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Study of antioxidant and antimicrobial activity of *Goniothalamus sesquipedalis* in ethanol extract

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Abstract

Goniothalamus sesquipedalis plant extract used to assess its antimicrobial and antioxidant activity. Extract was made by soaking the dried plant powder in ethanol. After comparing with the standard Ciprofloxacin we found that Ethanol extract of sample gave the activity against all the experimented microbes of ZI (zone of inhibition) against *E. coli* and *B.subtillis*. After performing the DPPH assay of plant extract of sample plant we saw that it has a good ability of inhibiting free radicals.

Keywords: *Goniothalamus sesquipedalis*, antioxidant, antimicrobial activity, *E.coli*, *B.subtillis*, DPPH

Introduction

Even before the understanding of microbes existed, the idea that specific plants contained healing properties was well established. Those plants hold what we now a days refer to as antimicrobial properties [6]. The practice of using these medicinal plants has been there for a long time; which is commonly known as traditional medicine. The knowledge of traditional medicine has been used to heal people and has been developed through generations by cultural beliefs and experimental theories [3]. Plants are used as the most general form of medicine for various indigenous people [4]. Different component of plants like leaves, twigs, roots, barks and stem are often used for conventional medicines. The worldwide demand for the practice of traditional medicine is escalating yearly. The reason behind it is that the medicines that the modern world offers right now are becoming resistant at a very high rate. Soon there will be no way out for us than to use the methods that have been used by our ancestors to heal our bodies. Around 60% of the pharmaceuticals are manufactured from plant origin. Our study includes the antimicrobial and antioxidant assay of extract from the plant *Goniothalamus sesquipedalis* to investigate whether it is effective against certain microbes and if it has health benefits or not. In Bangladesh, medicinal plant holds an important part in the health care system.

The genus *Goniothalamus* belongs to a prehistoric taxon of flowering plants: the Annonacea. It is said to consist 160 species of archaic shrubs and saplings, which grow in the shaded regions of primary rainforest located in tropical Asia. Numerous of the *Goniothalamus* species have been utilized for timber, as sources of fiber, for decorative ornaments as well as medicinal purposes: especially in relation with post-partum and abortion [5]. *Goniothalamus sesquipedalis* is of Indian origin [9]. Only 6 species out of 160 of *Goniothalamus* have been applied as ethno medicines in Asia [10]. *Goniothalamus sesquipedalis* is moderately branched and grows to become 50 to 120 cm. Leaves are oval shaped with sharp ends. The flowers are usually greenish or yellowish in color and are solitary and axillary. The pedicles are 0.2 to 0.4cm in length. Calyx is made up of three sepals, each with length of 0.4 cm and shining interior. The corolla consists of two sequences of glabrous three petals [1]. Plants of this family have reported to hold substances with cytotoxic, abortifacient, antitumor, pesticidal, teratogenic and embryotoxic activities [2]. Some chemical constituents together with one new and two known phenanthrene lactams have been isolated from the leaves and twigs of *G. sesquipedalis* [8].

To the extent of our knowledge, little work has been done with *Goniothalamus sesquipedalis*. Thus, we have performed experiments to look into its antimicrobial and antioxidant properties.

Methods and Materials

Collection of plant materials

The leaf part of *Goniothalamus sesquipedalis* plant was collected in July, 2018 from Chittagong hill tract. After collection, the National Herbarium Bangladesh (NHB), Mirpur, and Dhaka authenticated the plant material and provided a plant identification number, which were 42930.

Preparation of the extract

At first, the leaves part was washed with fresh water to remove the unwanted dust particles and plant scrap. After that, the cleaned leaves were dried under the sun for a day. Then the leaves were again dried for 1 hour at 30-40°C in hot air oven. By using a high capacity grinding machine, the dry and crusty leaves were ground. After that, at a normal ambient temperature (22-25 °C) around 900 g of ground powder was soaked in 2.5 L of ethanol for a period of 2 days with occasional stirring. With the help of cotton filter (pore size: 110mm) filtration was done and rotary evaporator was used at 100 rpm at 30 °C to evaporate the maximum amount of solvent. For vaporizing the solvent completely from the extract, the leaf extract was kept under laminar airflow cabinet. Moreover, it was used to avoid any possibility of microbial growth in the extract while drying. Finally, 20.1 g of plant leaf extract was obtained and kept in dry and cool place and proper labeling was done. After that, this extract was used to conduct antioxidant and antimicrobial activity studies.

Chemicals

The chemicals were gallic acid [Sigma-Aldrich, USA], Folin-Ciocalteu reagent [Sigma-Aldrich, USA], 2,2-Diphenyl-1-picrylhydrazyl (DPPH) [Sigma-Aldrich, USA], sodium carbonate [Merck, India] and ascorbic acid (ASA) [Merck, India]. All the chemicals used in this study were of analytical grade.

Anti-oxidant activity

Total phenolic content (TPC)

The phenols were oxidized by Folin-Ciocalteu in ionic phenolic solution. When the solution became yellow to dark blue, it is understood that the oxidation has been completed. After that, this color changed mixture measured in 760 nm in UV spectrophotometer. Finally, the value of the absorbance plotted in gallic acid calibration curve and data was evaluated as gallic acid equivalents (GAE).

Total Flavonoid content

Aluminum chloride was used to determine the total amount of flavonoids. Firstly, 0.5 ml of plant extract was made up to 1 ml of final volume with reaction medium (MeOH/H₂O/CH₃COOH=14:5:1) which was then mixed with Aluminum chloride reagent (4 ml, 133 mg of AlCl₃ × 6 H₂O and 400 mg of CH₃COONa dissolved in 100 ml H₂O). After 5 minute, the absorbance was measured at 430 nm. Based on the calibration curve, total flavonoid content was calculated and it was expressed as gram equivalents.

DPPH free radical scavenging assay

The antioxidant activity *Goniothalamus sesquipedalis* was determined by performing DPPH free radical scavenging assay. To run this assay, different concentrations of plant extracts were mixed with 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. In methanol or aqueous solution, free

radicals were generated due to delocalization of the free electrons and a deep purple colored solution is produced. Then absorbance of different concentration solutions was measured at 517 nm in UV spectrophotometer. The decreasing value of DPPH at 517 nm is directly proportional to the radical scavenging activity.

Percentage of inhibition of DPPH free radical (1%) was calculated by using the following equation:

$$(1\%) = (\text{Absorbance of blank} - \text{Absorbance of sample}) / \text{Absorbance of blank} \times 100$$

50% of inhibition of the extract concentration was calculated from the graph and the percentage of inhibition was plotted against extract concentration.

Antimicrobial assay

Disc Diffusion Assay Method

In recent years, different studies are developing as antimicrobial agents to fight antibiotics resistance from different sources and highest concentration has given to screen and evaluate the antimicrobial activity. By using disc diffusion assay method, antimicrobial activity of *Goniothalamus sesquipedalis* was evaluated. *E. coli* bacteria (gram negative) and *Bacillus Subtilis* bacteria (gram positive) were used in this study. Mular Hinton agar (MHA) was used as media in this assay. Firstly, every petri dish was autoclaved for sterilization and 20 ml of MHA was poured in every petri dish. After that, the plates were kept for a time being to be settled. With the help of cotton swab, the nutrient broth of bacterial strains was incubated in MHA. Small disc of filter paper was made by using paper punch machine and then different concentrations of plant extract (200 mg/mL and 400 mg/mL) were used to swallow that filter paper. When the discs become dry, they were transferred to the petri dishes and kept in incubator for 24 hours at 37°C. After 24 hours the zone of inhibition were calculated and for keeping the contamination limited, whole experiment was done under laminar flow.

Result and discussion

Antioxidant activity

Total phenolic content (TPC)

In total phenolic content test, Gallic acid was used as standard and methanol extract of leaves which was used as a sample. The absorbance of the sample plotted in Gallic acid calibration curve. The absorbance of the plant extract was found 0.491 and TPC value was 60.70 GAE/g against that absorbance which indicates that the plant has antioxidant activity.

Total Flavonoid content

The content of total flavonoid of the plant extracts was measured spectrophotometrically by using the aluminium chloride colorimetric assay. The flavonoid content of the extracts was expressed as mg quercetin equivalent per gram of the extract and it is 209.35 QE/g against the absorbance of 0.401 that indicates the presence of flavonoid content.

DPPH free radical scavenging assay

It is known that DPPH free radical scavenging activity is increasing along with increasing concentration of the ethanol extract. As the reference standard, ascorbic acid was used in this experiment for which IC₅₀ value was 75.688 µg/ml. On the other hand, the IC₅₀ value of the methanol extract of the

sample plant was 501.62 µg/ml. this result indicates the presence of antioxidant activity which is less significant.

Table 1: Evaluation of DPPH free radical scavenging activity of ethanolic extract of *Goniothalamus sesquipedalis*

	R ² value	IC ₅₀
Standard	0.6277	75.688
Sample (ethanol extract)	0.4215	601.2

Table 2: Antimicrobial activity of ethanolic extract of *Goniothalamus sesquipedalis*

Group	Inhibition zone (mm)	
	Gram (-ve) bacteria (<i>E. coli</i>)	Gram (+ve) bacteria (<i>B. subtilis</i>)
Control	0	0
Standard	17.33333 ± 1.527525	22 ± 1
Plant extract (200mg/mL)	0	0
Plant extract (400mg/mL)	13.71 ± 1.15	17.23 ± 1.15

Conclusion

To conclude this can be said that *Goniothalamus sesquipedalis* ethanolic extract has potential antioxidant and antimicrobial activity. Further study can be done to explore the other medicinal use of this medicinal plant.

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