Phytochemical Analysis and Antimicrobial Activity of Leaf and Stem Extracts of *Priva cordifolia* (L.f.) Druce

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Authors’ contributions

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ABSTRACT

In current environment increasing resistance to existing antimicrobial agents and herbal drugs are being investigated as important sources for new agents for testing various diseases related to bacterial infections. The present work is aimed at exploring the preliminary phytochemical screening and antimicrobial activity of leaf and stem extracts of *Priva cordifolia* belongs to family Verbenaceae. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, anthraquinones and glycosides. The antimicrobial activity was studied using various organisms by means of a disc diffusion method. Susceptibility of some Gram positive organisms (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative organisms (*Salmonella typhi* and *Escherichia coli*) were tested against leaf and stem extracts (water, methanol and ethanol). Among the various extracts, water stem extract was more effective (19.66±1.52 mm) and ethanol stem extract found to be less effective (1±1) against all the bacteria. It is concluded that the plant extract showed antimicrobial activity because of the presence of these phytochemicals. Further studies are recommended for drug development to treat various infectious diseases.

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1. INTRODUCTION

Preliminary screening of phytochemical is an important step in the finding of the bioactive principles present in medicinal plants and next may lead to drug discovery and development. Medicinal plants and herbal preparations are now a day’s gaining interest in the scientific communities due to their pharmacological actions and affordability makes them effective in control of various diseases. India has been considered as a source for large number of medicinal and aromatic plant species [1]. The Ministry of environment and forests has identified and documented over 9500 plant species as important for pharmaceutical industry. Medicinal and aromatic plants species are still gathered and collected from natural areas only a few are cultivated.

Plants have been used for medicinal functions long before prehistoric period [2]. Ancient Unani, manuscripts, Egyptians papyrus and Chinese writings described the use of herbs [3]. A conventional system of medicine continues to be extensively practiced on many accounts. Population rises, inadequate supply of drugs, prohibitive cost of treatment, side effects of several synthetic drugs and development of resistance to drugs for infectious diseases have led to the increased importance on the use of plant material as a source of medicines for a number of human ailments.

The potential of higher plants as a source of new drugs is still largely underutilized. It is estimated that among the 2,50,000-5,00,000 plant species, only 15-20% has been examined phytochemically. Thus, any phytochemical investigation of a plant will disclose only a narrow spectrum of its components. Historically pharmacological screening of compounds from natural or synthetic origin has been the source of numerous therapeutic agents. Random screening as a tool in ascertaining new biologically active molecules has been active in the area of antibiotics. Currently, different to common belief, drugs from superior plants continue to reside in an important niche in modern medicine. On a global basis, at least 130 drugs, all chemical entities extracted from higher plants, or modified further incorrectly, are currently in use, even if some of them are now being made unnaturally for economic motives [4].

A broad range of medicinal plant components is used to extract natural drugs and they hold different medicinal properties. The different part used include root, stem, flower, fruit, twigs exudates and modified plant organs. Whereas some of these natural drugs are composed in larger quantities and skilled in the market as the raw material for many herbal industries, even if hundreds of plant species have been tested for antimicrobial properties, the vast preponderance of plants have been effectively evaluated [5].

Aromatic plants are a class of plants used for their aroma and flavor. The aroma characteristics are due to the presence of volatile compounds known as essential oils. Many are exclusively used for medicinal purpose in aromatherapy as well as in various system of medicine. Aromatic plants also possess odorous volatile essences which occur as essential oil, gum exudates, balsam and oleoresin in one or more parts, namely root, wood, bark, stem, foliage, flower and fruits.

Aromatic and medicinal plants such as Aloe, Tulsi, Neem, Turmeric and Ginger cure several common ailments. These are considered as home remedies in many parts of India. Aromatic plants are considered as a rich resource of phytochemicals which can be used in drug development pharmacopoeial, non-pharmacopoeial or synthetic drugs. Some plants are considered as important source of nutrition and as a result of they are recommended for their therapeutic values. Some of the plants and their derivatives are measured as essential source for active ingredients which are used in aspirin and toothpaste.

Recently WHO (2006) estimated that 80% of people worldwide rely on aromatic and medicinal plants for their medicines for some aspects of their primary healthcare. According to the WHO 21,000 plant species have the potential for being used as medicinal plants. Resistance against antibiotics of abundant bacteria is accumulating; thus researches for inventive substances with antimicrobial activity have become an adamant necessary. Medicinal plants are frequently used in trendy medicine as therapy for many infectious diseases.

The employ of antimicrobial medicines to treat infection is identified as antimicrobial chemotherapy. The uses of substances with
antimicrobial property are common in practice since from 2000 years ago. “Ancient Egyptians and ancient Greeks” used specific molds and plants extracts to treat infection. Microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between bacteria. Plant antimicrobials fond potential agent to contract with the hazard of biological warfare [6] deliberation to finding of novel plant antimicrobials must be rewarded in these new anti-infective agents. Most of the antibiotics that were used in 1940’s and 1950’s is no longer in use because the resistance of bacteria to the antibiotics has increased [7].

The difficulty with microbial resistance is increasing and the position for the employ of antimicrobial drugs in the prospect is still doubtful. Therefore, actions must be taken to reduce this problem. For example, to control the use of antibiotics, develop research to better understand the genetic mechanisms of resistance, and to persist studies to develop new drugs, either synthetic or natural. The definitive goal is to propose suitable and competent antimicrobial drugs to the patient. For a extensive period, plants have been a precious source of usual products for continuing human health, particularly in the last decade, with more rigorous studies for natural therapies. The use of phytochemicals for pharmaceutical functions has regularly increased in country. The aim of this study was to Analysis on the plant extracts for presence of phytochemicals such as alkaloids, carbohydrates, glycosides, reducing sugars, proteins and tannins and as a valuable source of bioactive compounds.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves and stem of Priva cordifolia (Fig. 1) which are free from disease were selected from in and around Mysore city. Leaves were thoroughly washed 2-3 times in running tap water and once with sterile distilled water. The plant material was air dried on sterile blotter in shade at room temperature. After drying plant material was powdered using mechanical blender and stored in air tight containers for the further use.

Fig. 1a. Leaves of Priva cordifolia

Fig. 1b. Shade dried of leaves

Fig. 1c. Shade dried of stem
2.2 Preparation of Extracts

Dried and weighed plant material powder of plant material was soaked in 3 different solvents i.e. ethanol, methanol and water and kept for 24-48 hrs at 370C. The solvent extracts obtained were filtered using Whatman filter paper number 42 and solvents were removed under reduced pressure using vacuum drying and air dried.

2.3 Antibacterial Activity by Sterile Disk Diffusion Method

The methanol, ethanol and water extracts of the plant were subjected to antimicrobial activity assay, by disk method. The bacterial inoculum was spread uniformly on the sterile nutrient agar plates with a sterile swab moistened with the bacterial suspension. Each organic solvent extract of *Priva cordifolia* (10, 20, 30, 40, 50 µl from 100 mg/ml stock solution) was taken up in a micropipette separately and loaded on to the sterile disks. Disks impregnated with organic solvent extracts at different concentrations, positive control [standard streptomycin sulphate 50 µl (1mg/ml)] along with negative control were aseptically placed over bacteria seeded medium using a sterile forceps firmly. The plates were incubated in an upright position at 370C for 24 hrs and the zone of inhibition was measured and expressed in millimeters. Antimicrobial activity was recorded for each concentration of different extracts including positive control by measuring the zone of inhibition in diameter surrounding the discs. Each experiment was performed in triplicates.

2.4 Phytochemical Tests

Different extracts of *Priva cordifolia* were subjected to phytochemical analysis to detect the presence of chemical constituents. Chemical tests were carried out to identify the presence of Phytochemicals in methanol, ethanol and water extracts of *Priva cordifolia* using standard procedure of Ganesh et al. [7]. Qualitative test were conducted in the laboratory to confirm the presence or absence of Phytochemicals.

2.4.1 Test for tannins

**Iodine test:** Leaf and bark extract were treated with diluted iodine solution (1%). Appearance of transient red color indicates presence of tannins.

**Nitric acid test:** Leaf and bark extract were treated with diluted nitric acid. The formation of reddish to yellowish color indicates the presence of tannins.

2.4.2 Test for flavonoids

**Alkaline test:** A small quantity of extract was added to a lead acetate solution. After few a minutes- the appearance of a yellow color indicative of presence of flavonoids.

2.4.3 Test for alkaloid

**Mayer's test:** A few drops of Mayer's reagent to the solution. A white / creamy colour is obtained if alkaloids are present.

2.4.4 Test for carbohydrate

**Fehling's test:** The plant extract was dissolved in 5 ml of distilled water and filtered. The filtrate was treated with 1 ml of Fehling’s solution (A&B) and heat in a boiling water bath. A reddish orange color is obtained.

2.4.5 Test for reducing sugar

**Benedicts test:** Extract filtrates were treated with equal volume of Benedict's reagent. The mixture was boiled for 5-10 min in a water bath. Appearance of green-yellow / red depending on the concentration of reducing sugar present in solution.

2.4.6 Test for glycoside

0.2 g of extract is dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. Then 1 ml of concentrated sulphuric acid is slowly added. A brown ring obtained at the interface indicates the presence of glycosides.

2.4.7 Test for anthraquinone

About 0.5 g of extract was transferred into test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with an equal volume of 10% ammonia solution. A pink violet / red color in the ammonia layer indicates the presence of anthraquinone.

2.4.8 Test for proteins

**Biuret method:** Leaf and bark extract were treated with 1 ml NaOH (10%) solution and 1-2 drops CuSO4 solution is added. A violet color indicates the presence of proteins.
2.4.9 Test for saponins

0.5 g of the plant extract was vigorously shaken with water (10-15 ml) in a test tube and then heated to boiling point. Frothing was observed which was taken as a preliminary evidence for the presence of saponins.

3. RESULTS

In the present study the results were recorded for the antibacterial activity of Priva cordifolia.

3.1 Antibacterial Activity of Priva cordifolia by Disk Diffusion Method

The antibacterial activity of Priva cordifolia was carried out by disk diffusion method, against four bacterial strains viz., B. subtilis, S. aureus were gram positive E. coli and S. typhi gram negative bacteria.

Antimicrobial activity against all the bacteria was conducted using three different solvents namely methanol, ethanol and water extract. The inhibition zone of three different extracts of Priva cordifolia were observed at various concentration (10, 20, 30, 40, 50 µg/ml) after 24 hrs of incubation. No inhibition zone was noted in blank solvents i.e. negative control. An antibiotic Streptomycin was used as control; 0.1 g was dissolved in 15 ml sterile distilled water.

3.2 Antibacterial Activity of Priva cordifolia of Different Solvents against B. subtilis

In the methanol leaf extract, 50 µl concentrations showed high potential growth of inhibition zone of 15.33 mm and in stem extract 16.66 mm was recorded. In 40 µl concentration 11.33 mm in leaf extract and 8.33 mm in stem extract. In the lowest concentration i.e. 10 µl, the leaf extract showed 5.33 mm and in stem extract 11.66 mm inhibition.

In the ethanol leaf extract, 50 µl concentrations showed high potential growth of inhibition i.e. 13 mm, in 40 µl concentration 10.33 mm inhibition was observed. But in stem extract only 50 µl concentration showed the inhibition zone of 4.33 mm. In the water leaf extract, it showed maximum inhibition of 21 mm in 50 µl concentration and in the lowest concentration 10 µl showed 10.33 mm inhibition. Also in stem extract in 50 µl concentration 17.66 mm and in 10 µl it showed zero inhibition (Fig. 2 & Table 1).
Fig. 2. Antibacterial activity of Priva cordifolia against Bacillus subtilis

Table 1. Antibacterial activity of Priva cordifolia against Bacillus subtilis

| Sl. no | Extract used | Zone of inhibition in mm/conc µg/ml |
|--------|--------------|-------------------------------------|
|        | Positive control streptomycin | 10 | 20 | 30 | 40 | 50 |
| 1 Leaf | Methanol     | 23.66±0.57 | 5.33±0.57 | 10.33±0.57 | 10±00 | 11.33±0.57 | 15.33±0.57 |
| 2 Leaf | Ethanol      | 20.66±0.57 | 2.33±0.57 | 7±1 | 6.66±0.57 | 10.33±0.57 | 13±1 |
| 3 Leaf | Aqueous      | 23.33±1.15 | 10.33±0.57 | 7.83±0.28 | 7.66±0.57 | 9.66±0.57 | 21±1 |
| 4 Stem | Methanol     | 25.66±0.57 | 11.66±0.57 | 3.66±0.57 | 4.33±0.57 | 8.33±0.57 | 16.66±1.15 |
| 5 Stem | Ethanol      | 24.33±0.57 | 00±00 | 00±00 | 00±00 | 00±00 | 4.33±0.57 |
| 6 Stem | Aqueous      | 22.66±0.57 | 00±00 | 4.33±0.57 | 6±00 | 13±1 | 17.66±0.57 |

Note: Values are represented as mean diameter of zone of inhibition in triplicates ± Standard deviation

3.3 Antibacterial Activity of Priva cordifolia of Different Solvents against Staphylococcus aureus

In the methanol leaf extract, 50 µl concentrations showed the highest growth of inhibition of 8.66 mm and the same inhibition zone 40 µl concentrations. In the 10 µl concentration 2.33 mm inhibition was observed. In the stem methanol extract 50 µl concentration showed 7.66 mm, but in the 10 µl concentration no inhibition was recorded.

In the ethanol leaf extract, 50 µl concentrations showed high potential growth of inhibition zone which was 14.33 mm. In the 40 µl concentration the inhibition zone was 10 mm and in 10 µl concentrations zero inhibition was observed. In the ethanol stem extract of 50 µl concentration showed 10 mm inhibition, 40 µl concentrations showed 15 mm inhibition, 30 µl concentrations showed 15.66 mm inhibition, 20 µl concentration showed 10.66 mm inhibition and 10 µl concentration showed 11.33 mm inhibition of growth.

In the water leaf extract, 50 µl concentration showed potential growth of inhibition of zone of 20.66 mm. In 40 µl concentration the inhibition zone was 8.66 mm and in 10 µl concentration the zone of inhibition was 6.33 mm. In water stem extract the 50 µl concentration showed potential growth of inhibition zone of 27.66 mm. In the 10 µl concentration 19.66 mm growth of inhibition was recorded (Fig. 3 & Table 2).
Table 2. Antibacterial activity of Priva cordifolia against Staphylococcus aureus

| Sl. no | Extract used    | Positive control streptomycin | Zone of inhibition in mm/conc µg/ml |
|--------|-----------------|--------------------------------|-------------------------------------|
| 1      | Leaf Methanol   | 23.66±0.57                     | 5.33±0.57 10.33±0.57 10±00 11.33±0.57 15.33±0.57 |
| 2      | Leaf Ethanol    | 20.66±0.57                     | 2.33±0.57 7±1 6.66±0.57 10.33±0.57 13±1 |
| 3      | Leaf Aqueous    | 23.33±1.15                     | 10.33±0.57 7.83±0.28 7.66±0.57 9.66±0.57 21±1 |
| 4      | Stem Methanol   | 25.66±0.57                     | 11.66±0.57 3.66±0.57 4.33±0.57 8.33±0.57 16.66±1.15 |
| 5      | Stem Ethanol    | 24.33±0.57                     | 0±00 0±00 0±00 0±00 4.33±0.57 |
| 6      | Stem Aqueous    | 22.66±0.57                     | 0±00 4.33±0.57 6±00 13±1 17.66±0.57 |

*Note: Values are represented as mean diameter of zone of inhibition in triplicates ± Standard deviation*

3.4 Antibacterial Activity of Priva cordifolia of Different Solvents against Salmonella typhi

In the methanol leaf extract, 50 µl concentrations showed a potential growth of inhibition zone of 10.66 mm and in the 10 µl concentration. It showed the 6.33 mm inhibition in the stem methanol extract 50 µl concentration showed the inhibition of 8.66 mm, in 40 µl concentration 5 mm inhibition was observed and in the 10 µl concentration no inhibition of growth was evident.
In the ethanol leaf extract, 50 µl concentration showed potential growth of inhibition zone of 12 mm. In 40 µl concentration the inhibition zone was 10.66 mm and in 10 µl concentration 1.66 mm inhibition was recorded. In the stem ethanol extract, 50 µl concentrations showed 13.33 mm growth inhibition; in 10 µl concentration the zone of inhibition was 5.33 mm.

In the water leaf extract, 50 µl concentrations showed 8 mm of growth inhibited zone and in the 10 µl concentration no inhibition of zone was observed. In the stem extract, 50 µl concentration showed 13.33 mm zone of inhibition and in the 10 µl concentration the zone of inhibition zone was 5.33 mm (Fig. 4 & Table 3).

**Fig. 3. Antibacterial activity of Priva cordifolia against Staphylococcus aureus**

**Fig. 4. Antibacterial activity of Priva cordifolia against Salmonella typhii**
### Table 3. Antibacterial activity of *Priva cordifolia* against *Salmonella typhii*

| Sl. no | Extract used | Zone of inhibition in mm/conc µg/ml | Positive Control Streptomycin |
|-------|--------------|-------------------------------------|-------------------------------|
|       |              | 10       | 20       | 30       | 40       | 50       |
| 1     | Leaf Methanol | 23.33±1.15 | 6.33±0.57 | 5.66±0.57 | 06±00 | 7±1 | 10.66±0.57 |
| 2     | Leaf Ethanol  | 20.33±0.57 | 1.66±0.57 | 5.66±0.57 | 8.33±0.57 | 10.66±1.15 | 12±01 |
| 3     | Leaf Aqueous  | 21.33±1.15 | 00±00 | 00±00 | 7.33±1.15 | 7.66±1.52 | 8±1.73 |
| 4     | Stem Methanol | 24.66±2.30 | 00±00 | 00±00 | 3.66±0.57 | 5±1 | 8.66±0.57 |
| 5     | Stem Ethanol  | 26.66±1.15 | 5.66±0.57 | 6.66±0.57 | 8.33±0.57 | 11±1.73 | 13.33±1.52 |
| 6     | Stem Aqueous  | 16±1 | 5.33±0.57 | 4±00 | 4.66±0.57 | 12±00 | 13.33±1.52 |

*Note: Values are represented as mean diameter of zone of inhibition in triplicates ± Standard deviation*

### 3.5 Antibacterial Activity of *Priva cordifolia* of Different Solvents against *E. coli*

In the methanol leaf extract, 8.66 mm zone of inhibition was recorded in 50 µl concentration in 10 µl concentration no inhibition was found. In the stem extract 50 µl concentration showed the growth inhibition zone of 19.33 mm, in 20 µl concentration 7.33 mm of growth inhibition and in 10 µl concentration no inhibition was observed.

In the ethanol leaf extract, 50 µl concentration had growth inhibition zone of 14.33 mm and in the 10 µl concentration 1 mm of inhibited growth zone was recorded.

In the water leaf extract, 50 µl concentration showed 11.66 mm growth inhibition, but in the 10 µl and 20 µl concentration no inhibited growth was found. In the stem water extract only 50 µl concentration showed the inhibition activity of 3.66 mm, other concentration did not exhibit an inhibited growth zone (Fig. 5 & Table 4).

### 3.6 Phytochemical Screening of *Priva cordifolia*

Phytochemical screening of *Priva cordifolia* shown in Table 5.
4. DISCUSSION

Herbal preparations can supplement other systems of medicine for the treatment of disease caused by pathogenic bacteria [8]. Considering the potential of plants as basis for antibacterial drugs with references to antibacterial agents, an organized examination was commenced to
monitor the flora of India for antibacterial activity of *Priva cordifolia*. Compounds present in *Priva cordifolia* are known to be biologically active and therefore aid the antibacterial activities. A cold extraction method was carried out for dried powdered leaves of *Priva cordifolia* by soaking the powder in three different solvents viz., methanol, ethanol and water. The present study had showed significant antibacterial activity against bacteria. Antibacterial effect of the extracts was different, depending on the type of microorganisms, thus the gram-positive bacteria had higher sensitivity compared to gram-negative bacteria.

Priya et al. [9] showed that gram negative bacteria are less susceptible to plants extracts of Tulsi. But gram positive bacteria i.e. *S. aureus* and *B. subtilis* possess antibacterial activity. The methanol extract had high inhibition zone against all the pathogenic bacterial strains but, ethanol extract showed negligible effect i.e., 13 mm *Pseudomonas aeruginosa* shows the maximum inhibitory zone of 15.6 mm in the methanol extract Adiguzel et al. [10].

According to our observation data water stem extract showed inhibitory activity against *Staphylococcus aureus* with 27.66 mm at 50 µg of concentration, followed by *B. subtilis* by showing inhibition zone of 21 mm in concentration of 50 µg of water leaf extract.

The stem and leaf methanol extract of antimicrobial activity against *B. subtilis* was 15.33 mm at 50 µg concentration. According to result of this research ethanol leaf extract also shows significant results. The maximum inhibitory zone was 14.33 mm at 50 µg concentration against *Staphylococcus aureus*. According to Thiripura et al. [11,12] the antibacterial activity of methanol extract in *Vitex negundo* against *Staphylococcus aureus* (gram positive bacteria) shows 12 mm zone of inhibition, as per the antibacterial activity revealed 8.66 mm zone of inhibition against *S. aureus* as compared with *V. negundo*, the *P. cordifolia* showed less effect. Analysis conducted on the plant extracts revealed the presence of phytochemicals such as alkaloids, carbohydrates, glycosides, reducing sugars, proteins and tannins. The results obtained in this study suggested that identified phytochemical may be bioactive and that *Priva cordifolia* proving to be valuable source of bioactive compounds.

### 5. CONCLUSION

Plants have been used for traditional medicines as they contain components of therapeutic value. Plants are natural sources of antimicrobial agents. They contain a range of metabolites that can be extracted treat infectious and chronic diseases. Most of the aromatic and medicinal plants components, also known as herbal drugs that are primarily used for therapeutic, aromatic and culinary purposes as compounds of cosmetics, medicinal products, health foods and other natural health products.

Despite the different approaches for the discovery of therapeutics and natural products, plants still remain one of the best sources of new bioactive compounds. These plant compounds used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds. *Priva cordifolia* proved to possess antibacterial properties through the presence of bioactive compounds which were detected by phytochemical screening.

As human have reached the peak in the technology, herbs have proven to be a natural product which is free from side effects to cure
many life style disorders and infectious diseases. There is a need to promote the herbal products to save human lives, so *Priva cordifolia* is one of the aromatic plants.

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**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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