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# Synthesis, Spectroscopic Investigation of Phytochemicals for antibacterial efficacy of Gardenia latifolia

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## ABSTRACT

**Introduction**: Gardenia *latifolia*, commonly referred to as Indian boxwood or Ceylon boxwood, is a traditional medicinal plant historically used in India to treat various human diseases. From the past few decades it has been traditionally recognized that Gardenia *latifolia* acts as an effective agent in such as snake bites, skin issues, stomach pains, inflammatory discomfort, dental caries, bleeding in humans, and ephemeral fevers in livestock along with it acts as an excellent painkiller as well as an antiseptic for healing wounds. In the present study we investigated the phytochemicals of bark plant extract of Gardenia *latifolia* and its antibacterial activity.

**Materials and Methods**: The present work deals with synthesis and characterization of important organic constituents of Gardenia *latifolia*. The first step of synthesis process involves the collection of plant. For the selection of solvent systems for phytochemicals extraction from *G. latifolia* has been done using hexane, chloroform, ethyl acetate and methanol.

**Results**: Bioactive flavonoids, saponins, terpenoids and phenols have been isolated from the stem bark extract Gardenia *latifolia* for phytochemical investigation. Phytochemical structures were elucidated from obtained spectral data by FTIR spectra and UV-Visible spectra and anti-bacterial study. In FTIR Spectra shows the absorption bands pertinent to -OH stretching showed the presence of hydroxyl groups, the presence of phenolic compounds in methanolic and ethyl acetate extract of *G. latifolia.* UV-Vis spectroscopic confirms the presence of tannins and flavonoids in the methanolic and ethyl acetate extract of *G. latifolia*.

**Conclusion**: The methanol extract of *G. latifolia*, with bioactive compounds, is a promising source of lead compounds for future drug development.

***Keywords:*** *Phytochemical investigation, isolation of bioactive photochemical, methanol extract*

## 1. INTRODUCTION

India has one of the oldest, 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 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 phyto-pathology [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 traditionalmedicines to meettheir basic health needs. The medicinal value of plants lies in certain chemical components that have specific physiological effects on the human body [7]. Gardenia *latifolia*, commonly referred to as "Cape jasmine" or "Gardenia," is a member of the Rubiaceae family. This plant is indigenous to regions in Asia, Africa, and the Pacific Islands. Renowned for its aromatic blossoms,Gardenia *latifolia* has been utilized in traditional medicine for various therapeutic purposes. Gardenia *latifolia*, also known as Indian Boxwood, has a long history of use in traditional medicine for alleviating pain and treating various health issues. Its barks and bark have been utilized to

address ailments such as abdominal discomfort, fevers, and skin disorders. It is an excellent painkiller and acts as an antiseptic for healing wounds [8].

The most important raw material of traditional medicine is obtained from medicinal plants. 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, antibiotics are increasingly used for the prevention of microbial 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, urgent need to determine the preliminary phytochemical, spectroscopic characterization and antimicrobial properties of G. *latifolia* bark.

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, Odisha and Haryana in India.

Gardenia *latifolia* bark of the stem 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 bark 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 bark is said tobe crushed and used for breast diseases and applied to the forehead as a fever reducer. The bark 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. The 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.

G. *latifolia* is a little deciduous tree or huge shrub. Root is used as a remedy for treatment for indigestion in children. Barks are used in effective action of the mammary glands. Pounded pulp is put on the forehead in fever. Stem and bark is very effective for stomach pain. Bark 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. [13, 14]. 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 [15-16]. Therefore, this study reported the preliminary phytochemical, spectroscopic characterization and antimicrobial properties of G. *latifolia* bark.

## 2. MATERIALS AND METHODS

The present work deals with synthesis and characterization of important organic constituents of Gardenia *latifolia*. The synthesis process as follows:

### Collection of Plant Material

Bark of Gardenia *latifolia* Ait was collected and 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 air 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

Solvent based 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 Watman 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 gummy concentrate was obtained which was designated as methanol crude extract of G. *latifolia*. Investigation of separation of phytochemicals has been evaluated by FTIR, UV-Visible spectra and anti-bacterial studies.

### Phytochemical Screening of *G. latifolia* Extract

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.

**Table 1- Preliminary phytochemical analysis of G. *latifolia* extracts**

| Solvent Extract | Phytochemicals | | | | | |
|---|---|---|---|---|---|---|
| | Alkonides | Phenols | Flavonoids | Saponins | Glycosides | Terpenoids |
| **Hexane** | - | - | - | - | - | - |
| **Chloroform** | - | + | + | - | - | - |
| **Ethyl acetate** | - | + | + | - | + | + |
| **Methanol** | + | ++ | + | + | + | +++ |

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

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*hasbeen done using methanol. Phenolic compounds are important class of secondary metabolites in plants that predominantly help in defense against pathogens, parasites, and predators.Researchers reported in several papers that the phenolic compounds possess antioxidants, anti-bacterial, anti-atherosclerotic, anti-cancer, anti-viral and anti-inflammatory activities. Supplementing phenolic rich diet reduces the risk of cardiovascular disease. Flavonoids showed anti-allergic, anti-inflammatory, anticancer, antithrombotic, antimicrobial, antiviral, and hepato-protective properties owing to their ability in scavenging the free radicals effectively. Terpenoids have been reported with antibiotic, antiseptic, anti-helminthic and insecticidal activities.

**Instrumentation**

***Ultraviolet Visible Spectrophotometer*** - One g of plant powder was boiled with 10 mL of distilled water and then filtered. An aliquot of the filtered sample was scanned using UV- Visible Spectrophotometer (Thermo Fisher Scientific**)**, at a range of 200-800 nm, to detect the characteristic wavelength of the plant extract.

***Fourier Transform Infra-Red Spectrometer*** - The plant sample was dried at 40°C and ground to fine powder through mortar and pestle. The sample was mixed with KBr (FT-IR grade) at a ratio of 1:100 and pressed to a pellet. The pellet was immediately put into the sample holder of Perkin Elmer Spectrophotometer (Spectrum RX1, FT-IR V.2.0) and operated in the range 4000–480 $cm^{-1}$ Antibacterial activity. From the spectral data obtained, the functional groups were detected.

**Antibacterial studies-**

Antibacterial study of the methanolic extract was performed on selected microorganism such as gram-positive bacteria: *Bacillus subtilis* and gram-negative bacteria: *E coli*. 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. Preparation of different concentration of the methanolic extract 1000, 500, 250, 150, 75 µg/ml was done 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 forcep. 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.

## 3. RESULTS AND DISCUSSION

**FTIR Analysis** -. Figure 1 displays the FTIR spectra of the extracts of methanol and ethyl acetateused to identify the functional groups of the phytoconstituents present in the extract on the basis of peak value in the infrared region (**Table 1**). The extract of *G. latifolia* presents the characteristic of absorption bands at 3274 $cm^{-1}$ (-OH stretching), 2923 $cm^{-1}$ and 2457 $cm^{-1}$ (aromatic -CH stretching), 1520 $cm^{-1}$ (C=O stretching), 1242 $cm^{-1}$ (C=C stretching), 1047 $cm^{-1}$ (C-O-C stretching

vibration). The characteristic absorption band at 3285 $cm^{-1}$ pertinent to -OH stretching showed the presence of hydroxyl group, which is common in all phenolic compounds and implies the presence of phenolic compounds in methanolic and ethyl acetate extract of *G. latifolia*.

Table 1. FTIR values and functional groupsbark extract of G.*latifolia*

| **Peak Values ($cm^{-1}$)** | **Functional Groups** |
|---|---|
| 3285 | -OH stretching |
| 2923 | Aromatic -CH stretching |
| 2457 | Aromatic -CH stretching |
| 1520 | C=O stretching |
| 1242 | C=C stretching |
| 1047 | C-O-C stretching vibration |

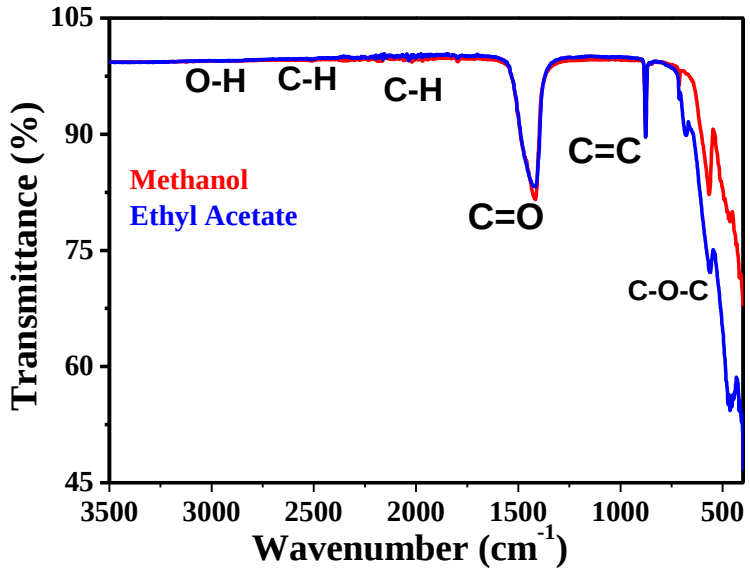


**Fig 1. FTIR spectrum of methanolic and ethyl acetatebark extract of *G. latifolia***

**UV-VIS Spectra-** In the UV-Vis spectra of *G. latifolia*extract were recorded in two extract methanol and ethyl acetate in the region from 200 to 800 nm (**Figure 2**) .The absorption spectra have large number of bands. These bands were centered at 280, 422, 450, 541, 650, 810 and 858 nm, respectively. This confirms the presence of organic chromospheres within the extract of *G. latifolia*extract. Two major bands were recorded at 280 and 422 nm with absorbance values of 0.8 and 0.9, respectively. The spectra for phenolic compounds (tannins) and flavonoids typically lie in the range of 280 - 422 nm. The result of UV-Vis spectroscopic analysis confirms the presence of tannins and flavonoids in the methanolic and ethyl acetate extract of *G. latifolia*. Nevertheless, the use of UV-visible spectrophotometry in the analysis of complex media is limited by the inherent difficulties in assigning the absorption peaks to any particular constituents in the system.

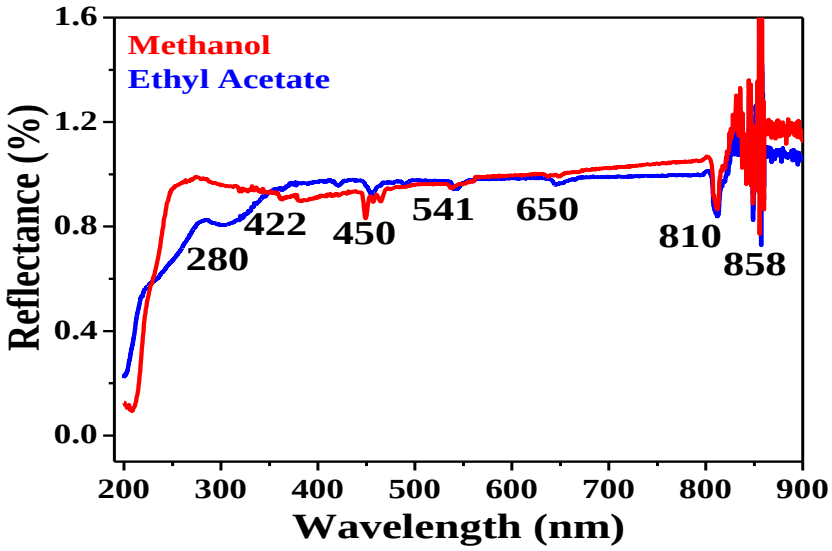


**Fig. 2 UV-VIS spectrum of methanolic and ethyl acetatebark extract of *G. latifolia***

**Antibacterial Studies**

The methanolic extract of Gardenia *latifolia* Ait showed inhibitory effects on the selected microorganism gram-positive bacteria: *Bacillus subtilis* and gram-negative bacteria: *E coli*. The zone of inhibition has been measured by Disc Diffusion Method. Diameter of zone of inhibition for antibiotic amikacin was 27.5, 20.5, 18.7and 17.5 mm against the antibiotic concentration 2500, 250,125 and 62.5 µg/ml as shown in Table 2. The results are given in Table3. The methanolic extract showed zone of inhibition on *B.subtilis*, at 16.25, 14.9, 12.5 and 7.5 mm against concentrations 1000, 200, 40 and 8 µg/ml, respectively (Table 4). The methanolic extract showed zone of inhibition on E. coli at 18.45, 15.4, 13.3 and 5.5 mm against *E coli*concentrations 1000, 200, 40 and 8 µg/ml, respectively. The results are depicted in Figure 3.The graph representsthat the zone of inhibition is proportionally dependent on concentration of them thereby demonstrating its antibacterial activity shown in Figure 4 & 5.

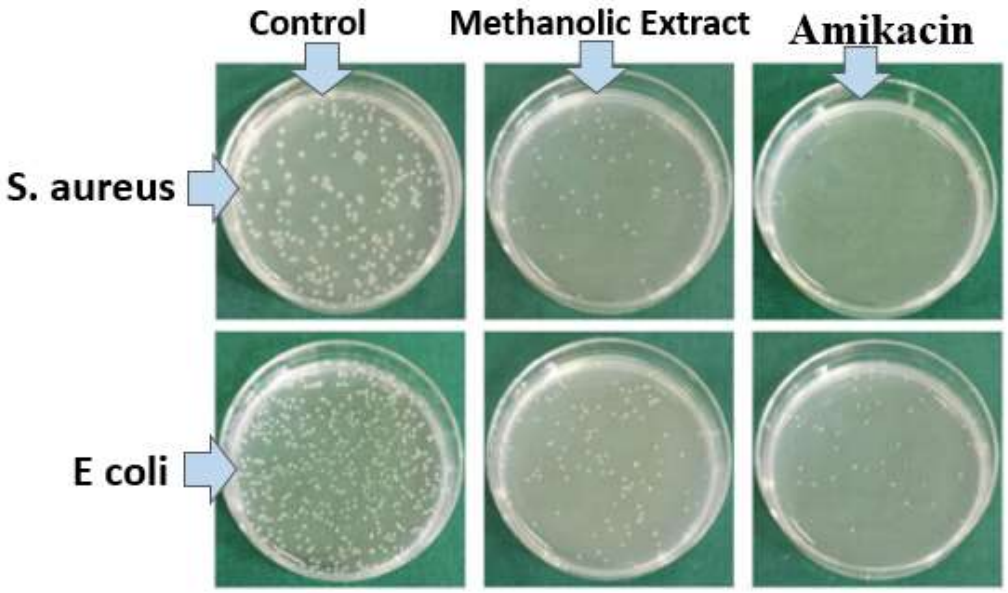


**Fig. Antibacterial properties of Gardenia *.latifolia* Ait**

**Table 3: Zone of inhibition for antibiotic Amokacin at different concentrations against *Bacillus subtilis***

| **Concentration of** Amokacin **(µg/ml)** | **Log Concentration of** Amokacin **(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 |

**Table 4: ZZone of inhibition for methanolic of G. *latifolia* Ait at different G. *latifolia* Ait extract 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 | 28.1 |
| 500 | 3.8 | 23.4 |
| 250 | 3.0 | 19.4 |
| 150 | 2.8 | 14.5 |
| 75 | 1. 8 | 10.2 |

**Table 5: Zone of inhibition for methanolic of G. *latifolia* Ait at different G. *latifolia* Ait extract concentrations against against *E coli***

| **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 | 25.9 |

| | | |
|---|---|---|
| 500 | 3.8 | 20.8 |
| 250 | 3.0 | 17.1 |
| 150 | 2.8 | 12.2 |
| 75 | 1. 8 | 7.9 |

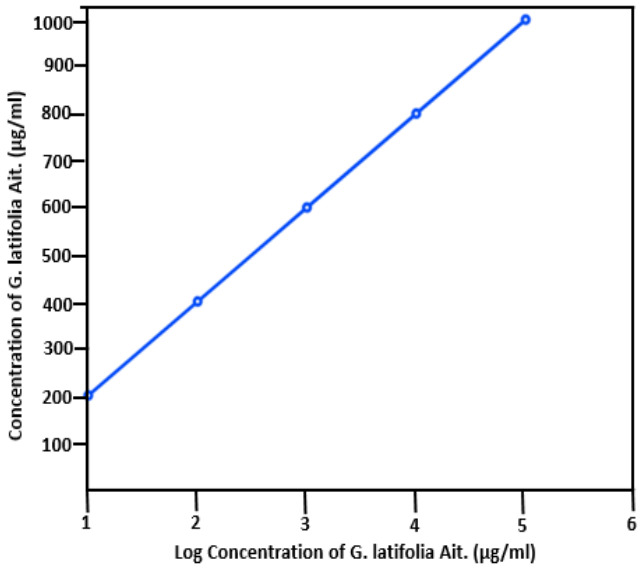


**Fig 4. Zone of inhibition for antibiotic *Amoxicillin* different concentrations against *S.***

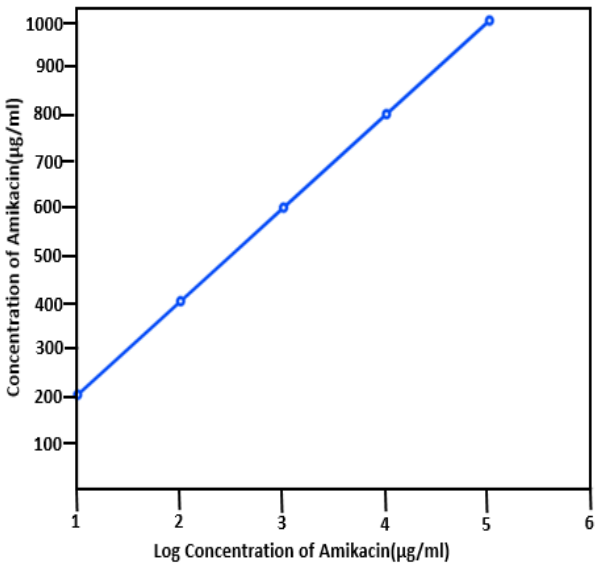


**Fig 5. Zone of inhibition for G. *latifolia* (Amikacin) at Ait at different concentrations against *aurens S.aurens***

## 4. CONCLUSION

The present study concluded that the phytochemical composition of the Gardenia *latifolia* extracts varied depending on the solvent used for extraction. In particular, the total flavonoid and terpenoids content varied extensively with different solvents. Methanol extracts of Gardenia *latifolia* bark stem contained more unique compounds than extracts obtained with the other solvents. The characterization of G. *latifolia* Ait was investigated such as FTIR Spectra, UV-Visible Spectra in methanol and ethyl acetate extract, but promising results are obtained in Methanol extract. In FTIR Spectra shows the absorption band at 3285 $cm^{-1}$ pertinent to OH stretching showed the presence of hydroxyl group, which is common in all phenolic compounds and implies the presence of phenolic compounds in methanolic and ethyl acetate extract of *G. latifolia.* A similar result as presented in UV-Vis spectroscopic analysis confirms the presence of tannins and flavonoids in the methanolic and ethyl acetate extract of *G. latifolia*. The methanol extract of *G. latifolia*showing promising result, we have chosen this for further study.Further studied the isolate constituents, responsible for the antibacterial properties against gram-positive bacteria: *bacillus subtilis*and gram-negative bacteria *E. coli*. The methanolic extract of the plant was found to be inactive against the bacteria tested. Thus, this study affords a good foundation for further investigations of the biochemical and phytochemical functions of the various compounds identified, and the findings propose that the methanol extract using bark of *G. latifolia is novel study and showed that*, it has numerous bioactive compounds, is a promising source of lead compounds for future drug development.

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