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Studies on the phytochemistry and antibacterial efficacy of *Gardenia latifolia*

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Abstract
The bark of *Gardenia latifolia* Ait. An important medicinal plant was analysis of phytochemical Screening and antibacterial investigation. The Preliminary phytochemical studies of bark extracts reflect the presence of the bioactive compounds such as alkaloids, flavonoids, phenolic compounds, saponins, steroids and tannins in the bark. Methanol extracts of stem bark showed significant antibacterial activity against *Bacillus subtilis*. The methanolic extract of the plant was found to be inactive against the bacteria tested. This might be due to the selection of the solvent system. Bioactive compounds responsible for these antimicrobial activities could be isolated and identified to develop a new drug of pharmaceutical interest.

Keywords: *Gardenia latifolia*, antibacterial activity, methanolic extract

Introduction
India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants [1]. Plants have been found to contain medicinal components with potentially important therapeutic uses against bacteria, fungi and viruses [2]. The use of phytochemicals as natural antimicrobial agents, commonly referred to as “biocides”, is gaining popularity [3]. The most important of these bioactive plant components are alkaloids, tannins, flavonoids and phenolic compounds. Many indigenous medicinal plants are used as spices and foods. Plant constituents have found applications as natural antimicrobial agents in the fields of preservation, medicine, and phytopathology [4]. The ineffectiveness of chemotherapy and the increasing antibiotic resistance of pathogenic microbial agents have led to the screening of medicinal plants for their potential antimicrobial activity. There are several reports on the antimicrobial activity of crude extracts obtained from plants [5]. Some of the active constituents of bioactive compounds are preferred for therapeutic purposes as single entities or in combination to inhibit microbial life processes [6]. Most industries have recently focused on utilizing natural materials for preservation. According to world health organization, more than 80% of the world's population relies on traditional medicines to meet their basic health needs. The medicinal value of plants lies in certain chemical components that have specific physiological effects on the human body [7]. The most important raw material of traditional medicine is obtained from medicinal plants. Plants have existed for thousands of years and are highly valued all over the world as a rich source of medicinal plants to fight diseases and illnesses [8]. Every plant is like a laboratory that can produce unlimited number of medicine components are present. This medicinal components are very complex and presently the research on this work never done till date. The structure of these components will forever remain beyond our imagination. This plant based medicinal component are very effective for Antibacterial, antioxidant, anticancer, antimalarial, immunomodulatory and other activities. The plant shows the phytochemical substances such as volatile oils, alkaloids, flavonoids, terpenoids and phenolic compounds etc. This leads to the elimination of the disease. Today, plants and herbs used in medicine are increasingly used for the prevention of diseases. This is due to the development of microbial resistance to antibiotics, as well as the emergence of negative side effects and high prices in the market. The development of resistance to many antibiotics leads to serious problems in the treatment of infectious diseases [9]. Therefore, this study aims to determine the preliminary phytochemical and antimicrobial properties of *G. latifolia* bark.

***Gardenia latifolia* Ait**

Rubiaceous plants extravagant a widespread range of chemical components. This family is well known since old centuries, as a source of alkaloids, which form the major bulk of chemical substances investigated so far. *Gardenia latifolia*, commonly known as Indian boxwood or Ceylon boxwood, is a small tree with dense leaves found throughout India, growing in deciduous forests along rivers [10]. It is a small deciduous shrub in the Rubiaceae family. It is native to tropical and subtropical regions of Africa, South Asia and Australia. It is found in the forests of Madhya Pradesh, Orissa and Haryana in India. It is also widely cultivated elsewhere, where it is valued for both its fruits and shade. The bark of the stem and fruit has been reported to be used to treat various diseases such as snakebites, skin diseases, colic, tooth decay, bleeding in humans, rheumatism, cuts, wounds, diarrhea, dysentery, and as a remedy for indigestion in children and transient fevers in livestock [11]. The fruit is used to make perfume for herbal cosmetic applications [12]. Various tribes in the Baragarh district of Odisha believe that the plant is useful in treating rheumatism. The pulp of the fruit is said to be crushed and used for breast diseases applied to the forehead as a fever reducer.

The fruit extract is also reported to be used in the treatment of snake bites, ulcers of the hands and feet, and abdominal pain.

The Gond tribe of the Bhandara district of Maharashtra consumes the powdered seeds along with Piper nigrum to regulate the irregular menstruation. Tribal communities in Bangladesh living in the Chittagong and Hill Tract regions use a decoction of the bark of the tree to treat dental caries.

Medicinal Properties of *G. latifolia*

G. latifolia is a little deciduous tree or huge shrub. Root is used as a remedy for treatment for indigestion in children. Fruits are used in effective action of the mammary glands. Pounded pulp is put on the forehead in fever. Stem and fruit is very effective for stomach pain. Fruit extract is applied to treat the snake bites, sores of hand and feet, stomach ache and wounds. For wound treatment, stem bark are cursed and boiled in the water and after obtained a saturated extract are applied to affected area of wound. Bark of this plant can used in skin diseases. The many components are present in bark and wood such as beta-sestorol, hederagenin, Me-esters of oleanic and gypsogenic acids. Root also present gardenins. Saponins are obtained from bark is used to decreased the formations of histamine and may be find to use in treatment of asthama. The available drug consisted expectorant properties did not very effective spasmolytic for effective treatment of asthma. The stem bark contains very effective components such as Hederagenin, D-mannitol, Sitosterol and Siaresinolic, episiaresinolic, Oleanolic and Spinosis acid. Considering the growing importance of medicinal plants, the need of the day as to promote more and better organized studies, the present investigation is designed to explore the preliminary phytochemical analysis of *Gardenia latifolia* of bark for analysis of their Antimicrobial properties.

Materials and methods

Collection of plant material

- Bark of *Gardenia latifolia* Ait was collected from plant. After collection of bark of *G. latifolia* were rinsed with running tap water followed by sterile distilled water to remove the dirt on the surface and cut into small pieces.
- After that dried at temperature not exceeding 35 to 50 °C and followed by the grinding using Herbs Grinding Machine. It was stored in desiccator till the further study.

Preparation of Extracts

For the selection of solvent systems for phytochemicals extraction from *G. latifolia* has been done using hexane, chloroform, ethyl acetate and methanol. The process has been same except solvents has to be change for evaluation of more phytochemicals present in different solvent.

- The powdered material was subjected to hot extraction with hexane/chloroform/ethyl acetate and methanol by the Soxhlet apparatus for 10h.
- The extraction was carried out for about 10 h and the extract was filtered through a cotton plug followed by what-man filter paper no. 1.
- The extract was then concentrated by evaporating the solvent bellow 45 °C temperature. The concentrated extract was stored at 4 °C until further analysis.
- After evaporation of the solvent, a concentrate was obtained which was designated as methanol crude extract of *G. latifolia* (MGL).

Antibacterial studies

Antibacterial study of the methanolic extract was performed on selected microorganism Such as gram-positive bacteria: *Bacillus subtilis*. Amikacin (500 mg) was the commercially available antibiotic used as standard medicine for the antibacterial study. An *in vitro* antibacterial activity of the extract was evaluated by determining the zone of inhibition. The zone of inhibition of the test samples was performed by disc-diffusion method.

Nutrient agar media are prepared in conical flask. To prepare the different concentration of the methanolic extract 1000, 500, 250, 150, 75 µg/ml by diluting the crude extract. Similarly we have prepared different concentration of the antibiotic (Amikacin) 1000, 500, 250, 150, 75 µg/ml respectively. Agar media was transferred to the petri dishes in order to allow to solidify. Broth culture of *Bacillus subtilis* was spread over the petri dishes containing nutrient agar. Petri dishes were divided in four quadrants. The sterilized discs were dipped into the different concentration of antibiotic and plant extract and placed into the pre-determined quadrant of Petri dishes one by one with a sterilized forceps. One Petri dish was kept blank for control. Plates were incubated for 24 hrs. The next day, we have measured the zone of inhibition in nm scale.

Phytochemical screening of *G. latifolia* extract

Among various solvents evaluated in the study, methanolic extract showed presence of alkaloids, saponins, glycosides, flavonoids and particularly phenols and terpenoids. In hexane, no compounds were present, while chloroform manifested the presence of phenols and flavonoids. Ethyl acetate showed presence of phenols, flavonoids, glycosides and terpenoids shown in Table 1.

Presence of majority compounds in methanolic extract implies that the solvent is having potential owing to its higher efficiency and solubility of phytochemical compounds. Hence, characterization of phytochemical compounds from *G. latifolia* has been done using methanol. Phenolic compounds are important class of secondary metabolites in plants that predominantly help in defense against pathogens, parasites, and predators.

Table 1: Preliminary phytochemical analysis of *G. latifolia* fruit extracts.

Solvent Extract	Phytochemicals					
	Alkaloids	Phenols	Flavonoids	Saponins	Glycosides	Terpenoids
Hexane	-	-	-	-	-	-
Chloroform	-	+	+	-	-	-
Ethyl acetate	-	+	+	-	+	+
Methanol	+	++	+	+	+	+++
Petroleum ether extract	-	-	-	-	-	+
Aqueous extract	+	+	+	-	-	+

"+" indicates the presence of constituents "-" indicates the absence of constituents

Antibacterial studies

The methanolic extract of *Gardenia latifolia* Ait. Showed inhibitory effects on the selected microorganism. Diameter of zone of inhibition for antibiotic Amikacin is 27.5, 20.5, 18.7 and 17.5 mm against the antibiotic concentration 2500, 250, 125 and 62.5 µg/ml respectively. The results are given in Table: 2. The methanolic extract showed zone of inhibition on *B. subtilis*, at 16.25, 14.9, 12.5 and 7.5 mm against concentration 1000, 200, 40 and 8 µg/ml respectively. The results are depicted in Table: 3. The results are graphically represented in Fig 1 and 2. The graph depicts that the zone of inhibition is proportionally dependent on the concentration of them thereby demonstrating its antibacterial activity.

Table 2: Zone of inhibition for antibiotic Amoxicillin in different concentrations against Bacillus subtilis.

Concentration of Amikacin (µg/ml)	Log Concentration of Amoxicillin (µg/ml)	Diameter of Zone of Inhibition (mm)
1000	5.6	27.5
500	2.4	21.2
250	2.0	18.4
150	1.8	17.5
75	0.8	9.2

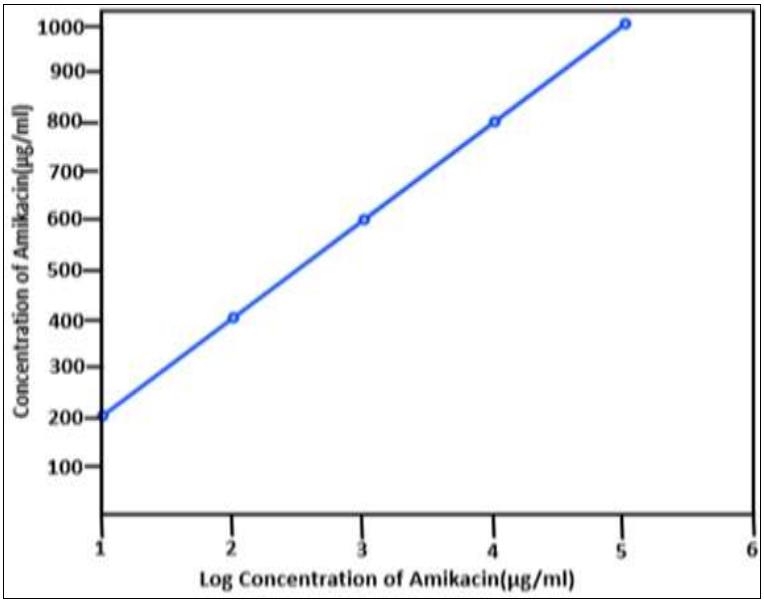

Fig 1: Represent the zone of inhibition Zone of inhibition for antibiotic Amoxicillin in different concentrations.

Table 3: Zone of inhibition for methanolic extract of *G. latifolia* Ait in different concentrations against *Bacillus subtilis*.

Concentration of *G. latifolia* Ait. (methanolic extract) (µg/ml)	Log Concentration of *G. latifolia* Ait. (methanolic extract) (µg/ml)	Diameter of Zone of Inhibition (mm)
1000	4.6	27.5
500	3.8	21.2
250	3.0	18.4
150	2.8	17.5
75	1. 8	9.2

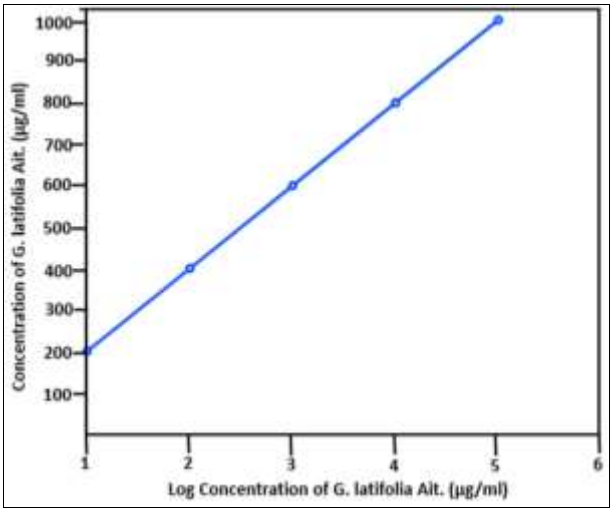

Fig 2: Represent the zone of inhibition Zone of inhibition for *G. latifolia* Ait in different concentrations.

Conclusions

The present study was carried out to detect the phytoconstituents in extract of *G. latifolia* Ait and the antibacterial efficacy of the methanolic extract of against gram-positive bacteria: *Bacillus subtilis*. Further study is required to isolate the constituents responsible for the antibacterial properties and find other pharmacological activities that can be utilized in new drugs for the therapy of various diseases. It is also essential to evaluate its activity against other gram-positive and gram-negative bacteria along with toxicity studies. The methanolic extract of the plant was found to be inactive against the bacteria tested. This might be due to the selection of the solvent system. However, selection of the dosage and solvent system is recommended for further studies against microbes. The study leads to a broader perspective for industries, in usage of a new class of preservative agent for food, pharmaceutical and leather industries.

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