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Antibacterial Activity and Phytochemical Screening of *Goniothalamus sesquipedalis* (Wall.) Hook. f. & Thomson Extracts from Manipur, North East India

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SSN and KSP designed the experiment and managed the analysis of the study. Author SCK wrote the protocol, performed the experiment and wrote the first draft of the manuscript. Author SSN performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To screen the phytochemical constituents and study the antibacterial properties of the *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson used in the traditional medicine in the North East India.
Place and Duration of Study: Plant samples were collected from different parts of Manipur during May 2013 to February 2014. Experiments were performed at Department of Life Sciences, Manipur University, Canchipur, Imphal.
Methodology: Antibacterial activities were analyzed by well diffusion method against the pathogen *Bacillus subtilis* and *Escherichia coli* by using different concentrations of methanolic extracts. Phytochemical screening was performed on the extracts of different solvents viz. chloroform, ethanol, methanol, petroleum ether and water.
Results: Methanolic extract exhibited higher inhibition zones in *Escherichia coli* with 10.03, 12.01,

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13.04, 14.04, 15.03 and 16.04 mm as compared to *Bacillus subtilis* which showed 3.00, 4.04, 6.03, 7.04, 8.03 and 10.01 mm against extract concentrations of 20, 40, 60, 80, 100 and 120 µl respectively. Alkaloids, flavonoids and terpenoids were detected in all the solvents used. Glycosides were not detected in chloroform extracts while phenols and tannins were absent in water extract. Phytosterol and saponins were detected in ethanol, water and petroleum ether extracts.

Conclusion: The present study showed that the *Goniothalamus sesquipedalis* is potential source of antibacterial agents and reaffirms its importance in traditional medicine.

Keywords: Goniothalamus sesquipedalis; antibacterial activity; phytochemical screening; North East India; traditional medicine.

1. INTRODUCTION

Medicinal plants are important component in the traditional medicine which have been practiced in many developed and developing countries [1]. Plants associated with ethnomedicinal practices are the potential target for drug discovery programmes with the anticipation that these plants might possess bioactive compounds or potential lead compounds. However, intense studies of these ethnomedicinal plants are required to determine whether these usage are actually related to their medicinal properties or placebo effects associated with their folklore. For validating the traditional uses as well as providing insights in drug discovery programmes, biochemical screening of ethnomedicinal plants used by different communities remain significant.

The genus *Goniothalamus* Hk. f. et Thoms. (family: Annonaceae) consists of about 130 shrubs and tree species growing in the rainforest of tropical Asia [2]. This genus has wide distribution covering Eastern India, Myanmar, Bangladesh and Malayan archipelago [3-5]. Many species of the genus are used in fiber, timber source, ornamental and medicinal purposes in different countries. Species of this genus are known for wide ranging biological activities such as immunosuppressive and anti-inflammatory, anti-malarial, anticancer and larvicidal activity [6]. Root of *G. cheliensis* from the Yunnan province of China were used for treatment of cancer, malaria, edema, rheumatism and as pesticide [7,8]. Seeds of *G. amuyon* [9] from Taiwan was used in the treatment of edema and rheumatism. Stem bark decoction of *G. laoticus* in Thailand were used as febrifuge. In Malaysia, *G. scortechinii* was used in abortion, post-natal treatment and insect repellents [10] while *G. macrophyllus* was used in colds, fever, malaria, cholera and post-partum treatments [11,12]. Roots of *G. giganteus* were also used for treatment of colds, swellings and as abortifacient [2]. In Indonesia, an infusion of the roots of *G. tapis* was used to treat typhoid fever [13]. Out of 130-140 species of the genus, about thirty seven species of the genus have been studied for phytochemical and pharmacological properties [14]. Because of the presence of cytotoxic acetogenins and styryl-lactones in the genus, the various species are considered to be potential source of anticancer and antibacterial drugs [2].

In the North East India, various species of the *Goniothalamus* are available. Out of these species, *Goniothalamus sesquipedalis* (Wall.) Hook.f. & Thomson is one important species that is used for various utilities. This glabrous shrub is sparingly branched and extends up to 50 to 120 cm [5,15]. Leaves are oblong, acuminate, coriaceous and minutely pellucid punctuate [3,5,15]. The flowers are solitary and axillary, often greenish yellow in colour [3-5,15] with pedicels 0.2-0.4 cm long [5]. Flower buds are triquetrous and valvate. Calyx consists of three sepals, each with 0.4 cm length [3,5] and shining interior [15]. The corolla consists of two series of three petals which are glabrous [3]. The androecium comprises of many stamens [3] and the gynoecium consists of carpels which are ovoid, glabrous, very short stalked orange red with 5 in number varying from 3-4 or 8-10 [3,4,15]. Fruit orange red with short stalked, 3-4 or 8-10 cm, mucronate, granulate [3,5]. Ovaries are golden strigose, narrow, and cylindrically recurved [4]. Flowering and fruiting occur between May and September [5].

G. sesquipedalis has wide ranging applications in traditional medicine in the North East Indian region. Traditional applications ranged from cough and urinary problems [16], insecticide and blood purifier [17], sleep inducer and asthma [18], post-natal treatments [19] to leucorrhoea [20]. Moreover, *G. sesquipedalis* along with another species of Lamiaceae, *Isodon ternifolius* were used in Manipur, one of the states of the

North East India, in pre and post-natal care as well as in traditional fumigation (Fig. 1). Traditional people believed that both of these species have properties that act against pathogens and possess other disease causing materials. Considering the phytochemical and pharmacological properties of other species of the genus and the traditional uses associated with this plant, it is highly probable that *G. sesquipedalis* might have possess antibacterial properties. With this perspective, an attempt has been made to study the antibacterial properties of the *G. sesquipedalis* and characterize the phytochemical constituents.

2. MATERIALS AND METHODS

2.1 Plant Collection and Storage

G. sesquipedalis plants were collected from different parts of Manipur, India from May 2013 to February 2014. Plants were identified and deposited (Voucher No. SCK-012) in the Department of Life Sciences, Manipur University, Canchipur, India. The plant materials were washed properly and grind to powder after drying. Then, the materials were stored in closed containers at room temperature until used.

2.2 Preparation of Extracts

The extracts were prepared by soaking 5 g of the powdered materials in 50 ml each of different solvents, viz. chloroform, ethanol, methanol, petroleum ether and water for 24 hr. The extracts were then filtered using Whatman Filter Paper which was concentrated to half of the volume. Filtrates were centrifuged at 1000 rpm for 30 min. It is again filtered, concentrated to dried residue and then stored in airtight containers separately till use [21].

2.3 Phytochemical Analysis

Extracts of different solvents were analyzed for the detection of various constituents. Test for alkaloid was done by using Dragendroff and Wagner Reagent method [21], Glycosides Salkowski's Test was also performed to determine the presence of phytosterol and terpenoids [22]. Froth Test and Foam Test were conducted for the presence of saponin with slight modification [21]. Estimation were also done for the presence of glycosides [1,23,24], flavonoids, phenol and tannins [25]. Estimations were done in triplicates.

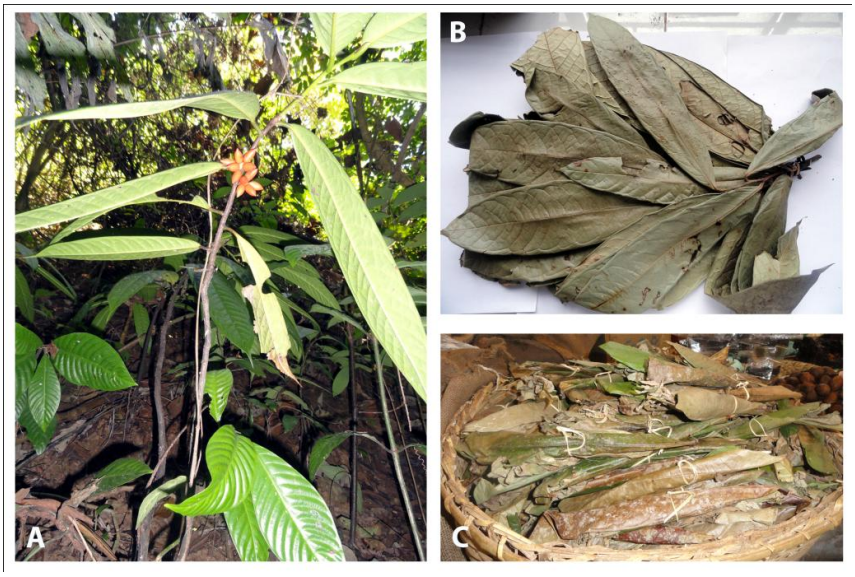

Fig. 1. (a) *Goniothalamus sesquipedalis* growing in the wild, (b) Dry leaves of *G. sesquipedalis* and (c) dry leaves of *G. sesquipedalis* and *Isodon ternifolius* selling in the market of Manipur, North East India

2.4 Bacterial Strains

The antibacterial activity of *G. sesquipedalis* leaf extract was tested against gram positive *Bacillus subtilis* (MUBS052) and gram negative *Escherichia coli* (MUEC045). The strains were provided by the Plant Pathology Laboratory, Life Sciences Department, Manipur University and were maintained in the Mueller Hinton agar medium at 4ºC. Inoculums were prepared by growing cells in Mueller Hinton broth (MHB) for 24 hours at 37ºC.

2.5 Determination of Antibacterial Activity

The antibacterial activity was evaluated in the methanolic extracts by the agar well diffusion method using Muller-Hinton agar plates [26]. The agar plates were swabbed with *B. subtilis* and *E. coli* by using sterile cotton swab and wells of 6 mm diameter were punched in each plate using a sterile cork borer. Then, 0.3 g of dried methanolic extract was dissolved in 1.5 ml of DMSO (dimethyl sufoxide) which served as the stock solution. The extracts were then transferred into the wells with different concentrations of 20, 40, 60, 80, 100 and 120 µl and incubated at 37°C for 24 h. After incubation, the diameter of the zone of growth of inhibition of the plant extracts were compared with that of DMSO as negative control [26,27].

2.6 Statistical Analysis

Experiments were carried out in triplicates and results are expressed as the mean ± SD (standard deviation). ANOVA tests were performed to test the significance difference between the different means at p=0.05. Statistical analyses were done in SPSS Version 19.0.

3. RESULTS AND DISCUSSION

Methanolic extract of the leaves of *G. sesquipedalis* have been tested against the *B. subtilis* and *E. coli*. The results from the present study showed that the extracts have antibacterial activities against both tested organisms. However, the activities differed according to the tested organisms and the concentrations of extracts. Methanolic extract showed less activities in gram positive *B. subtilis* as compared to gram negative *E. coli* (Table 1).

Methanolic extract of the *G. sesquipedalis* inhibited the growth of two tested organisms in dose-dependent manner (Fig. 2). It was observed that zone of inhibition at different well increased markedly corresponding to drug concentration. These inhibitions were observed to significantly different at p = 0.05 in both the organisms.

Table 1. Antibacterial activity of methanolic extract of *G. sesquipedalis* leaves against *E. coli* and *B. subtilis*

Organisms	Zone of Inhibition (in mm)
Escherichia coli	10.03 ± 0.05
Bacillus subtilis	3.00 ± 0.01

The results of phytochemical screening of *G. sesquipedalis* showed the presence of alkaloids, flavonoids and terpenoids in all the extracts used (Table 2). Glycosides were detected in all extracts except in chloroform. Phenols and tannins were not detected in water extract. Phytosterols and saponins were found to be present in ethanol, water and petroleum ether extracts.

In the present study, methanolic extract had been used for testing the antibacterial properties of the species. This organic solvent has been selected because of its capability of solubilizing the various active components belonging to alkaloids, flavonoids, glycosides, phenols, tannins and terpenoids [28]. The *G. sesquipedalis* methanolic extract has shown to exhibit antibacterial activities against two test organisms. These bioactivities can be compared to similar activities reported in other species [2,29]. Methanolic extracts of *G. scortechinii* exhibited mean zone of inhibition 20 mm and 13 mm against *Bacillus sp.* and *Escherichia coli* in Disc diffusion method [2], while *G. umbrosus* exhibited mean zone of inhibition 15 mm in dichloromethane extract [6] while using 20 mg/ml of the extracts.

Application of plant extract with known antimicrobial properties are significant for further therapeutic treatments. Antimicrobial activities of the *G. sesquipedalis* could be ascribed to the presence of different compound such as goniopedaline, aristololactam A-II, taliscanine, aurantiamide acetate, beta sitosterol [30]. Among the phytochemicals present, alkaloids were known for antibacterial properties in many plants. Presence of alkaloids were also characterized from other species of the genus such as *G. griffithii* [31] and *G. laoticus* [32] which also exhibited antimicrobial properties.

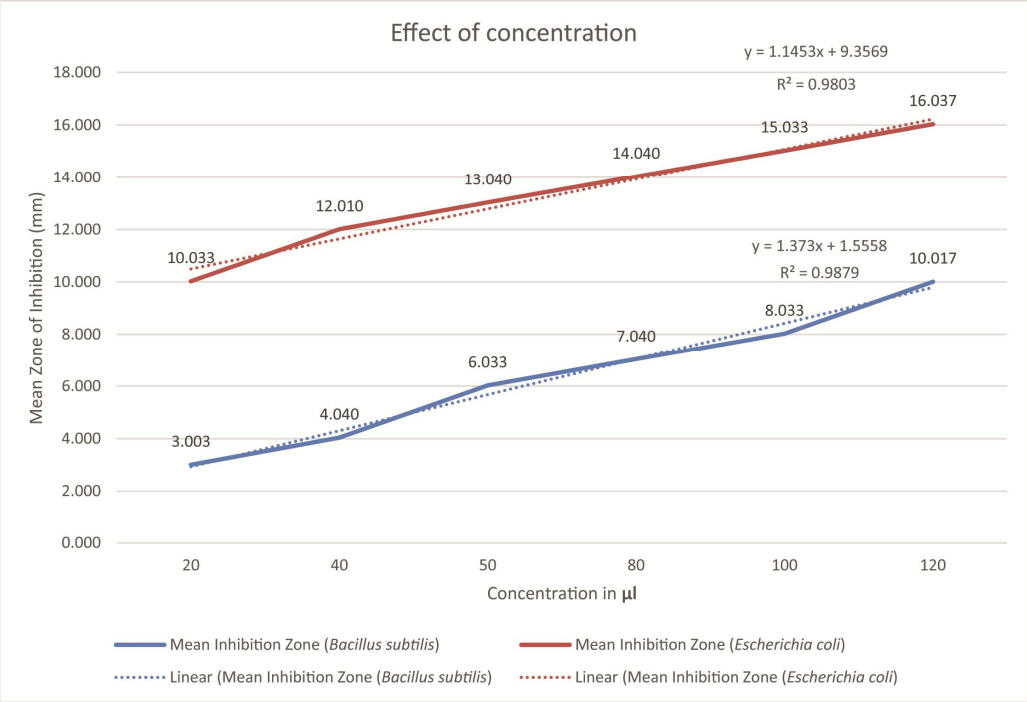

Fig. 2. Effect of concentration of crude extract on inhibition zone

Table 2. Phytochemical groups present in different extracts of *G. sesquipedalis*

Solvents	Alkaloids	Flavonoids	Glycosides	Phenols	Phytosterols	Saponins	Tannins	Terpenoids
Ethanol	+	+	+	+	+	+	+	+
Water	+	+	+	-	+	+	-	+
Methanol	+	+	+	+	-	-	+	+
Petroleum ether	+	+	+	+	+	+	+	+
Chloroform	+	+	-	+	-	-	+	+

Presence of flavonoids in the methanolic extract might be another result as these compounds are known to possess anti-allergic, anti-inflammatory, anti-microbial and anti-cancer properties [33]. Flavonoids can act as bacteriostatic compounds by restricting the number of viable colonies and can also act as energy metabolism inhibitor. Terpenoids present in the methanolic extract might have also contributed to the antibacterial properties as these compounds are active against bacteria and fungi [2].

With regard to antibacterial property, one compound altholactone, a styryl-lactone isolated from *G. mayalayanus* was observed to be effective against both Gram positive and Gram negative bacteria [34]. This compound is yet to be isolated from *G. sesquipedalis*, though many species of this genus are believed to possess different compounds belonging to stylryl-lactone [35,36]. Exact mechanism of the antibacterial activity in this species are yet to be confirmed as there are chances of synergistic relationship between different compounds [29].

4. CONCLUSION

The present study affirmed the antibacterial activity of *G. sesquipedalis* species growing in North East India. These antibacterial activities which might be the reason for preference of this species as fumigants in traditional post-partum management. The study also highlighted the possible utilization of this species in future drug discovery processes from the natural resources from Manipur. Future direction calls for isolation and characterization of the individual components present in this species for drug discovery processes.

ETHICAL APPROVAL

No human and animal subjects were used in the experiment. Authors have followed National and International ethical standard while performing the experiment and writing the manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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