International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X
Impact Factor: RJIF 5.22
www.pharmacyjournal.in
Volume 3; Issue 6; November 2018; Page No. 19-22

Study of Cytotoxic and thrombolytic activity of *Goniothalamus Sesquipedalis* in different extracts

Nahid Akter[1*], Nur Akter Habiba[2], Tanjina Afrin[3], Mahia Ferdushi[4], Shakera Islam[5], Kazi Nuruddin Al Masud[6*]
[1] Research Lab, Department of Pharmacy, BRAC University, Dhaka, Bangladesh
[2] Physical Pharmacy and Pharmaceutical analysis Lab, Department of Pharmacy, BRAC University, Dhaka, Bangladesh
[3-7] Department of Pharmacy, BRAC University, Dhaka, Bangladesh

Abstract
In this study the thrombolytic activity and cytotoxic activity of *Goniothalamus Sesquipedalis* was evaluated. The plant is associated to the Annonaceae family and locally used for tumors, boils and buboes treatment. The thrombolytic property and the cytotoxic activity of *Goniothalamus Sesquipedalis* was evaluated by using the aqueous, methanolic, ethanolic, ethyl acetate, chloroform and their cyclohexane soluble partitioning materials and also extracts of *Goniothalamus Sesquipedalis*. As standard for the thrombolytic activity evaluation Streptokinase was used and % of clot lysis was 86.31 which is very high. For Cytotoxic activity Vincristine sulphate was used as standard and IC50 value of the methanolic extracts was 25.19µg/ml which was comparatively best.

Keywords: *Goniothalamus Sesquipedalis*, cytotoxic activity, thrombolytic activity, different extracts

Introduction
From the beginning of the modern era traditional medicinal plants play a significant part in maintaining healthcare system in most countries. These medicinal plants are the great sources for therapeutically active compounds. In addition, for treating various types of diseases therapeutic active compounds have been used [1]. Medicinal plants have different types of pharmacological and biological properties [2]. Such as antimicrobial and antioxidant activity is provided by Achillea wilhelmsii [3]. *Nitraria schoberi* L. shows antioxidant properties [4]. Some important properties like anti-dermatophyte, anti-fusarium and cytotoxic activity is provided by plant extracts of Piper genus [5]. Some other plants showed antifungal, antiparasitic activity [6, 7].
Goniothalamus Sesquipedalis is associated to Annonaceae family. Almost, 150 genera and 3000 species are beloned to Annonaceae family [8]. In traditional medicine, mainly against fever, cough, colds, snakebite, pains, and infectious and inflammatory diseases some of these species have been used. Antimicrobial, anti-inflammatory, antioxidant, and antitumor properties have been showed by Annonaceae species chemical compounds like flavonoids, steroids, phenolic glucosides, simple phenolics, quinones, xanthones [9]. "*Goniothalamus Sesquipedalis*" is the common name of the *Goniothalamus Sesquipedalis* which is located to the tropic areas like Bangladesh, India etc. Medicinal plants are great source of killing cancer cells that means they have cytotoxic activity [11]. However, pharmacological properties can be showed by almost every part of the plant like leaf, stem and bark which will be very helpful to deal with various disease conditions [12]. Moreover, thrombolytic activity is also very important property where compounds able to destroy blood clots [10]. So, the current study was taken on to discover cytotoxic and thrombolytic activities of *Goniothalamus Sesquipedalis* plant and to make some contribution to medical science.

Materials and Methods
Collection of the seeds: *Goniothalamus Sesquipedalis* plant was selected during this research work for investigating different pharmacological activities. The fresh leaves of the plant were collected from Chittagong during June 2017. The plant sample was then sent to National Herbarium of Bangladesh (NHB), Mirpur for identification where they identified the plant and provided a verification number 42930.

Extraction of seed Material: The whole plants were dried under sun for a few days and finally oven dried to remove all the moisture content. Then the seeds were crushed to coarse consistency. The coarse grains were extracted in a decreasing polarity order. The coarse plant material (900g) was taken and soaked with 1500 ml of methanol for 3 consecutive days at 25ºC. The extract was filtered and the filtrate was kept for further extraction. In the same manner the filtrate was soaked in different solvents by polarity decreasing order.
Aqueous > Methanol > Ethanol > Ethyl Acetate > Chloroform > Cyclohexane
For every case, the extract was preserved and solvent evaporation was done by using rotary evaporator. Finally, all the extracts of *Goniothalamus Sesquipedalis* was kept under laminar airflow for protecting it from any type of contamination.

Drugs and chemicals: Most of the chemicals used in this research were purchased from well-known vendors and some of them were received as gifts from different leading pharmaceutical companies in the country. First of all,

lyophilized Altepase (Streptokinase) was obtained from Sanofi Bangladesh Ltd whereas Beacon Pharmaceuticals Limited, Bangladesh provided Vincristine sulphate. Methanol was purchased from a reputed chemicals supplier known as Active Fine Chemicals Limited, Bangladesh. Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific, UK.

Cytotoxic activity: The cytotoxic potentialities of all the extracts of *Goniothalamus Sesquipedalis* were performed on brine shrimp nauplii by following a method described by Meyer which is also a valid method to determine other pharmacological activities, e.g. antimicrobial property, antiviral property, pesticides and tumor-resistant property, etc. [13. 14]. At the very beginning, 38gm of salt was mixed thoroughly in one liter of water in a container to prepare artificial sea water which is simply 3.8% NaCl solution [15]. Collected egg of brine shrimps (*Artemia salina*) were hatched in the artificial sea water which were afterward used in the Brine Shrimp Lethality Bioassay test. The test sample were prepared by dissolving 4mg of crude extract in DMSO and serial dilution technique was followed to prepared varied concentration of sample. The test solutions were then introduced into the labelled vials containing 10 live brine shrimp and incubate d for 24 hours. When the incubation for 24 hours were done, all the vials were examined using magnifying glass to count the survived brine shrimp nauplii and the data were used to calculate the cytotoxicity potential of the plant *Goniothalamus Sesquipedalis.*

Thrombolytic activity

Standard drug and test solution preparation: Lyophilized Altepase, 30,000 IU Streptokinase (Streptase®, by Sanofi-Aventis) vials were used as standard drug to compare the thrombolytic property of *Goniothalamus Sesquipedalis.* On the other hand, test sample were prepared by dissolving crude extracts in methanol with vigorous shaking and kept overnight to decant to release the supernatants which were further reformed into 2mg/ml. 4mg/ml, 6mg/ml, 8mg/ml and 10mg/ml concentration by following serial dilution method. Those methanolic supernatants were formerly filtered through 0.22microne syringe filter to get the clear test solution which were used to observe the thrombolytic activity of the plant.

Thrombolytic analysis: Prasad et.al illustrated a method which was followed as a standard method to observe the thrombolytic activity of the plant [16]. Blood sample from healthy persons were collected who do not have any history of oral contraceptives, anticoagulants, and drug history in recent past. 5 ml of blood were then dispersed into different micro centrifuge tubes (0.5 ml/tube) which were then incubated at room temperature for 45 minutes. As a result, blood cells clotted at the bottom while serum floated at the top of the tubes which were then removed from the tube without irrupting the clots at the bottom. Weights of the clots were measured and test samples were introduced into the tubes congaing clots with proper labeling. Simultaneously, streptokinase as a positive control and distilled water as negative control were also added in separate tubes. All the tubes were then incubated for 90 minutes at 37°C temperature. When the incubation was complete, fluid in each tube was taken away and weight variation of clots between after and before of lysis was calculated and represented as the percentage of lysis.

wt. of tube and clot – wt. of tube tube = wt. of clot
wt. of tube and clot – weight after lysis = wt. of lysis

$$\frac{wt.of\ lysis}{wt.of\ clot} \times 100 = \%\ of\ lysis$$

Statistical Analysis

Every analysis was performed in triplicate under strict aseptic conditions to ensure consistency of all findings. For each extract triplicate data was taken and the final data was taken by the triplicate data's mean ± SD (Standard Deviation), which was analyzed by Microsoft excel.

Results and Discussion

Cytotoxic activity

Brine shrimp lethality bioassay: Lethal Concentration (LC50) is a standard measure of the toxicity of the substance that kills half of the test nauplii at a specific time which refers to the presence of cytotoxicity activity.

Table 1: LC50 values of the test samples of whole plants of *Goniothalamus Sesquipedalis*

Sample Name	Regression line	R2	LC50 (µg/ml)
VS	y = 30.799x + 60.653	0.973	0.45
WE	y = 0.0867x + 29.341	0.3537	128.28
ME	y = 0.1417x + 47.848	0.5116	25.19
EE	y = 0.1275x + 40.266	0.4511	63.35
AE	y = 0.1014x + 40.874	0.7292	40.6
CHE	y = 0.161x + 36.911	0.6875	61.29
CYE	y = 0.1267x + 29.344	0.5234	203.03

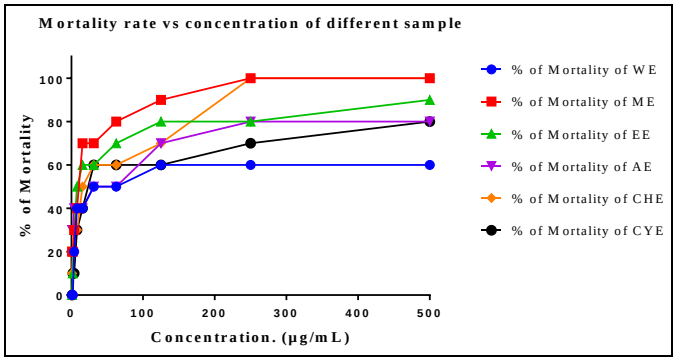

Fig 1: Graphical representation of % of mortality and concentrations of different sample.

In this study, vincristine sulphate used as a standard showed that the LC50 value was 0.45 µg / ml. compared to the standard among extractive ME, it showed the highest lethality with a value of 25.19 µg / ml. The LC50 values of WE, EE, AE, CHE and CYE were respectively 128.28 µg / ml, 63.35 µg / ml, 40.6 µg / ml, 61.29 µg / ml and 203.03 µg / ml (Table 1). After this analysis, it was clarified that the plant *Goniothalamus Sesquipedalis* has a cytotoxic activity. Each sample regression line provided in Figure 1. Therefore, this

plant can be used as a cytotoxic agent with adequate purification and isolation.

Thrombolytic activity: To identify blood thinning medications from plant source different extractives of *Goniothalamus Sesquipedalis* were studied for thrombolytic activity. All the results were displayed in the table 2.

Table 2: % of clot lysis of different extract and standard.

Sample	% of clot lysis
Blank	5.076
Aqueous	86.31
Methanol	64.17
Ethanol	55.58
Acetone	49.17
Chloroform	36.75
Cyclo-Hexane	30.57
Pet Ether	24.94
Streptokinase	71.83

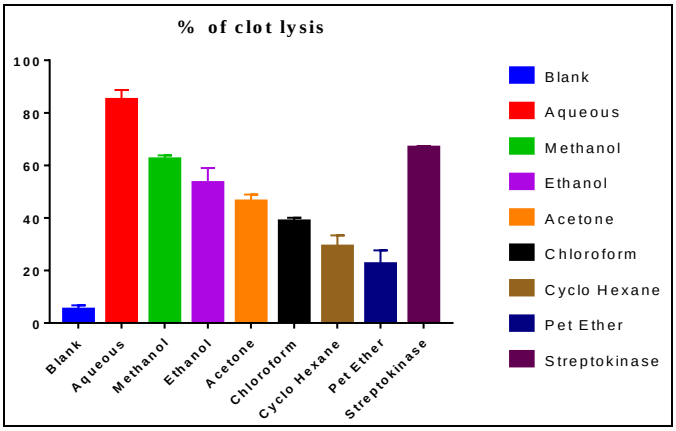

Fig 2: % of clot lysis of different extract of *Goniothalamus Sesquipedalis* and Standard.

The expansion of 100 µl of Streptokinase, a positive control (30,000 i.u. units), coagulation and subsequent incubation for one and a half hours at 37 ° C indicated a clot lysis of 71.83%. On the other hand, purified water used as negative control which showed insignificant rate of clot lysis 5.07%. It was found that the average distinction in the clot lysis rate between positive and negative control is exceptionally critical. In this study, aqueous extractive showed very high and surprising thrombolytic activity 86.31%. Moreover, other extractive like methanol, ethanol, acetone, chloroform, cyclo-hexane and pet ether showed clot lysis respectively 64.17%, 55.58%, 49.17%, 36.75%, 30.57% and 24.94%. From this experiment, it can be said that some of the *Goniothalamus Sesquipedalis* extracts showed gentle to direct lysis of the clot. Therefore, this plant can be used as some thrombolytic agents with its best pharmaceutical possibilities.

Conclusion

The lethality assay of the brine shrimp indicated that there was significant cytotoxic activity in the different extractives of the *Goniothalamus Sesquipedalis* plant. Among them methanol extract showed the most potent cytotoxic property. Moreover, this plant showed significant level of thrombolytic activity so it can be a good source of anti-thrombolytic agents. All the experiments performed in this study are based on crude extracts and are considered preliminary and a more sophisticated investigation is needed to reach a concrete conclusion on the results of this study.

Reference

1. Kirtikar KR, Basu BD. Indian Medicinal Plants. International Book Distributors, Allahabad. 1996; 3:2247.
2. Akter R, Uddin SJ, Grice ID, Tiralongo E. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. Journal of Natural Medicines. 2014; 68:246-252.
3. Alfatemi SMH, Rad JS, Rad MS, Mohsenzadeh S, da Silva JAT. Chemical composition, antioxidant activity and in vitro antibacterial activity of Achillea wilhelmsii C. Koch essential oil on methicillin-susceptible and methicillin-resistant Staphylococcus aureus spp. 3 Biotech. 2015; 5:39-44.
4. Sharifi Rad J, Hoseini-Alfatemi SM, Sharifi Rad M, Iriti M. Free radical scavenging and antioxidant activities of different parts of *Nitraria schoberi* L. Journal of Biologically Active Products from Nature. 2014; 4:44-51.
5. Tangarife-Castaño V, Correa-Royero JB, Roa-Linares VC, Pino-Benitez N, Betancur-Galvis LA, Duráncd DC *et al*. Anti-dermatophyte, anti-Fusarium and cytotoxic activity of essential oils and plant extracts of Piper genus. Journal of Essential Oil Research. 2014; 26:221-227.
6. Bhagwat MK, Datar AG. Antifungal activity of herbal extracts against plant pathogenic fungi. Archives of Phytopathology and Plant Protection. 2014; 47:959-965.
7. Horn D, Duraisingh MT. Antiparasitic chemotherapy: From genomes to mechanisms. Annual Review of Pharmacology and Toxicology. 2014; 54:71-94.
8. "The Plant List - A Working List for All Plant Species." Home - The Plant List, www.theplantlist.org/browse/A/Gesneriaceae/Boeica/
9. Verdan, Maria Helena, and Maria Elida Alves Stefanello. "ChemInform Abstract: Secondary Metabolites and Biological Properties of Gesneriaceae Species." ChemInform, vol. 44, no. 12, 2013, doi:10.1002/chin.201312269.
10. Bekker J, Ploem S, Jong KP. Early Hepatic Artery Thrombosis after Liver Transplantation: A Systematic Review of the Incidence, Outcome and Risk Factors. American Journal of Transplantation, 2009; 9(4):746-757.
11. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medicinal plants using brine shrimp (Artemia salina) lethality assay. International Journal of Applied Science and Engineering. 2005; 3:125-134.
12. Omale, James, Okafor, Polycarp Nnacheta. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissus multistriata. African Journal of Biotechnology. 2008; 7(17):3129-3133.
13. Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, J. (1982).Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. Planta Medica, 45(5), 31–34.https://doi.org/10.1055/s-2007- 971236

14. McLaughlin JL, Rogers LL, Anderson JE. The Use of Biological Assays to evaluate Botanicals. Drug Information Journal. 1998; 32(2):513-524. https://doi.org/10.1177/009286159803200223
15. Ali R, Marjan H, Jannatul FR, Hasanuzzaman M, Islam M. Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds. Avicenna Journal of Phytomedicin. 2014; 4(6):430-6.
16. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thromb J. 2006; 4(14):1-4.