Research Article

Investigation of Antioxidant, Thrombolytic, Cytotoxic and Antimicrobial Activities of *Goniothalamus sesquipedals* (wall.) Hook.f. & Thomson

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ABSTRACT

Goniothalamus sesquipedalis (Wall.) Hook.f. & Thomson is a medicinal plant that came from Annonaceae family. The leaves of the plant were exerted in this research to investigate several pharmacological actions. This investigation included evaluation of antioxidant feature by total phenolic content (TPC) test and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, thrombolytic activity, cytotoxicity and antimicrobial activity assay. This was a self-funded study. The investigation revealed that in TPC test, methanol plant extract of leaves unveiled 90.53 mg of gallic acid equivalents/g of extract and in DPPH free radical scavenging assay, IC_{50} value of positive control (ascorbic acid) was 75.69 µg/mL and for methanol extract of leaves, it was 53.59 µg/mL. The thrombolytic study unveiled 77.25% clot lysis for methanol extract of the leaves where positive control (streptokinase) unveiled 74.70% clot lysis. The cytotoxic study unveiled LC_{50} value of 17.2 µg/mL for methanol extract of leaves whereas positive control (vincristine sulfate) gave 12.07 µg/mL value of LC_{50}. The antimicrobial test also revealed that plant extract at the concentration of 400 mg/mL showed better efficacy in suppressing microbial growth. All the findings of the investigation support the acceptable existence of antioxidant activity, thrombolytic activity, cytotoxicity and antimicrobial activity.

Keywords: Antioxidant, Cytotoxicity, Thrombolytic, Antimicrobial.

INTRODUCTION

Globally researchers are showing notable interest in medical plants because of its pharmacological valuation. Investigation on the possible therapeutic application of plants has been flowed over the years and quantities of documented scientific information brought out substantial possibilities for medicinal plants' application in the treatment of numbers of diseases.[1] Statistically, 87% of drugs are derived from the natural source and used for the treatment of human diseases and plants are used in 25% of recommended drugs as an active ingredient.[2] As a natural resource, medicinal plants are safer and with the passage of time, their antioxidant, hypoglycaemic, biological and antimicrobial effects have been examined and frequently found to play the dynamic role in modern medicine.[3] Besides that, healthier compatibility with the human body, fewer side effects as well as better cultural satisfactoriness made herbal plants as a safe option in the developed countries for the last few years. Moreover, the alarming incidence of antimicrobial resistance associated adverse effects like hypersensitivity, allergic reactions, and immune-suppression forced scientists to figure out new and effective therapeutic agents.[4] The study was designed to discover antioxidant, cytotoxicity, thrombolytic and antimicrobial properties of the leaves *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson. The genus *Goniothalamus* Hk. f. et Thoms. (Family: Annonaceae) comprises of about 130 shrubs and tree species rising in the tropical forest of Asia. This genus has extensive distribution covering Bangladesh, Eastern India, Myanmar, and Malayan archipelago.[5] Five species of *Goniothalamus* used for the medical purpose in the Asian system for a long period of time and mostly connected with abortion, fever and childbirth.[6]

MATERIALS AND METHODS

Collection of plant material

In May 2018, from hill tracts of Chittagong division of Bangladesh, the leaf part of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson plant was collected and sent to the national herbarium Bangladesh (NHB), Mirpur, Dhaka, Bangladesh for verification and was authenticated by NHB and the plant accession number was also provided (DACB-42930).

Preparation of the extract

To remove the plant scrap and dust particles, the leaves were washed with clean water. Then the leaves were allowed to dry under the sun and were dried in hot air oven at 30-40°C for 1 hour. Afterwards, the dry and crusty leaves were grinded in coarse powder using a high capacity grinding machine. About 900 g of powder was allowed to be absorbed in 2.5 L of methanol for the time being of 2 days at a normal ambient temperature (22-25°C) with time to time stirring. Filtration was done afterwards using the cotton filter having the pore size of 110 mm and then evaporation of maximum amount of solvent was done using rotary evaporator at 100 rpm at the temperature of 30°C. The extract of the leaves was then placed under laminar airflow cabinet to vaporize the solvent completely from the extract. This method of vaporization was used to avoid any chance of microbial growth in the extract while

drying. Lastly, the obtained 22.4g of plant leaf extract was kept in a dry and cool place with accurate labelling.

Chemicals

All the chemicals named gallic acid [Sigma-Aldrich, USA], sodium chloride [Sigma-Aldrich, USA], Folin-Ciocalteu reagent [Sigma-Aldrich, USA], vincristine sulfate [Sigma-Aldrich, USA], sodium carbonate [Merck, India] and ascorbic acid (ASA) [Merck, India], dimethyl sulfoxide (DMSO) [Fisher Scientific, UK] were purchased. All the chemicals used for the study purpose were of lab grade.

Drugs

Glibenclamide (Square Pharmaceuticals Ltd., Dhaka, Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Dhaka, Bangladesh), Streptokinase (Healthcare Pharma Ltd., Dhaka, Bangladesh), Vincristine sulfate (Cristone, Beacon Pharmaceuticals Ltd., Dhaka, Bangladesh), Streptomycin disc (WELL's Health Care, Spain).

Antioxidant activity (Total phenolic content (TPC))

Folin-Ciocalteu chemical is used for the oxidation of phenolic content. The phenolic content could be oxidized easily by adding the chemical to the ionic phenolic solution.[7] Yellow colouring chemical changed into dark blue after completion of the oxidation process. This changing colour mixture was measured in a 760 nm in ultraviolet (UV) spectrophotometer. Absorbance values have been plotted in the gallic acid calibration curve and assessment of the data has been done as gallic acid equivalents (GAE).[8]

DPPH free radical scavenging assay

To determine the antioxidant activity of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was accomplished. Methanol plant extract disclosed DPPH free radical scavenging activities on stable radicals which were dimensioned for this test. Plant extract was compounded with DPPH solution at various concentrations. Violet coloured DPPH radical reduced to yellowish coloured diphenyl-picrylhydrazine radical by accepting the electron from the antioxidant compound.[9] Calculation of the absorbance values at different concentration was done at 517nm and the alleviating value of DPPH at 517 nm is directly proportional to the radical scavenging activity.[10]

Percentage of inhibition of DPPH free radical (I%) was calculated by utilizing following equation:

I% = ((Absorbance of blank − Absorbance of sample)/ (Absorbance of blank)) × 100

From the graph, calculation of 50% of inhibition (IC_{50}) of extract concentration was done; where the percentage of inhibition (I %) was plotted against extract concentration.

Thrombolytic activity

From the pharmacological point of view, thrombolysis is the breakage of blood clots and generally known as clot busting. Principally, thrombolysis covers the application of thrombolytic drugs that work to dissolve blood clots. Derivation of the drug was made both from Streptococcus and at present by using recombinant biotechnology. Some generally used anti-thrombolytic agents are streptokinase and urokinase.[11] For the experiment purpose, the blood sample was collected from healthier persons who did not have any history of taking anti-coagulant, oral contraceptive. Sample blood was preserved in three pre-measured sterile microbes. Then the blood sample was incubated at 37°C for 45 minutes. After completion of clotting, formed plasma materials were withdrawn from the micro tubes and then micro tubes were weighted with blood. Streptokinase, an anti-thrombolytic agent was used as positive control (standard) and water was used as negative control (blank). Each test tube was filled with 100 µl of plant extract at 37°C and then micro tubes were incubated for one and half hour (90 minutes). After that, the liquid was withdrawn from the clot and re-weighted the tubes in order to evaluate weight differentiation when the clot interference happened.[12]

Percentage of clot lysis was counted by the subsequent equation:

(%) of clot lysis = ((released clot weight)/ (clot weight after clot disruption)) × 100

Cytotoxic activity (Brine shrimp lethality assay)

The brine shrimp cytotoxicity assay is the well-thought-out consideration for primary assessment of toxicity[13]. *Artemia salina* shrimp was used in this lethality assay and to grow nauplii. Preparation of sample in the desired concentration was done by dilution through the addition of calculated volume of dimethylsulfoxide (DMSO). Through visual examination, nauplii positioned in vials containing around 5mL stimulated seawater was counted. The sample of various concentrations was placed in the different test tube with the help of micropipette and 10 nauplii were added in each test tube where vincristine sulfate was used as positive control. After that, test tubes were placed in a dry place at room temperature for 24 hours. As a conclusive step, survivors were counted after passing 24 hours.[14]

Percentage (%) of mortality was calculated by using following equation:

Percentage (%) of mortality = ((Number of nauplii taken − Number of nauplii alive) /(Number of nauplii taken)) × 100

From the graph, 50% of lethal concentration (LC_{50}) of extract concentration was counted and plotted percentage of mortality against concentration.

Antimicrobial activity (Disc diffusion assay method)

In current years, a rising interest has been found in studying and developing new antimicrobial agents to fight antibiotics resistance from different sources. As a result, the highest concentration has been bestowed to the screening and evaluation methods of antimicrobial

activity. The antimicrobial activity of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson was assessed by using disc diffusion assay method. *E.coli* and *Bacillus subtilis* are gram negative and gram positive bacteria respectively used in this regard. Mular hinton agar (MHA) was used as the media in this process. At first, 20 mL of MHA was poured into every petri dish after autoclaving it for sterilization. Then, plates with media were kept for a while to be settled. Afterwards, nutrient broth cultured bacterial strains *(E.coli and Bacillus subtilis)* were incubated in MHA using the cotton swab for overnight. Filter papers were used to make small disc by using a paper punch machine which was swallowed at the different concentrations of plant extract (200 mg/mL and 400 mg/mL), negative control and positive control (streptomycin). Then discs were taken out and kept till dry. After that, they were transferred to the Petri dishes. Finally, all the dishes were kept in the incubator at 37°C for 24 hours. At 24 hours, the zone of inhibition (ZI) was measured.[15] Whole process was done in the laminar flow for limiting the contamination.

Statistical analysis

All the obtained data of antioxidant activity and cytotoxic activity were presented through graphical presentation via Graph pad Prism version 7.0. Rest of the data were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

Antioxidant activity

In this study, the methanol extract of experimented plant's leaves was examined with accuracy through TPC and DPPH assay. Antioxidant activity is very essential in order to prevent free radical reaction as they can their radical activity can neutralize free radical property.[16]

Total phenolic content (TPC)

In TPC test, the methanol extract of the experimented plant leaves disclosed presence of phenolic compound that was recorded in Table 1. For the test purpose, gallic acid considered as a positive control (standard) and the absorbance of methanol extract was plotted in gallic acid calibration curve. The absorbance of plant extract was 0.834 and acquired TPC value was 90.53 mg of the gallic acid equivalent (GAE)/g of extract. The documented TPC value expressed the presence of the antioxidant characteristic in the experimented plant.

Table 1: Determination of the total phenolic content of methanol extract of leaves of of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson

Extract name	Absorbance of extract	Total phenolic content (GAE/g)
Methanol extract of the leaves	0.834	90.53

Determination of DPPH radical scavenging activity

Among the number of methods that could be used to define the radical scavenging properties of antioxidant, the DPPH method is the most favoured method as it is easy, trustworthy and fast and does not need any distinct reaction and device.[17] Here, ascorbic acid was used as reference positive control (standard) and the obtained IC_{50} value was 75.69 µg/mL. Percentage of inhibition of positive control and sample (methanol extract) at various concentration (0.977 µg/mL to 500 µg/mL) were documented (Figure 1) and highest percentage of inhibition of methanol extract (94.48947) was recorded at the highest concentration (500 µg/mL). In context, IC_{50} value of methanol extract of leaves was 53.59µg/mL. Through the presence of DPPH radical scavenging activity, this finding implied that existence of antioxidant property in the leaves of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson.

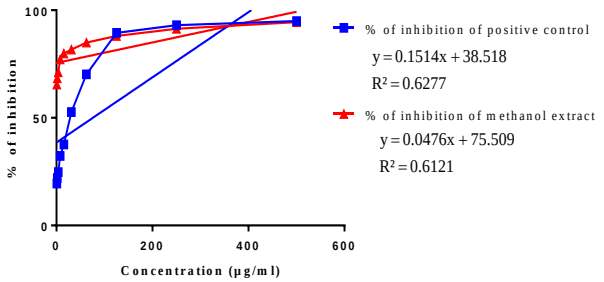

Figure 1: Comparison of percentage (%) of inhibition and predicted linear regression line of positive control and methanol extract of leaves of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson

Thrombolytic activity

The study revealed that methanol extract of the plant had higher level thrombolytic activity than the positive control (standard) (Figure 2). Here, streptokinase, an anti-thrombolytic agent was used as positive control and obtained % of clot lysis was 74.70%. Distilled water which was used as negative control (blank) gave 13.72% clot lysis. In context, % of clot lysis of methanol extract of the experimented plant was 77.25%. By comparing % of clot lysis results of positive control and plant extract, it is quite clear that experimented plant has the higher level of thrombolytic activity. The calculation of the percentage of clot lysis was done based on the obtained value of Table 2.

Table 2: Determination of the thrombolytic activity of methanol extract of leaves of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson

Group	W1	W2	W3	W4	W5
Negative control	0.783	1.637	1.534	0.751	0.103
Positive control	0.831	1.563	1.250	0.419	0.313
Plant extract	0.791	1.539	1.213	0.422	0.326

Here, W1 = Micro-tube weight, W2 = Clot with micro-tube weight, W3 = Clot with micro-tube weight after clot disruption, W4 (W3-W1) = Clot weight after clot disruption, W5 (W2-W3) = Released clot weight

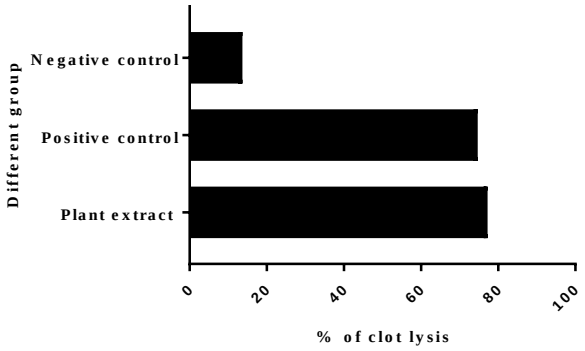

Figure 2: Percentage (%) of clot lysis of negative control, positive control and methanol extract of the leaves of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson

Cytotoxic activity (Brine shrimp lethality assay)

This assay technique was applied in order to determine the cytotoxic property of methanol extract of leaves. At various concentrations, positive control (standard) and methanol extract showed a different percentage of mortality (Figure 3). Here, vincristine sulfate was used as positive control and obtained LC_{50} value was 12.07 µg/mL and methanol extract provided LC_{50} value was 17.2 µg/mL. The methanol extract showed the highest lethality (80%) at the highest concentration (500 µg/mL). The gradation of mortality was directly proportional to the concentration of the methanol extract. This finding revealed that experimented plant has moderate cytotoxic activity.

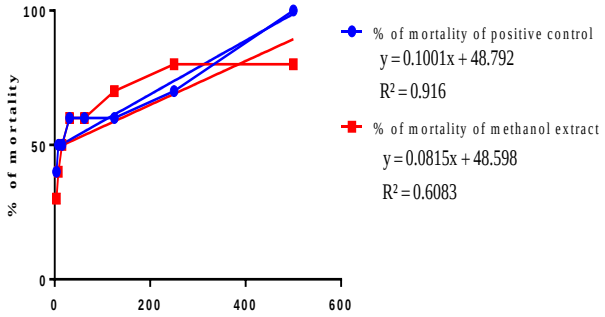

Figure 3: Comparison of percentage (%) of mortality and predicted linear regression line of positive control and methanol extract of leaves of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson

Antimicrobial activity (Disc diffusion assay method)

The experimented plant was researched to evaluate its antimicrobial activity against Gram (+ve) *(E.coli)* and Gram (-ve) *(B. subtilis)* bacteria using disc diffusion method. Assessment of antimicrobial activity of this plant was documented in Table 3. The result revealed that the plant extract was moderately effective in suppressing the antimicrobial growth of Gram (+ve) and Gram (-ve) bacteria with volatile potency. Plant extract at the concentration of 400 mg/mL has been shown most effectiveness in clogging microbial growth of all bacteria. The test support existence of moderate antimicrobial property of the methanol extract of the leaves.

Table 3: Determination of the antimicrobial activity of the leaves of Goniothalamus *sesquipedalis* (Wall.) Hook.f. & Thomson

Group	Inhibition zone (mm)	
	Gram (+ve) bacteria *(E.coli)*	Gram (-ve) bacteria *(B. subtilis)*
Negative control	0.00	22.34±0.58
Positive control	19.67± 1.52	14.34±0.58
Plant extract (200mg/mL)	7 ± 6.08	14.34±0.58
Plant extract (400mg/mL)	13.34± 0.58	17.67± 1.16

Data are average of three replicates (n=3) ± standard deviation

CONCLUSION

The methanol extracts of the leaves of *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson were studied to determine pharmacological features. The investigation unveiled that experimented plant leaves has various pharmacological potentials like mild to severe antioxidant features, the higher level of thrombolytic activity, moderate level of cytotoxicity, mild antibacterial activity. We still don't mark out which elements have the above dominations. Advanced investigations are required to make it as a new and serviceable agent in the field of medicine.

REFERENCES

1. Bello I, Bakkouri AS, Tabana YM, Al-Hindi B, Al-Mansoub, MA, Mahmud R and Asmawi MZ, Acute and sub-acute toxicity evaluation of the methanolic extract of alstonia scholaris stem bark, Medical Sciences, 4, 2016,4.
2. Chakma U, Morshed Z, Islam MN, Ahmed T, Deeba MT, Masud K N Al and Islam R. In-vitro investigation of antioxidant activity and phytochemical screening of Persicaria glabra, Journal of Medical Plants Studies, 6, 2018, 237-242.
3. Hossain MS, Uddin MS, Kabir MT, Akhter S, Goswami S, Mamun AA, Herrera-Calderon O, Asaduzzaman MD and Abdel-Daim MM, In vivo screening for analgesic and anti-Inflammatory activities of Syngonium podophyllum L.: A remarkable herbal medicine, Annual Research and Review in Biology, 16, 2017, 1-12.
4. Parekh J, Jadeja D and Chanda S, Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity, Turkish Journal of Biology, 29, 2005, 203-210.
5. Konsam SC, Ningthoujam SS and Potsangbam KS, Antibacterial activity and phytochemical screening of Goniothalamus sesquipedalis (Wall.) Hook. f. & Thomson Extracts from Manipur, North East India. European Journal of Medicinal Plants, 8, 2015, 142-148.
6. Wiart C. Goniothalamus species: A source of drugs for the treatment of cancers and bacterial infections? Evidence-based Complementary and Alternative Medicine, 4, 2004, 299-311.

7. Singleton VL, Orthofer R, Rosa M. and Raventós L, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in Enzymology, Elsevier, 299, 1999, 152-178.

8. Johari MA and Khong HY, Total phenolic content and antioxidant and antibacterial activities of Pereskia bleo, Advanced in Pharmacological Sciences, 2019, 1-4.

9. Pavithra, K and Vadivukkarasi, S. Evaluation of free radical scavenging activity of various extracts of leaves from Kedrostis foetidissima (Jacq.) Cogn. Food Science and Human Wellness, 4, 2015, 42-46.

10. Brand-Williams W, Cuvelier ME, Berset C, Use of a free radical method to evaluate antioxidant activity, Food Science and Technology, 28, 1995, 25-30.

11. Chowdhury TA, Kamal ATMM, Chowdhury KAA, Jahan A, Hossain MS, Mamur A, Hasan M and Hossain J, Cytotoxic & thrombolytic activity of methanolic extract of Macaranga denticulata Bark, Pharma Innovation Journal, 4, 2015, 36-39.

12. Al Masud KN, Shishir TA, Mahbub N, Hossain M, Islam N and Alam F, Study of cytotoxic and thrombolytic activity of Boeica filiformis in different extracts, International Journal of Research in Pharmacy and Pharmaceutical Science, 3, 2018, 22-25.

13. Baravalia Y, Vaghasiya Y and Chanda S, Brine shrimp cytotoxicity, anti-inflammatory and analgesic properties of Woodfordia fruticosa Kurz flowers. Iranian Journal of Pharmaceutical Research, 11, 2012, 851-861.

14. Ghosh T, Masud KN Al, Islam MN, Shishir TA, Morshed Z, Islam R and Hossain MR, In vitro anti-microbial and cytotoxic activity analysis of cissus adnata in different fractions, World Journal of Pharmacy and Pharmaceutical Science, 7, 2018, 162-172.

15. Balouiri M, Sadiki M and Ibnsouda SK, Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6, 2016, 71-79.

16. Ahmad S, Rehman T, Abbasi W and Zaman M, Analysis of antioxidant activity and total phenolic content of some homoeopathic mother tinctures, Indian Journal of Research in Homoeopathy, 11, 2017, 21.

17. Aksoy L, Kolay E, Aĝilönü Y, Aslan Z and Kargioĝlu M, Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic Thermopsis turcica, Saudi Journal of Biological Sciences, 20, 2013, 235-239.

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