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Screening of antibacterial properties of crude extract of *Gardenia latifolia* Ait. Bark

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Abstract
The bark of *Gardenia latifolia* Ait**.,** an important medicinal plant was subjected to phytochemical screening and antibacterial investigation. The Preliminary phytochemical studies of bark extracts revealed 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 bioactive compounds responsible for these antimicrobial activities could be isolated and identified to develop a new drug of pharmaceutical interest.

Keywords: *Gardenia latifolia* Ait., antibacterial activity, *Bacillus subtilis*

1. Introduction
The most important source of raw materials for traditional medicine is obtained from the medicinal plants. Plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments [1]. Each plant is like a laboratory capable of synthesizing unlimited number of chemical substances. The substances are highly complex and the structures of such compounds if not studied otherwise would escape our imagination forever. The medicinal activity like antimicrobial, antioxidant, anticancer, antimalarial, immunomodulatory, etc. shown by plants is basically due to the presence of an array of bioactive constituents like essential oil, alkaloids, flavonoids, terpenoids, phenolic compounds etc. These bioactive substances have been found to produce definite physiological action on the human body and hence lead to the elimination of the ailment [2, 3].

The use of plant and plant derived substances to fight against microorganisms is now on the increase. This is due to the development of resistance against chemical antibiotics by microbial strains accompanied with occurrence of undesirable side effects along with high price prevailing in the market. The development of resistance to many antibiotics has led to serious clinical problems in the treatment of infectous diseases [4]. This in turn has prompted the search for antibiotics which largely depends on medicinal plants as raw materials [5]. Therefore, this study is aimed at determining the Preliminary phytochemical screening and antibacterial properties of *G. latifolia* bark.

1.1. *Gardenia latifolia* Ait
belonging to the family Rubiaceae is a small deciduous ornamental tree. It is native to tropical and subtropical regions of Africa, Southern Asia and Australia. It is found in the forests of Madhya Pradesh, Orissa in India and is widely cultivated elsewhere where the tree is highly valued for both its fruit and shade. Different parts of *G. latifolia* are used traditionally for the treatment of various ailments like rheumatism, cuts, wounds, diarrhea, dysentery, and remedy for indigestion in children etc. [6]. The different tribes of Baragarh district of Odisha believe that the plant is good for treating rheumatism [7]. Pounded pulp of the fruits is reported to be used in affections of the mammary glands and is also applied to forehead in fever. Fruit extract is also reported to be used in treating snake-bite, sores of hand as well as feet and stomach ache [8]. The Gond tribes of Bhandara district in Maharashtra consume the powder of the seeds along with *Piper nigrum* for regularizing irregular menstruation [9]. The tribal communities of Bangladesh living in Chittagong and Hill Tract districts use the decoction of the stem bark to treat dental caries [10].

2. Materials and method
2.1 Plant Material: The fresh bark was collected from the well grown and matured trees from Tirumala, Andhra Pradesh during March 2017.

The bark was authenticated by the Botanist Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Part of the fresh bark was shade dried, followed by milling in to coarse powder and stored in an airtight container till further successive extraction.

2.2 Test organisms: The bacteria cells used for this study were *Bacillus subtilis* (MTCC 441).It was collected from MTCC, Chandigarh.

2.3 Preparation of extracts: The dried powdered plant material was first defatted with petroleum ether (40 – 60^{0}C). It was then successively extracted with chloroform, methanol and water using soxhlet apparatus for eight hours. The extracts were filtered and concentrated under reduced pressure to dryness [11.12].

2.4 Preliminary phytochemical studies: The chloroform, methanol and aqueous extracts were subjected to qualitative chemical tests. Standard procedures were used. The study was carried out to identify presence of different class of phytoconstituents in the bark sample [6, 13].

2.5 Antibacterial studies: Antibacterial study of the methanolic extract was performed on selected microorganism. The microorganism used for this study included gram-positive bacteria: *Bacillus subtilis*. Amikacin (500 mg) was the commercially available antibiotic used as standard for the study. An in-vitro antibacterial activity of the extract was evaluated by Determining the Zone of Inhibition [14].

2.5.1 Determination of Zone of Inhibition: The zone of inhibition of the test samples was performed by disc-diffusion method [15]. Conical flasks containing nutrient agar media, filter paper disks placed in separate conical flasks, test tubes plugged and autoclaved at 121 °C (249 °F) for around 15–20 minutes at 15 Psi. Different concentration of the methanolic extract 1000,200,40,8 µg/ml was made by diluting the crude extract just prior to the experiment. Different concentration of the antibiotic (Amikacin) 2500, 250, 125, 62.5µg/ml was also prepared freshly. Hot nutrient agar media was transferred aseptically to the petri dishes and were allowed 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 forcep. One Petri dish was kept blank for control. Plates were incubated for 24 hrs. The next day, zone of inhibition was measured in mm scale.

3. Result & discussion

3.1 Preliminary Phytochemical Studies: The different extracts of the bark were found to contain alkaloids, steroids, phenolic compounds, tannins, flavonoids and terpenoids. Alkaloids were found only in the methanolic extract. Saponins on the other hand were present in the aqueous extract. Steroids were present both in petroleum ether and chloroform extracts. Phenolic compounds, tannins and flavonoids were present both in methanolic and aqueous extract. Terpenoids were present both in petroleum ether and chloroform extracts. The results are given in Table 1.

3.2 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 concentration of them thereby demonstrating its antibacterial activity.

Table 1: Preliminary phytochemical studies of different extracts of *G. latifolia*

Test for	Petroleum ether extract	Chloroform extract	Methanolic extract	Aqueous extract
Proteins& amino acid	-	-	-	-
Alkaloids	-	-	+	-
Carbohydrates	-	-	-	+
Saponins	-	-	-	-
Steroids	+	+	-	-
Phenolic compounds	-	-	+	+
Tannins	-	-	+	+
Flavonoids	-	-	+	+
Terpenoids	+	+	-	-

+ = present, - = absent

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

Concentration of Amikacin (µg/ml)	Log Concentration of Amikacin(µg/ml)	Diameter of Zone of Inhibition (mm)
2500	5.3	27.5
250	2.4	20.5
125	2.0	18.7
62.5	1.8	17.5

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

Concentration of extract(µg/ml)	Log Concentration of extract (µg/ml)	Diameter of Zone of Inhibition (mm)
1000	3	16.25
200	2.3	14.9
40	1.6	12.5
8	0.9	7.5

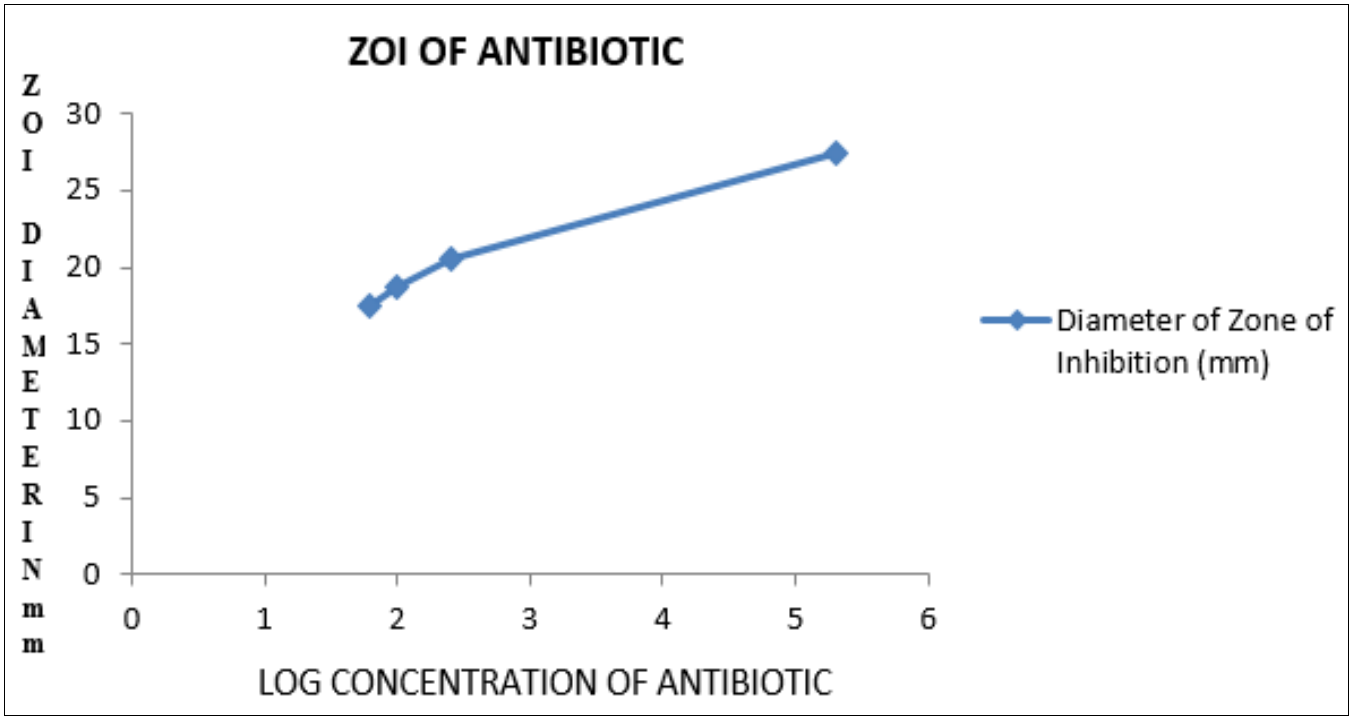

Fig 1: Zone of inhibition for antibiotic Amikacin against *Bacillus subtilis.*

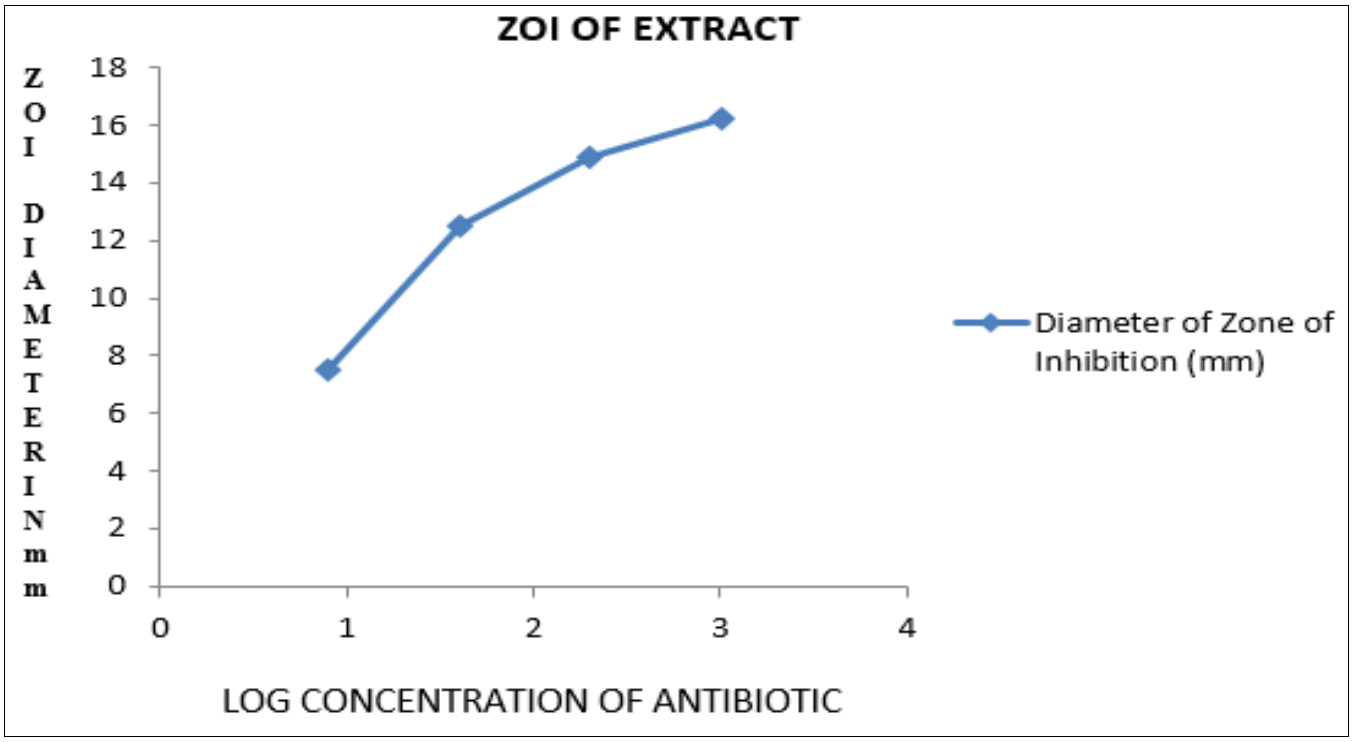

Fig 2: Zone of inhibition for methanolic extract of *G. latifolia* Ait. against *Bacillus subtilis*.

4. Conclusion

From the present study, the antibacterial activity of the bark of *Gardenia latifolia* Ait. is established 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.

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6. References

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