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***In vitro* antibacterial activity of leaf extracts of *Justicia gendarussa* wild**

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ABSTRACT

*Traditional knowledge and ethno-botanical use of plants have been widely acknowledged all over the world. In the present scenario, emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. *Justicia gendarussa* a traditional medicinal liana, used by rural peoples, villagers and tribals residing in different parts of Maharashtra, Tamil Nadu and Kerala, to treat various ailments. The Muthuvans, Chinnar tribe of Idukki district, Kerala is using stem bark of this plant as a remedy for inflammation. The study was carried out to ascertain the antibacterial properties present in different extracts of dried leaves of *Justicia gendarussa*. The Antibacterial testing of leaf extract *Justicia gendarussa* was evaluated by Agar well diffusion method using gram positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae*. Amongst the test extracts, the results suggested that, Ethyl acetate, Ethanol extracts of leaf showed significant antibacterial activity compared with standard drug.*

Keywords: *Justicia gendarussa*, Antibiotics, E.coli, Dimethyl sulfoxide (DMSO)

INTRODUCTION

The plants have been utilized for basic and curative health care since time immemorial. The use of plants as food and medicines started ever since man started life on the planet. The plant kingdom is a virtual goldmine of potential drug targets and other active drug molecules waiting to be discovered. During the last decade, use of traditional medicine has expanded globally and gained popularity [1]. Plant based drugs are having a revived interest now-a-days because of awareness of deleterious effects of modern synthetic drugs. Natural products can play a very crucial role in pharmaceutical industry as drug themselves or as drug carrier or bio-enhancers or excipients. India has a rich medicinal plant flora of some 2500 species, of these 2000 to 3000 atleast 150 species are used commercially on a fairly large scale. Foreign researchers have always appreciated the traditional Indian healers[2]. If the Indian herbal industry, is to survive in the domestic and international markets steps have to be taken to establish a good quality control mechanism, for which the government should consider assisting the standardization of drugs to meet International requirements in the coming years[9].

Justicia gendarussa belongs to the family *Acanthaceae*. The family *Acanthaceae* is a taxon of dicotyledonous flowering plants containing almost 250 genera and 2500 species. Most are tropical herbs, shrubs or twining vines,

some are epiphytes. Only a few species are distributed in temperate region. The four main centers of distribution are Indonesia, Malaysia, Africa, Brazil and Central America[10].

MATERIALS AND METHODS

Collection and Authentication: The plant collected from Chinnar, Idukki district of Kerala, during the month of March-April and authenticated at Taxonomy Division, Centre for Medicinal Plants Research (CMPR), Arya Vaidya Sala, Kottakkal, Kerala. The voucher specimen (Col. No. 5512) and raw drug (Col. No. CMPR/RD 201) were deposited at CMPR herbarium and raw drug depository respectively. One part of the stem bark were shade dried, powdered and passed through 20 mesh sieve and stored in an airtight container for further use.

Antibacterial Activity:

Preparation of Plant Extract: The leaves were thoroughly washed, dried in shade and powdered 100g of the dried leaves were successfully extracted using Soxhlet apparatus with ethanol, ethyl acetate as solvent [3][10]. After two days of extraction the solvent was evaporated using vacuum and the residue obtained was used for the studies. The extract was dissolved in Dimethyl sulfoxide (DMSO) and used for antibacterial screening at the concentration of 1g/5ml. The water extract was prepared by weighing 100mg powder of leaf by boil with 300ml of distilled water in a water bath for 24 hours, filtered and evaporated. The extract obtained was dissolved in sterile distilled water at a concentration of 1g/5ml and used for antibacterial screening [4].

Microorganisms: The test organisms included for study were gram positive bacteria like *Bacillus subtilis*, gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae*. All the bacterial strains were procured from Osmania University, Hyderabad, Andhra Pradesh. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

Bacterial Media: Muller Hinton Media was mixed with distilled water and then sterilized in autoclave at 15 lb pressure for 15 minutes. The sterilized media were poured into Petri dishes and allowed for solidification [6][9]. The solidified plates were bored with 5mm diameter cork borer. The plates with wells were used for the antibacterial studies.

Antibacterial activity of the plant extracts: Different leaf extract of *Justicia gendarussa* at a concentration of 500µg/ml, 750µg/ml, 1000µg/ml were tested against the gram positive bacteria like, *Bacillus subtilis*, gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae* by Well Diffusion Method [5,11].

Well Diffusion Method

Antibacterial activity of the plant extract was tested using Well diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 6mm cork borer. The dried extracts were dissolved in 95% of ethanol for preparation of different concentration ranges of extracts. The extracts were poured into the well using sterile syringe [7]. The plates were incubated at 37°C ± 2°C for 24 hours for bacterial activity. The plates were observed for the zone clearance around the wells. The extracts of the dried scale leaves were used for the study. The extracts were dissolved in sterile distilled water to form dilution such as 500µg/ml, 750µg/ml and 1000µg/ml. Each concentration of the extract was tested against different bacterial pathogens. Gentamycin at a concentration of 5µg/ml and 10µg/ml was used as standard antibacterial drug. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter [8]. The readings were taken in three different fixed directions in all three replicates and the average values were tabulated.

Table 1: Zone of inhibition shown by the Gentamycin and the Ethanol, Ethyl alcohol extracts of dried leaf of *Justicia gendarussa*

Micro-organisms	GENTAMYCIN		EXTRACTS (1000µg/ml)	
	5µg/ml	10µg/ml	Ethanol extract	Ethyl acetate extract
<i>Bacillus subtilis</i>	7.5 mm	9 mm	8 mm	7 mm
<i>Escherichia Coli</i>	7 mm	9 mm	6.5 mm	6 mm
<i>Klebsiella pneumoniae</i>	7 mm	9 mm	8 mm	7 mm



FIGURE 1 Zone of inhibition showed by ethanol and ethyle acetate of Leaf extract of *Justicia gendarussa* on *Bacillus subtilis* bacteria

RESULTS AND DISCUSSION

Antibacterial assay of the Ethanol, Ethyl acetate extracts of leaf of *Justicia gendarussa* exhibited dose dependent antibacterial activity against the tested microorganisms at three different concentrations of 500, 750 and 1000 μ g/ml. The potential sensitivity of the extracts was obtained against all the tested micro organisms and the zone of inhibition was recorded and presented in the table given below (Table 1). From the above study the zone of inhibition obtained was dose dependent and the activity shown by the Ethyl acetate, Ethanol extracts of leaf of *Justicia gendarussa* at a concentration of 1000 μ g/ml against gram positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, and gram negative bacteria like *Escherichia coli*, *Klebsiella pneumonia* strains involved in present study was more in comparison to Gentamycin, at a concentration of 5 μ g/ml. The antibacterial potential exhibited by stem extracts may be contributed to the presence of tannins, flavonoids, anthraquinones in preliminary phytochemical investigations. Further study is needed to characterize the active principles.

CONCLUSION

From the above study, it is concluded that the leaf of *Justicia gendarussa* may represent a new source of antibacterial with stable, biologically active components that can establish a scientific base for the use of this in modern medicine. These local ethnomedical preparations of plant sources should be scientifically evaluated and then disseminated properly. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology.

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