

**SCREENING OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES
OF SIDDHA HERBAL DRUG KODAGASALAI CHOORANAM
(*JUSTICIA PROCUMBENS*)**

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ABSTRACT

This present study aims to validate the antimicrobial activity of Siddha herbal drug Kodakasalai Chooranam (*Justicia procumbens*) commonly known as Water Willow belongs to Acanthaceae family. It is used in the form of decoction for treating variety of diseases including venereal diseases, boils, tumors, skin diseases, aphthous ulcer, cancer, diabetes, fever, headache, inflammation, arthritis and different gastrointestinal disorders. The dried and powdered plant material was extracted into aqueous, ethanol and chloroform fractions. The antimicrobial activity was evaluated against *Bacillus subtilis*, *Streptococcus pyogens*, *Staphylococcus aureus* (Gram positive), *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Proteus vulgaris* (Gram negative)

and one fungal species *Candida albicans* using the disk diffusion method and the broth dilution method. Ciprofloxacin (10µg) and Ketoconazole (10µg) were used as a positive control. It was observed that the aqueous, ethanol and chloroform extracts of *Justicia procumbens* presented marked antimicrobial activity against tested gram negative bacteria.

Ethanol and chloroform extracts showed moderate activity against gram positive bacteria. The antibacterial activity was more apparent in ethanol extract as compared to chloroform extract for all tested organisms. The aqueous and ethanol extracts showed no activity against the yeast *Candida albicans*. But Chloroform extract showed marked inhibitory effect against *Candida albicans*. It can be concluded that the results of this study support traditional use of *Justicia procumbens* for its antimicrobial activity in Siddha system of medicine.

KEYWORDS: Antimicrobial, *Justicia procumbens*, disk diffusion, broth dilution, Kodakasalai Chooranam, Siddha.

INTRODUCTION

The World Health Organization estimated that 80 percent of the world's population still uses traditional remedies, including plants, as the main tool for health care.^[1] Most new drugs (70 percent) presented in the United States are derived from natural products, mainly plants.^[2] TM can also have impact on infectious diseases. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants.^[3] Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived.^[4]

Traditional medical system especially Siddha system of medicine can also have impact on infectious diseases. Plants with antimicrobial properties, which help to strengthen the immune system of the body, are particularly remarkable. 60-80% of current antibacterial and anticancer drugs are derived from natural products.^[5]

In the perpetual struggle against microbial infections due to its antibiotic resistance through the various microorganisms and the side effects of modern synthetic drugs, new sources of antimicrobial products, especially plant sources, are being investigated. There are a number of scientific research confirmed the efficacy of Siddha herbal formulations in the treatment of various infections, including bacterial, fungal and protozoal origin.^[6-8] Numerous single herb and poly herbal formulations have a very good preventive therapeutic role in various infections.

The plant Kodakasalai (*Justicia procumbens*) commonly known as Water Willow belongs to the Acanthaceae family. It is a small perennial shrub, distributed throughout the world and

endemic to India. The plant is slender, stems diffuse, with many divaricate branches, rootings at the lower nodes, the flowers pale purple, glabrous or pubescent.^[9]

It is bitter in taste and can be used in the form of decoction for treating variety of diseases including venereal diseases, boils, tumors, skin diseases, aphthous ulcer, cancer, diabetes, fever, headache, inflammation, arthritis and different gastrointestinal disorders.^[10-11] Among the chemical compounds from the plant are reported: lignans and their glycosides, alkaloids, tannins, diterpenoids, cyanogenetic compounds and saponins.^[12-13] The purpose of this study was to evaluate the antimicrobial potency of aqueous extract of Kodakasalai Chooranam (*Justicia procumbens*) by diffusion method in agar medium.

MATERIALS AND METHODS

Plant material

The plant *Justicia procumbens* used in this present study was collected from in and around Yercaud hills in Salem district on March 15, 2015 and botanical identification of this plant was performed by the botanist and Gunapadam experts of Government Siddha Medical College, Arumbakkam, Chennai. A specimen sample was kept in the department for future reference. After identification, the whole plant material was washed in order to remove dust, debris and sand under running tap water. Then it was allowed to complete drying at room temperature for 7 days. After then, the dried plant material was powdered well by using stone mortar sieved as per classical method by thin white cotton cloth and stored in a hermetically sealed container.^[14]

Preparation of extracts

1. Aqueous extract: To make 10% plant extract, 20 gm of powdered plant material was suspended in 200 ml of distilled water and kept in a boiling water bath for 20 minutes and was filtered through Whatmann filter paper no.1, autoclaved at 121 C for 15 minutes. Then the extract was kept in clean and sterilized test tubes at 4 C. The extract of the drug was used for further studies.

2. Ethanol extract: Ethanol extract of the plant *Justicia procumbens* was carried out as per the standard protocol.^[15] Dried plant material was ground and extracted in a percolator with 95% ethanol. About 10 ml of ethanol per gram of plant sample was used. Then the extract was dried under a reduced pressure at 40°C. The dried extract was stored in sterile bottle which was used for further studies.

3. Chloroform extract: Chloroform extract of *Justicia procumbens* was carried out as per standard guidelines.^[16] The plant material (100 g) was extracted with chloroform using a Soxhlet extractor for continuously 10 h or until the solvent changed in to pure and colorless. The solvent was removed using a rotary vacuum evaporator at 40°C to give a concentrated extract, which was then frozen and freeze-dried until use.

Test microorganisms

Strains of microorganisms

The strains used in testing antimicrobial activity of JP were obtained from Microbial Type Culture Collection (MTCC). Bacteria used in this present work are *Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus* (Gram positive), *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Proteus vulgaris* (Gram negative) and one fungal species *Candida albicans*.

Antimicrobial screening

Antimicrobial susceptibility test of the isolated organisms was done by disc diffusion technique established by Kirby-Bauer^[17] and the minimum inhibitory concentration (MIC) values of were determined by using the micro-broth dilution assay method which was recommended by the guideline of National Committee for Clinical Laboratory Standards.^[18]

All the strains of micro-organisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37°C for 24 hours and were referred to as seeded broth. For bacterial strains, Muller Hinton Agar (Himedia, Mumbai, India) plates were prepared and the test microorganisms were inoculated by the spread plate method. Filter paper discs of 6mm in diameter were impregnated with 20µl of the *Justicia procumbens* extract and placed on the upper layer of the prepared agar plates. The agar plates were then incubated 24 hours at 37°C. For fungal strain, Sabouraud dextrose agar media was prepared and incubated 24 hours at 28°C. The stock cultures were maintained at 4°C. Ciprofloxacin (10 µg/disc) was used as a standard drug for anti-bacterial screening Ketoconazole (10 µg /disc) was used as a standard drug for anti-fungal screening. After incubation, anti-bacterial and anti-fungal activity of the test drug aqueous, ethanol and chloroform extracts of *Justicia procumbens* were assessed by measuring the inhibition zone formed around the discs. However, in case of inconsistent results among the two MIC values, a particular test experiment was repeated in triplicate under same incubation conditions. The

MIC was defined as the lowest concentration of the *Justicia procumbens* extract that inhibited the growth of microorganisms compared with standard drug.

RESULTS AND DISCUSSION

This present study aimed to validate the antimicrobial activity of aqueous, ethanolic and chloroform extracts of *Justicia procumbens* against the various pathogens include *Bacillus subtilis*, *Streptococcus pyogens*, *Staphylococcus aureus* (Gram positive), *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Proteus vulgaris* (Gram negative) and one fungal species *Candida albicans*. Anti-microbial experiment was designed in which compares the ability of these three plant extracts to inhibit the growth of microorganisms through disc diffusion technique, using the Ciprofloxacin and Ketoconazole as positive control which were presented in Table No.1, Figure 1 and Figure 2.

The aqueous extract of *Justicia procumbens* showed moderate activity against gram negative bacteria with MIC values ranging between 3.125 – 12.5 mg/ml and no activity against gram positive bacteria when compared to the standard drug Ciprofloxacin with MIC values ranging between 25 – 50 mg/ml.

The aqueous extract of *Justicia procumbens* showed no activity against *Candida albicans* (MIC - 50 mg/ml) and inhibition zone diameter of 0 mm. Ketoconazole was very effective in inhibiting *Candida albicans* used for this study (10 µg) and inhibition zone diameter of 22 mm. So the aqueous extract of *Justicia procumbens* has potent antibacterial effect against gram negative bacteria but not against gram positive and fungi.

Indeed, the ethonolic and chloroform extracts of *Justicia procumbens* showed a significant inhibitory effect against all the microorganisms studied. The antibacterial activity was more apparent in ethanol extract as compared to chloroform extract for all tested organisms. It was also revealed that the anti-microbial activity of ethonolic extract of *Justicia procumbens* was more apparent on both gram positive and gram negative bacteria compared with aqueous and chloroform extracts as depicted in Table 1. Ethanolic extract of JP with MIC values ranging between 0.78 to 6.25 mg/ml. In connection with anti-fungal activity, Chloroform extract showed marked inhibitory effect than ethanol extract of *Justicia procumbens* and inhibition zone diameter of 22 mm for Chloroform extract and inhibition zone diameter of 16 mm for ethanol extract).

Finally, the results obtained in this present study showed potent anti-bacterial activity against the gram negative bacterial strains and anti-fungal activity against *Candida albicans*. Most antimicrobial activity was presented by the ethanolic and chloroform extracts, because the biologically active principles present in the plant *Justicia procumbens* are best extracted with ethanol and chloroform. Lignans and their glycosides, alkaloids, tannins, diterpenoids, cyanogenetic compounds and saponins found in this plant may be responsible for antimicrobial activity. Most of the antimicrobial herbs have been attributed to their potency due to the presence of tannins.^[19] Several studies have shown that the saponins contain a steroidal or a triterpenoid aglycone present in the medicinal plants are endowed with a great antibacterial and antifungal power due to their nonionic detergent properties.^[20-21] In addition, the strong inhibitory power of *Justicia procumbens* against microorganisms is also due to the presence of lignans. Because various lignan compounds are the main active principles of medicinal herbs responsible for the antibacterial activity.^[22-23] The synergistic interactions between the lignans, tannins and saponins may be responsible for a much more pronounced antibacterial activity of Kodakasalai Chooranam.

Table-1. The antimicrobial activity of Kodakasalai Chooranam (*Justicia procumbens*) against various bacterial strains and yeast by disc diffusion and micro broth dilution methods

Bacterial strains	SOLVENT EXTRACTS						Positive control	
	Aqueous		Ethanol		Chloroform			
	Disc (mm)	MIC (mg/ml)	Disc (mm)	MIC (mg/ml)	Disc (mm)	MIC (mg/ml)	C	K
<i>Bacillus subtilis</i>	0	50	18	6.25	16		16	NT
<i>Streptococcus pyogens</i>	0	25	19	3.125	18	6.25	17	NT
<i>Staphylococcus aureus</i>	0	25	19	3.125	15	12.5	17	NT
<i>Klebsiella pneumonia</i>	14	6.25	20	1.56	17	6.25	22	NT
<i>Pseudomonas aeruginosa</i>	16	3.125	21	1.56	13	12.5	24	NT
<i>Escherichia coli</i>	14	12.5	19	3.125	14	12.5	21	NT
<i>Salmonella typhi</i>	12	12.5	18	3.125	15	12.5	20	NT
<i>Shigella flexneri</i>	11	12.5	21	0.78	11	12.5	19	NT
<i>Proteus vulgaris</i>	14	6.25	20	0.78	16	6.25	22	NT
YEAST <i>Candida albicans</i>	0	50	16	12.5	20	6.25	NT	22

The inhibitory diameter was measured by Himedia zone of inhibition scale. C: Ciprofloxacin 10 µg. K: Ketoconazole 10 µg. NT: Not Tested.

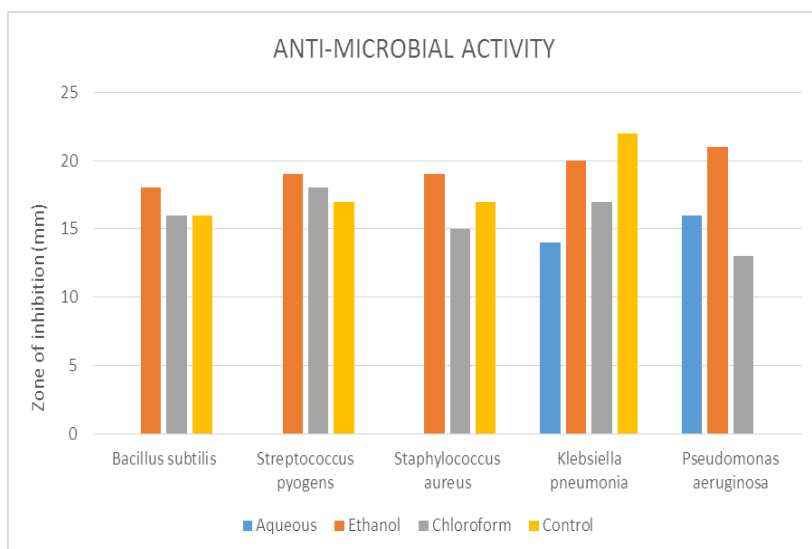


Figure 1: Average Zones of Inhibition of Kodakasalai Chooranam (*Justicia procumbens*). Control: Ciprofloxacin 10 µg / Ketoconazole 10 µg.

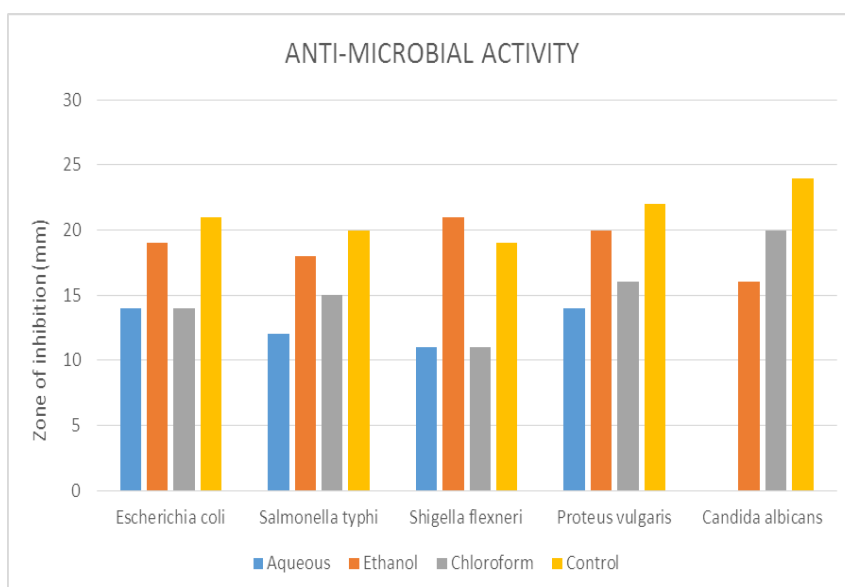


Figure 2: Average Zones of Inhibition of Kodakasalai Chooranam (*Justicia procumbens*). Control: Ciprofloxacin 10 µg / Ketoconazole 10 µg.

CONCLUSION

The aqueous, ethanol and chloroform extracts of *Justicia procumbens* presented marked antimicrobial activity against gram negative bacteria (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri* and *Proteus vulgaris*). Ethanol and chloroform extracts showed moderate activity against gram positive bacteria (*Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*). The antibacterial activity was more apparent in ethanol extract as compared to chloroform extract for all tested

organisms. The aqueous and ethanol extracts showed no activity against the yeast *Candida albicans*. But Chloroform extract of *Justicia procumbens* showed marked inhibitory effect against *Candida albicans*. It can be concluded that the results of this study support traditional use of Kodakasalai Chooranam (*Justicia procumbens*) for its antimicrobial activity. Further studies are needed to find out the chemical constituents responsible for inhibition of various microorganisms.

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