to be isolated from different species of the genus Macaranga. Flavonoids and stilbenes are regarded

as the major constituents and are most likely responsible for most of the activities found in the plants of this genus. The aim of our present study was to investigate the cytotoxic and thrombolytic activity of methanolic extracts of M. denticulata bark by using an in vitro procedure.

## 2. Materials and method

**2.1. Collection and identification of plant material:** The different parts of Macaranga denticulata were collected from comilla cantonment hilly area in November, 2014 then identified by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, and Bangladesh.

**2.2. Preparation of Extract:** The collected fruits were washed thoroughly with distill water, chopped, air dried for a week and pulverized in electric grinder (Miyako 3 in One blender, Miyako, China). The powder (500 g) obtained was successively extracted in methanol (55-60ºC) for 10 days with a 2 days interval. The filtrated supernatant was evaporated to dry using a rotary evaporator (RE200, BB Sterling, UK) under reduced pressure. The crude extract (22.5 g, blackish green semisolid, yield 4.5%) was preserved at 4ºC until further use.

**2.3 Brine shrimp lethality bioassay:** For the preparation of sea water 38 g of sodium chloride was weighed, dissolved in distilled water to make 1 liter solution and then filtered off to get clear solution. This simulated sea water was used for hatching of brine shrimp. The shrimp were allowed for two days to hatch and mature as nauplii (larvae). In a small beaker, measured amount of the sample was accurately weighed and dissolved in DMSO (Dimethylsulfoxide) to give a final concentration of 5 mg/ml (5 µg/µl). From the test tube containing brine shrimp nauplii, 6 test tubes were taken for the sample where each contained 5ml of seawater and 10 nauplii. These test tubes were marked from 1 to 6 for the sample. To these test tubes different concentrations (400 µg/ml, 200 µg/ml, 100 µg/ml, 75 µg/ml, 50 µg/ml, 30 µg/ml, 20 µg/ml and 10 µg/ml) of the sample were added. Then the samples were subjected to brine shrimp lethality evaluation [7]. In this case, only 50 µl DMSO was added in 5 ml sea water containing 10 nauplii. No extract was added to prepare control solution, vincristine sulphate was used as a standard and LC50 values were calculated.

**2.4 Sample preparation:** The crude extract was suspended in 10 ml distilled water and shaken vigorously on a vortex mixer. Then the suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a filter paper. The solution was then ready for in vitro evaluation of clot lysis activity.

**2.5 Streptokinase (SK) solution preparation:** To the commercially available lyophilized SK vial (PolaminWerk GmbH, Herdecke, Germany) of 15,00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000I.U) was used for in vitro thrombolysis.

**2.6 Specimen:** Whole blood (5 ml) was drawn from healthy human volunteers (n=10) without a history of oral contraceptive or anticoagulant therapy. 500 µl of blood was transferred to each of the ten previously weighed alpine tubes to form clots. 2.7 Thrombolytic assay: Experiments for clot lysis were carried as reported earlier [8]. Venous blood drawn from healthy volunteers was transferred in different pre-weighed sterile eppendorf tube (500 µl/tube) and incubated at 37 °C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube-weight of tube alone). Each eppendorf tube containing clot was properly labeled and 100 µl of plant extract was added to the tubes. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference in weight taken before and after clot lysis was expressed as percentage of clot lysis [9]. Streptokinase and water were used as positive and negative control, respectively. The experiment was repeated several times with the blood samples of different volunteers. % clot lysis = (Weight of the lysis clot /Weight of clot before lysis) × 100.

## 3. Result

### 3.1. Brine shrimp lethality bioassay

In brine shrimp lethality bioassay the methanolic extract of macaranga denticulata bark showed positive result in comparison to positive control vincristine sulphate. plotting the log concentration (log C) versus percent mortality for all test sample showed approximately a linear correlation. From the graph it stated that the methanolic extract of Macaranga denticulata bark showed a significant cytotoxic activity against brine shrimp nauplii ana $LC_{50}$ value was 57.25 µg/ml (Table 1 & fig 1)

**Table 1:** Cytotoxicity of methanol extract *Macaranga denticulata bark*

| Concentration | LogC | Total Naupli | No. of naupli dead | No. of naupli live | % of mortality | $LC_{50}$ |
|---|---|---|---|---|---|---|
| 10 | 1 | 10 | 1 | 9 | 10 | 57.25 µg/ml |
| 20 | 1.30103 | 10 | 2 | 8 | 20 | |
| 30 | 1.47 | 10 | 3 | 7 | 30 | |
| 50 | 1.69 | 10 | 4 | 6 | 40 | |
| 75 | 1.87 | 10 | 6 | 4 | 60 | |
| 100 | 2 | 10 | 7 | 3 | 70 | |
| 200 | 2.30 | 10 | 8 | 2 | 80 | |
| 400 | 2.60 | 10 | 10 | 0 | 100 | |

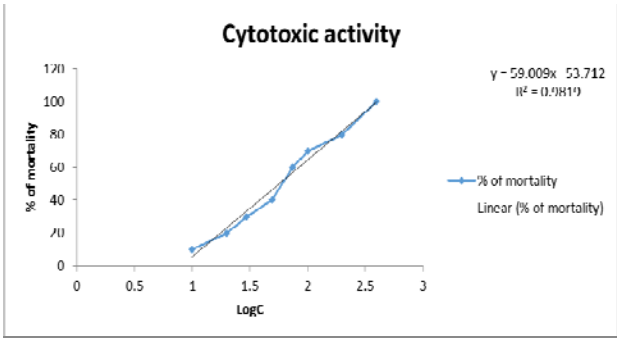


**Fig 1:** Determination of $LC_{50}$ value for extract of M.denticulata from linear correlation between logC versus % of mortality

### 3.2. Thrombolytic activity assay

Addition of 100 µl SK, a positive control (15,00,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 70% ± 1.88 % clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (2.60%). The *in vitro* thrombolytic activity study revealed that M.denticulata showed 31.59%.Statistical representation of the effective clot lysis percentage by our herbal preparation, positive thrombolytic control Streptokinase) and negative control (sterile distilled water) is tabulated in (Table 2 & fig 2)

**Table 2:** Thrombolytic activity of Macaranga denticulata bark

| No | Weight of the empty tube(A)gm | Weight of the tube with clot(B)gm | Weight of clot.(C) C=B-A | Weight of the tube with clot after lysis(D)gm | Weight of lysis(E) (B-D) | % of clot lysis | Average % of clot lysis |
|---|---|---|---|---|---|---|---|
| 1 | 0.80 | 1.10 | 0.3 | 1.03 | 0.7 | 23.33 | 31.59% |
| 2 | 0.83 | 1.11 | 0.28 | 1.00 | 0.11 | 39.28 | |
| 3 | 0.80 | 1.12 | 0.32 | 1.02 | 0.10 | 31.25 | |
| 4 | 0.82 | 1.22 | 0.4 | 1.10 | 0.12 | 37.5 | |
| 5 | 0.80 | 1.07 | 0.27 | 0.99 | 0.08 | 29.62 | |
| 6 | 0.80 | 1.15 | 0.34 | 1.04 | 0.11 | 32.35 | |
| 7 | 0.78 | 1.17 | 0.39 | 1.08 | 0.09 | 23.07 | |
| 8 | 0.79 | 1.22 | 0.43 | 1.11 | 0.11 | 32.35 | |
| 9 | 0.82 | 1.19 | 0.36 | 1.02 | 0.13 | 36.11 | |
| 10 | 0.81 | 1.30 | 0.45 | 1.15 | 0.14 | 31.11 | |

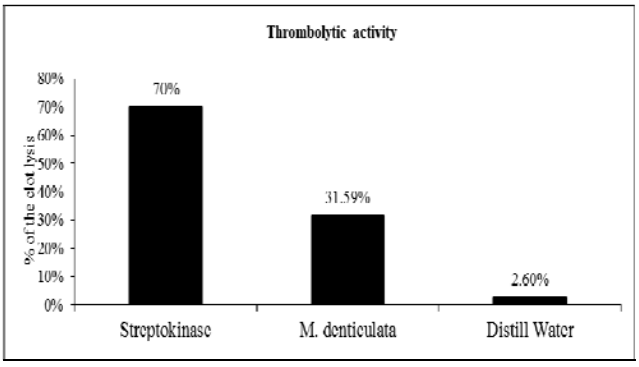


**Fig 2:** Clot lysis by Streptokinase, water and Macaranga denticulata

### 4. Discussion

The present study was undertaken to evalute the cytotoxic and thrombolytic activity of methanolic extract of Macaranga denticulata bark. To evalute cytotoxic activity brine shrimp lethality method was used. Brine shrimp lethality bioassay is an easy and straight forward bench top screening method for predicting important pharmacological activities like enzyme inhibition, ion channel interference, antimicrobial and cytotoxic activity [10-11]. The extract showed $LC_{50}$ at a very low concentration with very quick response indicating that the extract is significantly potent. Ideally, any agent useful in the treat men to 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 [12]. It is necessary to test this extract in low concentration to evaluate its potency and also against various cancer cell lines as normal cell lines to justify the potential to further investigate this plant for anticancer activity. Further investigation is required to find the responsible compound(s) for the cytotoxic activity observed for M. denticulata. A number of studies have been conducted by various researchers to find out the herbs and natural food sources and their supplements having thrombolytic (anticoagulant and antiplatelet) effect and there is evidence that consuming such food leads to prevention of coronary events and stroke [13-16]. In the thrombolytic bioassay result suggested that the extract showed very significant activity. The plant can be evaluated to further research for thrombolytic activity to a specific disease.

### 5. Conclusion

It can be concluded that Macaranga denticulata has got the potential as a candidate for future thrombolytic agent. It can also be investigated as a possible source of antitumor drugs. This is only a preliminary study and investigated be phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentials.

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