Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 3(6): 43-46
Received: 17-01-2015
Accepted: 30-01-2015

Abul Hasanat
Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazar, Chittagong-4203, Bangladesh

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

Md Ibrahim Tarek
Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazar, Chittagong-4203, Bangladesh

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

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

Md. Sekendar Ali
Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazar, Chittagong-4203, Bangladesh

Correspondence:
Md. Jakaria
Department of Pharmacy, International Islamic University Chittagong (IIUC), Chawkbazar, Chittagong-4203, Bangladesh

Bioassay of brine shrimp lethality and thrombolytic activity of methanolic extract of *Macaranga denticulata* leaves

Abul Hasanat, Md. Jakaria, Md Ibrahim Tarek, Tanvir Ahmad Chowdhury, Arifujjaman, Md. Sekendar Ali

Abstract
The study was aimed to investigate the brine shrimp lethality bioassay and thrombolytic activity of the methanolic extract of *Macaranga denticulata* leaves. 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. The cytotoxic activity of methanolic extracts of *M. denticulata* leaves was evaluated by Brine shrimp lethality bioassay. The brine shrimp lethality bioassay result was (LC_{50}=82.74 µg/ml) compared with standard vincristine sulphate (LC_{50}=0.839 µg/ml). It has significant thrombolytic activity (34.77%) compared to standard streptokinase (70%). The results of this study confirmed that this plant candidate for future research of anticancer and thrombolytic drugs.

Keywords: Brine shrimp, Thrombolytic, Cytotoxic, Clot lysis, *Macaranga denticulata.*

1. Introduction
Thrombolysis is the breakdown (*lysis*) of blood clots by pharmacological means. It is colloquially referred to as 'clot busting' for this reason. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator (tPA), the protein that normally activates plasmin. *In vitro* thrombolytic activity of crude extract was enumerated and was compared with streptokinase, which is a well known anticoagulant used in myocardial infarction [1]. Brine shrimp lethality bioassay is a bench top bioassay method for evaluating anticancer, antimicrobial and other pharmacological activity of natural products. Natural products extracts, fractions or pure compounds can be tested for their bioactivity by this method [2]. *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 [3]. In the mountains of Northern Thailand, *M. denticulata* is used as a fallow enriching species by Karen hill tribe farmers [4]. In folk medicine, traditional healers use fresh or dried leaves of some Macaranga species to treat swellings, cuts, sores, boils and bruises [5]. 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. An increasing number of phytochemical studies are being carried out on plants belonging to the genus Macaranga due to their various traditional uses. Thus, the isolated natural products from this genus have been reported to display interesting biological activities including antitumor, antioxidant, antimicrobial and anti-inflammatory [6].
This is a resourceful area of research as many species of Macaranga are used in traditional medicine as well as exhibits various pharmacological properties while their chemistry indicates varied chemical structures. The aim of our present work was to investigate the cytotoxic and thrombolytic activity of methanolic extracts of *M. denticulata* by using an *in vitro* procedure.

2. Materials and Method
2.1 Plant collection: The leaves of *M. denticulata* were collected from the Chittagong city area in front of Chittagong Medical college hostel gate of Bangladesh in October, 2014 then identified by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, and Bangladesh.

2.2 Extracts preparation: The collected plant was washed thoroughly with water and air dried for a week at 35 to 40 °C and pulverized in electric grinder. The obtained powder was successively added to methanol with vigorous shaking at 55 to 60 °C temperature. The extracts were made to dry by using rotary evaporator under reduced pressure. The extract was preserved at 4 °C for 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 (250 µg/ml, 200 µg/ml, 150 µg/ml, 100 µg/ml, 50 µg/ml and 25 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 LC_{50} 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. 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. Results

3.1 Brine shrimp lethality bioassay: In brine shrimp lethality bioassay, the methanolic extract of *M. denticulata* leaves showed positive result in comparison with the positive control vincristine sulphate. By plotting the concentration versus percent (%) of mortality for all test samples showed an approximate linear correlation. From the graph, the median lethal concentration (LC_{50}) was determined to check the toxic level of the extract. The crude extract of *M. denticulata* leave showed significant cytotoxic activity against brine shrimp nauplii and LC_{50} value was 82.74 µg/ml compared with standard vincristine sulphate (LC_{50}=0.839 µg/ml). (Table 1 & Figure 1). DMSO was used as a negative control to validate the test method.

Table 1: Brine shrimp lethality of *Macaranga denticulata*

Concentration (µg/ml)	Log C	Total nauplii	No. of nauplii Dead	No. of nauplii live	% of mortality	LC_{50}
25	1.39	10	02	08	20%	82.74 µg/ml
50	1.69	10	02	07	30%	
100	2	10	05	05	50%	
150	2.17	10	06	04	60%	
200	2.30	10	08	02	80%	
250	2.39	10	09	01	90%	

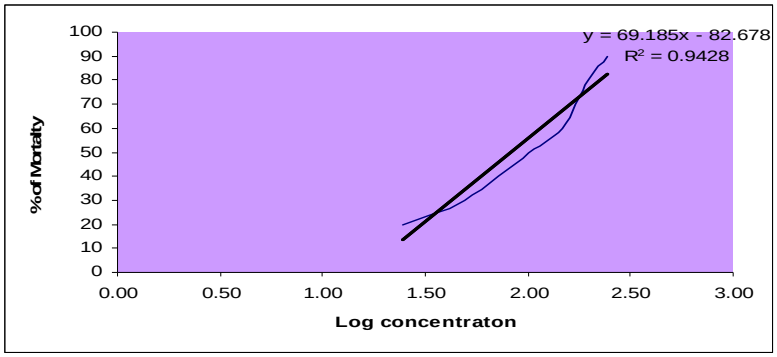

Fig 1: Determination of LC_{50} of methanol extract of *Macaranga denticulata*

3.2 Thrombolytic activity assay: 100 µl Streptokinase as a positive control (30,000 I.U.) was added to the clots along with 90 minutes of incubation at 37 °C, showed 70% clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (2.8%). The *in vitro* thrombolytic activity study revealed that *M. denticulata* showed 34.77% clot lysis. The percentage of weight, loss of clot after application of extract solution was taken as the functional indication of thrombolytic activity. % Clot lysis obtained after treating clots with different concentration of the sample was shown in (Table 2 & Figure 2).

Table 2: Thrombolytic activity of *Macaranga denticulata*

No.	Weight of empty tube (A) gm	Weight of clot tube with (B) gm	Weight of clot (C) (B-A) gm	Weight of tube with clot after lysis (D) gm	Weight of lysis (E) (B-D)	% of clot lysis	Average % of clot lysis
1.	0.85605	1.2178	0.36175	1.00115	0.21665	59.89	34.77%
2.	0.81830	1.1299	0.3116	1.09205	0.03785	12.15	
3.	0.82150	1.1881	0.3666	1.05705	0.13105	35.75	
4.	0.77820	1.1531	0.3749	0.97515	0.17795	47.47	
5.	0.80110	1.08195	0.28085	1.005	0.07695	27.40	
6.	0.83070	1.08135	0.25065	1.00075	0.08085	32.26	
7.	0.80330	1.0316	0.2283	0.9693	0.0623	27.29	
8.	0.80475	1.0233	0.21855	0.92755	0.09575	43.82	
9.	0.80875	1.0399	0.23115	0.9539	0.086	37.15	
10.	0.80925	1.0646	0.25535	1.0019	0.0627	24.56	

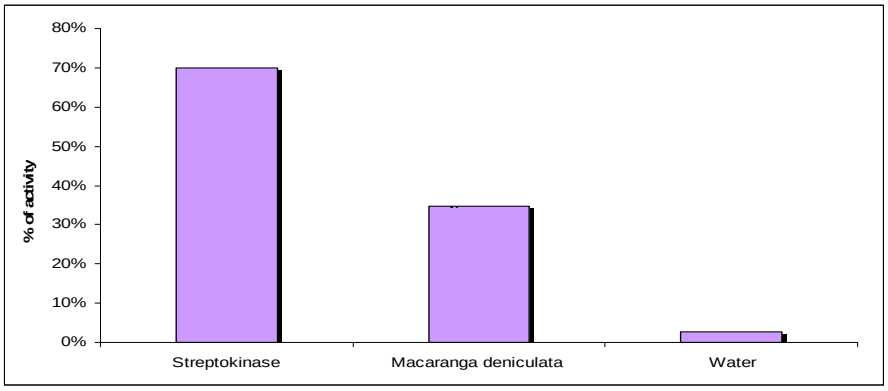

Fig 2: Clot lysis by streptokinase, *Macaranga denticulata* and water

4. Discussion

This study evaluated the brine Shrimp lethality and thrombolytic activity bioassay of methanolic extract of *M. denticulata.* the herbal preparations are used since ancient times for the treatment of diseases. Phytopharmacological and phytochemical evaluation lead to drug discovery. About 30% of the pharmaceuticals are prepared from plants worldwide [9-10]. 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[11-14]. Although there are several thrombolytic drugs, including those obtained by recombinant DNA technology, but side effects related to some of these drugs that lead to further complications have been reported [15-18]. 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 [19-21]. 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 [22]. 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*. In the thrombolytic bioassay result suggested that the extract showed very potent activity. The plant can be evaluated to further research for thrombolytic activity to a specific disease.

5. Conclusion

From our bioassay, we concluded that *M. denticulata* has got the potential as a candidate for future thrombolytic agent. It can also be investigated as a possible source of antitumour drugs. This is only a preliminary study and to make final comment the extract should thoroughly investigated be phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentials. This study may be helpful for further research works.

6. References

1. Sikri N, Bardia A. A history of streptokinase use in acute myocardial infarction. Tex Heart Inst J 2007; 34(3):318-327.

2. Dockey M, Tonkins S. Brine shrimp ecology. British Ecological Society.
3. Kerby J, Elliot S, Maxwell JF, Blakesley D, Anusansunthorn V. Macaranga denticulata. In: Tree Seeds and Seedlings for Restoring Forest in Northern Thailand. The Forest Restoration Research Unit. Biology Department, Science Faculty, Chiang Mai University, Chiang Mai, Thailand, 2000, 92-93.
4. Rerkasem K, Yimyam N, Kosamphan C, Thong-Ngam C, Rerkasem B. Agrodiversity lessons in mountain land management. Mountain Research and Development 2002; 22:4-9.
5. Nick A, Rali T, Sticher O. Biological screening of traditional medicinal plants from Papua New Guinea. J Ethnopharmacol 1995; 49:147-156.
6. Joseph J. Magadula Phytochemistry and pharmacology of the genus Macaranga: A review Journal of Medicinal Plant Research 2014; 8(12):489-503.
7. Mayer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Mclaughlin JL. Brine shrimp: a convenient bioassay for active plant constituents. Planta Med 1982; 45:31-34.
8. Sweta P, Rajpal SK, Jayant YD, Hemant JP, Girdhar MT, Hatim FD. Effect of Fagonia Arabica (Dhamasa) on *in vitro* thrombolysis. BMC Complementary and Alternative Medicine 2007; 7:36.
9. Leta GC, Mourao PAS, Tovar AMF. Human venous and arterial glycosaminoglycans have similar affinity for plasma low-density lipoproteins. Biochim Biophys Acta 2002; 586:243-253.
10. Gillman MW, Cupples LA, Gagnon D, Posner BM, Ellison RC, Castelli WP et al. Protective effect of fruits and vegetables on development of stroke in men. J of Ame Med Asso 1995; 273:1113-1117.
11. Ratnasooriya WD, Fernando TSP, Madubashini PP. *In vitro* thrombolytic activity of Sri Lankan black tea, *Camellia sinensis* (L.) O. Kuntze. J Nat Sci Found Sri Lanka 2008; 36:179-181.
12. Joshipura KJ, Ascherio A, Manson JE, Stampher MJ, Rimm EB, Speizer FE. Fruit and vegetable intake in relation to risk of ischemic stroke. J Am Med Ass 1999; 282: 1233-1239.
13. Liu S, Manson JE, Lee IM, Cole SR, Hennekens CH, Willett WC *et al*. Fruit and vegetable intake and risk of cardiovascular disease: the womenûs health study. Am J Clin Nutr 2000; 72: 922-928.
14. Bazzano LA, He J, Ogden LG, Loria CM, Vupputuri S, Myers L *et al*. Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first national health and nutrition examination survey epidemiologic follow-up study. Am J Clin Nutr 2002; 76:93-99.
15. Baruah DB, Dash RN, Chaudhari MR, and Kadam SS. Plasminogen activators: A comparison. Vascular Pharmacol 2006; 44:1-9.
16. Gallus AS. Thrombolytic therapy for venous thrombosis & pulmonary embolism. Bailliereûs Clin Haematol 1998; 11:663-673.
17. Wardlaw JM, Murray V, Berge E, Del Zoppo GJ. Thrombolysis for acute ischemic stroke. The Cochrane lib 2004; 35:2914-2915.
18. Capstick T, Henry MT. Efficacy of thrombolytic agents in the treatment of pulmonary embolism. Eur Respir J 2005; 26:864-874.
19. Cardellina JH, Fuller RW, Gamble WR, Westergaard C, Boswell J, Munro MHG *et al*. Evolving strategies for the selection dereplication and prioritization of antitumor and HIV inhibitory natural products extracts. In: Bohlin, L., Bruhn, J.G. (Eds), Bioassay Methods in Natural Product Research and Development. Kluwer Academic Publishers, Dordrecht 1999; 43:25-36.
20. Meyer BN, Ferrigni NR, Putnam JB, Jacobsen LB, Nichols DE *et al*. Brineshrimp: a convenientgeneral bioassay for active plant constituents. Planta Med 1982; 45:31-34.
21. Anderson JE, Goetz CM, McLaughlin JL, Suffness M. A blind comparison of simple bench top bioassay and human tumour cell cytotoxicities as antitumor pre screens. Phytochem Analysis 1991; 2:107-111.
22. Priestman T. Cancer Chemotherapy in Clinical Practice. London: Springer-Verlag, 2008, 130-136.