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# PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *JUSTICIA GLAUCA* ROTTLER

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## ABSTRACT

*Justicia glauca* Rottler, a medicinal plant belongs to dicotyledonous flowering plants family Acanthaceae. Plants from this family have been reported in the scientific literature to possess promising medicinal properties. The preliminary phytochemical analysis and antibacterial activity of *Justicia glauca* Rottler total plant extracts were carried out using different organic solvents against some pathogenic bacteria viz., *Escherichia coli, Klebsiella pneumonia, Bacillus subtilis* and *Staphylococcus aureus* by agar well diffusion method. Results indicated that the petroleum ether, chloroform and ethanol extracts of *Justicia glauca* Rottler showed significant growth inhibitory activity against *Bacillus subtilis*, *E. coli* and *Klebsiella pneumonia* at 100mg/ml concentration. The most active extracts were compared with the standard antibiotic Ampicillin. The highest zone of inhibition was recorded (13mm) at 100mg/ml concentration of petroleum ether and ethanol *Klebsiella pneumonia* and *Bacillus subtilis* respectively. This study revealed that the *Justiscia glauca* Rottler have a potential broad spectrum antibacterial activity. Combinations of extracts may be the best herbal antibiotics.

**KEYWORDS:** ***Justiscia glauca*** **Rottler, Phytochemicals, well diffusion, zone of inhibition, herbal antibiotics.**

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## INTRODUCTION

The medicinal property of the plants is contributed by the phytochemical constituents that cause definite pharmacological action on the human body[1]. Therefore, exploring phytochemical compounds from the medicinal plants has become desirable[2]. There are large number of medicinal plants as a source of herbal drugs still not yet explored. From the previous studies of research only a small percentage of plants have been investigated from the estimated 250,000-500,000 plant species, for their phytochemical compounds and they were subjected to screening pharmacological and biological activities even in smaller[3, 4]. Screening of herbal drugs on microorganisms lead to get higher rate of resistance strains evolutionally. Therefore, freshly collected clinical samples are preferable to screen antibacterial activity of such herbal drug because it is more potent[5]. Discovery of novel herbal drugs from the medicinal plants are immeasurable because the range of availability of chemical from the plants are matchless[6]. *Justicia glauca* Rottler (*J. glauca*), a medicinal plant belongs to family Acanthaceae. The investigations at a taxonomic level have been done to explore the floristic status of the family Acanthaceae. It is one of the advanced and specialized families with the majority of the herbaceous plants. A number of plant species have significant medicinal value[7]. Only a limited work has been recorded in phytochemical study and other medicinal properties. Therefore the present study has been extensively evaluated for phytochemical compounds and inhibitory activity of *J. glauca* plant extracts (chloroform, petroleum ether, methanol, ethanol and water) on human pathogenic microorganisms.

## MATERIALS AND METHODS

### *Collection of plant materials*

The fresh plant *Justicia glauca* Rottler was collected in and around the surroundings of main campus, Osmania University, Hyderabad, Telangana State, India. Genus of Justicia roughly contains 420 species.

**Figure 1**
***Flowering plant Justicia glauca Rottler***

**Figure 2**
***Justicia glauca Rottler flower***

Among these, *J. galuca* is an under shrub found in the foothills of Peninsular India. Leaves are elliptic-ovate, 3-5 cm long and 2-3 cm wide. Flower spikes are erect, about 10 cm long. Bracts are broadly ovate, and bracteoles are linear. Flowers are pink, with 5 lance-shaped sepals which are 3-nerved and have minute hair on them. Flowering between July to December months of every year. The taxonomical identification of the plant (Voucher No. 0458) was confirmed by Dr. Prathibha Devi, Head, Department of Botany, Osmania University.

***Extraction and Preparation of Plant Materials***

*Justicia glauca* Rottler, the whole plant material is washed thoroughly 2-3 times with running tap water and then with sterile water followed by shade dried, powdered and used for extraction. The extraction of plant material was done by modified method of dissolving 30g of the powder in 250ml conical flask with 120ml petroleum ether, chloroform, methanol, ethanol and water separately. They were kept at for three days at room temperature[8]. The suspension was filtered through a Whatman No.1 filter paper. The organic solvents (petroleum ether, chloroform, methanol and ethanol) and aqueous extracts were concentrated under reduced pressure using a rotary evaporator, while aqueous extract was dried using water bath[9]. Finally, the extracted semi gummy powder was re-suspended in the respective solvents at a concentration of 100mg/ml preserved at low temperature before it was tested for the phytochemical analysis and antibacterial activity[10, 11].

***Preliminary phytochemical screening***

The phytochemical studies were performed as described by Bhatt Shashank et al., (2012) and Priyanka et al., (2012)[12, 13]. The presence of alkaloids, flavonoids, carbohydrates, proteins, glycosides, saponins, tannins, phenolic compounds, terpenoids and sterols has given in Table 1.

***Preparation of bacterial cultures***

A total of 4 microorganisms was used to assess the antimicrobial activity. It includes two Gram- positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*; two Gram-negative bacteria, *Escherichia coli and Klebsiella pneumoniae*. The microorganisms were originally obtained from Department of Biochemistry, Osmania University, Hyderabad, India. They were maintained on a nutrient broth at $37^0$ C[3].

***Determination of antibacterial activity by agar well diffusion Method***

The antimicrobial activity of the plant extracts was determined with the agar well diffusion method by the following published (Geeta S *et al*., (2013); Saba I. *et al*., (2012) procedure with slight modifications[14,15]. Nutrient agar was inoculated with the given microorganisms by

spreading the bacterial inoculums on the media. Wells (6mm diameter) were punched in the agar and filled with plant extracts. Positive control wells containing antibiotic Ampicillin 10µg/ml. DMSO (Dimethyl sulfoxide) which showed no zone of inhibition acts as a negative control. A total of 100mg/ml concentration of 25µl and 50µl plant extracts was poured into wells. The plates were incubated at 37°C for 24 h and measuring the diameter of the zone of inhibition assessed the antibacterial activity.

## RESULTS AND DISCUSSION

The preliminary phytochemical screening of *J. glauca* obtained successively using petroleum ethers, chloroform, methanol, ethanol and water extracts of total plant were performed. Each extract of this plant was subjected to various qualitative tests to identify the phytoconstituents such as alkaloids, flavonoids, carbohydrates, proteins, glycosides, saponins, tannins, phenolic compounds, terpenoids, sterols were analysed and the results are given in Table 1.

**Table No 1**
***Phytochemical Screening of Justicia glauca Rottler***

| Name of the Phytoconstituents | Chloroform | Petroleum ether | Ethanol | Methanol | Aqueous |
|---|---|---|---|---|---|
| **Alkaloid** | | | | | |
| Iodine Test | - | - | - | - | - |
| Wagner's Test | + | + | - | - | + |
| Dragendorff Test | - | - | - | - | + |
| Mayer's Test | - | - | - | - | - |
| **Glycosides** | | | | | |
| Keller Kiliani Test | + | - | - | - | - |
| Conc. $H_2SO_4$ | + | - | - | - | + |
| Molisch's Test | - | - | + | + | + |
| **Tannins** | - | - | - | - | - |
| **Phenols** | | | | | |
| Phenol Tests($FeCl_3$ Test) | - | - | - | - | - |
| **Sterols** | | | | | |
| Salkowski Test | - | - | + | + | + |
| **Flavonoids** | | | | | |
| Pew's Test | - | - | - | - | - |
| NaoH Test | + | - | - | - | - |
| **Carbohydrates** | | | | | |
| Benedict's Test | - | + | - | - | - |
| **Proteins** | | | | | |
| Xanthoproteic Test | - | - | - | - | - |

**Table No 2**
***Antimicrobial activity of Justicia glauca Rottler***

| Name of the microorganisms | Zone of inhibition in mm Concentration of plant *J.glauca* extract (100mg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Standard antibiotic Ampicillin (10µg/disc) | DMSO | Petroleum Ether | Chloroform | Methanol | Ethanol | Aqueous |
| ***Bacillus subtilis*** | 14 | 0 | 10 | 10 | 6 | 13 | 2 |
| ***Staphylococcus aureus*** | 14 | 0 | 10 | 7 | 5 | 10 | 3 |
| ***Escherichia coli*** | 15 | 0 | 8 | 10 | 10 | 6 | 5 |
| ***Klebsiella pneumonia*** | 15 | 0 | 13 | 12 | 12 | 12 | 5 |

The antimicrobial activities of flowering plant *J.glauca* extract (100mg/ml) was evaluated against four bacterial strains. Among them two were Gram positive namely *Klebsiella pneumonia* and *Bacillus subtilis* bacteria and two were Gram negative bacteria namely *Staphylococcus aureus* and *Escherichia coli*. According to the results given in Table No 2, ethanolic extract of *J.glauca* showed highest antibacterial effect compared with other extracts against *Bacillus subtilis* with zone of inhibition diameter 13 mm, whereas chloroform and petroleum ether extracts showed 10mm zone of inhibition respectively. Ethanol and petroleum ether extracts showed 10mm zone of inhibition against *Staphylococcus aureus*, chloroform and methanolic extracts showed 10mm zone of inhibition against *Escherichia coli*. Similarly, all solvent extracts showed significant zones of inhibition (13mm and 12mm) on *Klebsiella pneumonia*. Aqueous extracts had the lowest zones of inhibition on all the test organisms. On the other hand, standard antibiotic ampicillin (10µg/disc) showed more significant antibacterial activity against all tested Gram positive and Gram negative bacteria showing the larger zone of inhibition in every case such as Staphylococcus with zones of inhibition diameter 14 mm, E. coli with zones of inhibition diameter 15 mm, Bacillus subtilis with zones of inhibition diameter 14 mm and Klebsiella pneumonia with zones of inhibition diameter 15 mm each respectively. In comparison with standard positive control, negative control DMSO has not shown zone of inhibition for respective organisms. Phytochemical compounds such as alkaloids, flavonoids, carbohydrates, proteins, glycosides, saponins, tannins, phenolic compounds, terpenoids, sterols and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other organisms[16, 11]. The present study carried out on the *J.glauca* plant extracts revealed the presence of medicinally active constituents. The phytochemical compounds of the plant investigated were summarized in Table-2. Analysis of plant extracts revealed the presence of alkaloid, glycosides, sterols, flavonoids, carbohydrates. In most of the selected plants which could be responsible for the observed antimicrobial property. Tannins and phenols were found to be absent from all the plant extracts studied. The bioactive compounds extracted from medicinal plants are known to act by different mechanism and exert antimicrobial action[11]. Hena et al., (2010)[17] reported that the presence of these constituents, plants have been accounting for the exertion of antimicrobial activity. Petroleum ether and ethanol plant extracts showed significant activity against *Bacillus subtilis* and *Klebsiella pneumonia*.

## CONCLUSION

*Justicia glauca* Rottler plant extract was tested against different pathogenic bacteria species such as *Staphylococcus aureus, Klebsiella*

*pneumonia, Escherichia coli and Bacillus subtilis*. The organic extracts were showed highly significant zone of inhibitions compared to aqueous extracts. Petroleum ether, chloroform and ethanol extracts were highly sensitive to the growth of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia.* From the results of this study it has been concluded that the *J. glauca* plant extracts may have great potential as a remedy for infectious disease caused by various pathogenic bacteria species. Antibacterial principle compounds need to be isolated from this plant for the production of new antibiotics.

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