



Screening of Antimicrobial Activity of Various Extracts of the Stem *Justicia gendarussa*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The present study was designed to check *in-vitro* efficacy of Antibacterial and antifungal activity of aqueous and hexane extracts of stem of *Justicia gendarussa* against selected bacterial and fungal strains. *Justicia gendarussa*, belonging to the family Acanthaceae, commonly known as "vatham kolli" was used in treatment of bronchitis, inflammation, eye diseases, ear ache, vaginal discharges, rheumatism, dysentery, eczema and jaundice.

Methodology: Hexane and aqueous extracts of *Justicia gendarussa* was used for antimicrobial screening. Antibacterial activity was tested against pathogenic bacterial strains *Escherichia coli* and *Staphylococcus aureus*. Also antifungal activity was tested against one human pathogenic fungal strain i.e. *Candida albicans*. Antibacterial and antifungal activities of *Justicia gendarussa* extract was carried out by using disc diffusion method.

Results: Aqueous extract of *Justicia gendarussa* showed the largest zone of inhibition (5mm) against *Escherichia coli*, at 50µg/ml and 3mm zone of inhibition against *S. aureus*, at 50µg/ml. Hexane extract does not showing activity against to human pathogenic *E. coli* and *S. aureus*, at 50µg/ml. Aqueous extract has shown more potent antibacterial activity against *Escherichia coli*.

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Aqueous extract was found to be more effective against human pathogenic fungus *Candida albicans* than hexane extract and standard drug. The zone of inhibition of Aqueous extract was 11mm and the zone of inhibition of hexane extract was 7mm and standard only 2mm. The phytochemical screening demonstrated the presence of different types of compounds like terpenoids, tannins, and flavonoids which may contribute for the anti-microbial action of this plant. **Conclusions:** These findings provide scientific evidence of traditional use of *Justicia gendarussa* and also indicate the potential of this plant for the development of antimicrobial agents.

Keywords: *Justicia gendarussa*; antibacterial activity; antifungal activity; *Escherichia coli*; *Staphylococcus aureus*; *Candida albicans*.

1. INTRODUCTION

Herbals have a great potential for producing of new drugs for the benefits of mankind. There are many approaches to search for biologically active principles in plants [1]. Medicinal plants are abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries [2]. Experts turned their concentration back towards obtaining advantages from medicinal plants after observing more side effects of synthetic drugs compared to their benefits [3].

It is estimated that about 35,000 to 70,000 plants species are used as medicinal plants out of 422127 reported worldwide plant species [4]. In the worldwide as well as in the developing countries, the most human died due to infectious bacterial diseases [5]. The bacterial organisms including Gram positive and Gram negative like different species of *Bacillus*, *Staphylococcus*, *Salmonella* and *Pseudomonas* are the main source to cause severe infections in humans. Because these organisms have the ability to survive in harsh condition due to their multiple environmental habitats [6]. The synthetic antibiotics have the following limitation: Firstly, these are costly and are out of range from the patient belonging to developing countries. Secondly, with the passage of time microorganism develop resistance against antibiotics. Therefore, after some time these antibiotics are not effective against the microbes [7-8]. Furthermore, the antibiotics may be associated with adverse effects on the host, including hypersensitivity, immune suppression, and also allergic reactions. On the other hand, natural products have got incredible success in serving as a guidepost for new antibacterial drug discovery. Moreover, antibiotics obtained in this way have biological friendliness nature [9,10]. As

is well known that the bioactive plant extracts is a promising source of majority of drugs [11]. For example, Quinine (*Cinchona*) and berberine (*Berberis*) are the antibiotics obtained from plants which are highly effective against microbes (*Staphylococcus aureus*, *Escherichia coli*) [12]. In India, a vast diversity of bioactive plants grown naturally.

Justicia gendarussa Burm, which belongs to the family of Acanthaceae is commonly called as "vatham kolli". Willow-Leaf *Justicia* is an erect, branched, smooth undershrub 0.8-1.5 m tall. The leaves are lance-shaped, 7-14 cm long, 1-2.5 cm wide, and pointed at the ends. The rather small flowers are borne in 4-12 cm long spikes, at the end of branches or in leaf axils. The teeth of the sepals cup are smooth, linear, and about 3 mm long. The flowers are about 1.5 cm long, white or pink, with purple spots. The capsule is club-shaped, about 1.2 cm long, and smooth. Willow-Leaf *Justicia* is found throughout India and SE Asia. This plant exhibits anticancer, antibacterial, hepatoprotective, antioxidant, anthelmintic and antiangiogenic activities. Conventionally this plant was used in treatment of bronchitis, diseases, ear ache, vaginal discharges, inflammation, eye, rheumatism, dysentery, eczema and jaundice [13]. The present study was designed to check *in-vitro* efficacy of *Justicia gendarussa* stem extracts against selected bacterial and fungal strains.

2. MATERIALS AND METHODS

2.1 Collection and Authentication

Justicia gendarussa Burm was collected from Vellapara, Palakkad, Kerala, India. The plant was identified and authenticated by the taxonomist Dr Kanakamany. M.T professor and Head, Office of AICRP on Medicinal and Aromatic Plants, Thrissur, Kerala. The authenticated specimen was deposited in the Department of Pharmacognosy, Sanjo College of

Pharmaceutical studies, Palakkad, the authentication specimen number is SCOPS/P.CO/004/2019. The Plant parts were separated.

The stem was broken into small pieces and was dried in room temperature for 2 months. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

2.2 Extraction of Plant Material

The extract was prepared by weighing 1 kg of the dried powdered stem was subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, benzene, Hexane, chloroform, ethanol and finally with aqueous. The extracts were filtered in each step using What man filter paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45°C) and pressure. These extracts used for screening of antimicrobial activity.

2.3 Methods

Strain, Culture media and Sterile discs: *Justicia gendarussa* Burm stem aqueous and hexane extracts were conducted for anti-bacterial and antifungal activity against to *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

Microbial cultures procured from government medical college from Tiruchirappalli, Tamil Nadu. Media used for microbial test was potato dextrose agar and Muller-Hinton agar media obtained from Himedia Pvt. Bombay, India.

Sterile discs used for antimicrobial activity procured from Himedia Pvt. Bombay, India.

2.4 Antibacterial Activity

Antibacterial activity of aqueous and hexane extracts was studied by using disc diffusion method. *Escherichia coli*, *Staphylococcus aureus*, inoculums were prepared by using nutrient broth media. Double strength sterile Mueller Hinton agar media were prepared by autoclaving 7.6 gm in 100ml. Inoculate the test microorganisms on the Mueller hinton agar plates by using sterile cotton swabs. Aqueous and hexane extracts of *Justicia gendarussa* stem were placed on sterile discs. Discs were dried aseptically under laminar air flow to remove solvents. Dried discs are placed on the surface of culture inoculated Mueller hinton agar plates and plates were incubated at 37°C for 24hr. Antibacterial activity was evaluated by using himedia zone reader.

2.5 Antifungal Activity

Antifungal activity of *Justicia gendarussa* stem aqueous and hexane extracts were studied by using disc diffusion method. *Candida albicans*, inoculum was prepared by using potato dextrose broth. Potato dextrose agar media were prepared by autoclaving 3.9 gm in 100ml. Inoculate the test microorganisms on the Potato dextrose agar plates by using sterile cotton swabs. Aqueous and hexane extracts of *Justicia gendarussa* Burm stem were placed on sterile discs. Discs were dried aseptically under laminar air flow to remove solvent. Dried discs are placed on the surface of culture inoculated potato dextrose agar plates and plates were incubated at room temperature for 48hr. Antifungal activity was evaluated by using Himedia zone reader [14-19].

3. RESULTS

Table 1. Antibacterial activity of *Justicia gendarussa* stem

S.NO	Extract/Standard drug	Zone of Inhibition (mm)	
		<i>Escherichia coli</i> (n=6)	<i>Staphylococcus aureus</i> (n=6)
1	Hexane	R	R
2	Aqueous	5±5	3±5
3	Cephalexin	2±5	22±5
4	Ampicillin	-	5±5

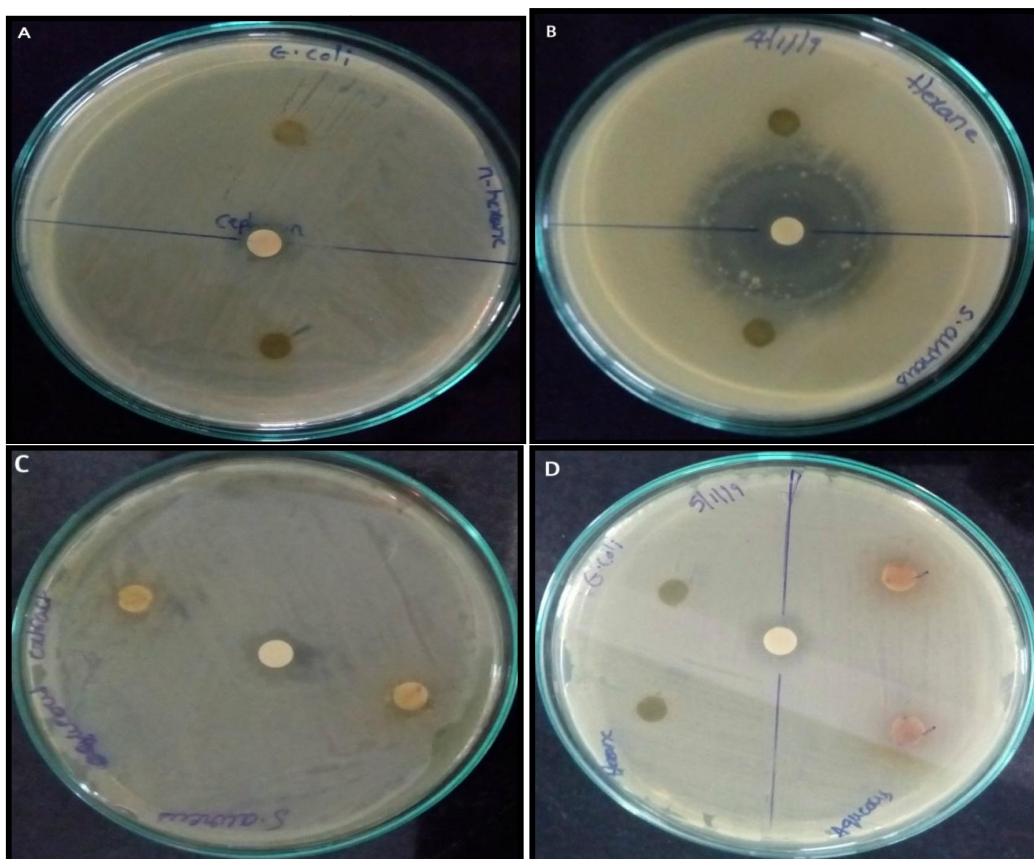
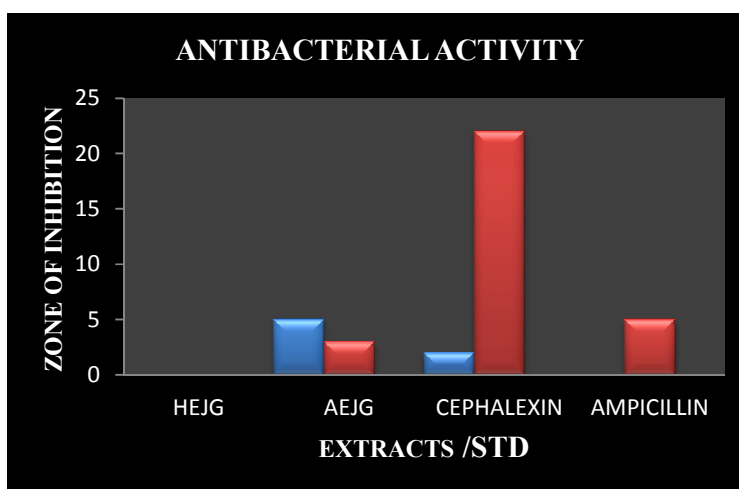


Fig. 1. Antibacterial activity of *Justicia gendarussa*

A: Hexane extracts not showing activity against to human pathogenic *E. coli*. **B:** Hexane extracts not showing significant activity against to human pathogenic *Staphylococcus aureus*. **C:** Aqueous extract showing activity against to human pathogenic *Staphylococcus aureus*. **D:** Aqueous extract showing activity against to human pathogenic *E. coli*



The results (Table 1) illustrated that aqueous extract of *Justicia gendarussa* showed the largest zone of inhibition of about 5 mm against *Escherichia coli*, at 50 µg/ml and about

3 mm zone of inhibition against *S. aureus*, at 50 µg/ml. Hexane extract does not showing antibacterial activity against to human pathogenic bacteria's like *E. coli* and *S. aureus*, at 50 µg/ml.

Aqueous extract has shown more potent antibacterial activity against *Escherichia coli*. The phytochemical screening demonstrated the presence of different types of compounds like terpenoids, tannins, and flavonoids which may contribute for the anti-bacterial action of this plant.

Antifungal activity of *Justicia gendarussa* stem

Antifungal activity of aqueous and hexane extracts was studied by using disc diffusion method.

The above results clearly demonstrates that aqueous extract had more potent antifungal activity against *Candida albicans* than hexane

extract of *Justicia gendarussa* and STD. The zone of inhibition of aqueous extract was 11mm and the zone of inhibition of hexane extract was 7 mm and standard only 2 mm. The phytochemical screening demonstrated the presence of different types of compounds like terpenoids, tannins, and flavonoids which may contribute for the anti-fungal action of this plant.

Table 2. Antifungal activity of *Justicia gendarussa* stem

S. NO	Extract/ Standard drug	Zone of Inhibition (mm) <i>Candida albicans</i> (n=6)
1	Aqueous	11±5
2	Hexane	7±5
3	Clotrimazole	2±5

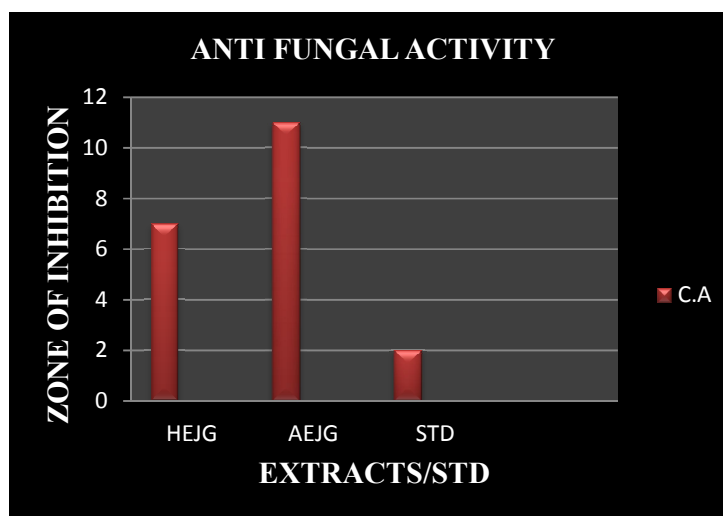


Fig. 2. Antifungal activity of *Justicia gendarussa* stem

4. DISCUSSION

In recent times there has been considerable significance in the use of plant material as an unconventional method to control pathogenic microorganism [20] and many components of plants products have been shown to be particularly targeted against resistant pathogenic bacteria [21]. The appearance of multi drug resistant strain of many pathogens is a severe threat and makes chemotherapy more difficult. Furthermore, the current price of most of the chemotherapeutic agents is intolerable to the public particularly in developing countries like India [22]. Therefore attempts must be directed towards the development of effective natural, non-toxic drug for treatment. Therefore the present work was carried out to explore the antimicrobial property of *Justicia gendarussa*. The aqueous extract of *Justicia gendarussa* stem showed the activity against *Escherichia coli*, and *S.aureus*. The plant based products have been effectively proven for their utilization as source for antimicrobial compounds. Aqueous extract was more effective against fungus *Candida albicans* than hexane extract and STD.

5. CONCLUSION

From the above study, it is concluded that the plant *Justicia gendarussa* has significant antibacterial activity against *Escherichia coli*, and *S.aureus*. Also the plant is found to be a potent antifungal agent against *Candida albicans*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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