

EVALUATION OF INVITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF WHOLE PLANT *JUSTICIA BETONICA*

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ABSTRACT

The ethanolic extract of whole plant *Justicia Betonica* is very commonly used in India and Sri Lanka as traditional medicine for the treatment of wide variety of diseases. The whole plant extract was tested for its antibacterial activity against several gram positive and gram negative bacteria such as *Klebsiella Pneumonia* (ATCC33495), *Pseudomons Aeruginosa* (ATCC10662), *Escherichia Coli* (ATCC10536), *Bacillus Subtilis* (ATCC11774), *Staphylococcus Aereus* (ATCCBAA1026), the zone of inhibitions was compared with standard drug Amikacin. Further the antioxidant activity using different methods like DPPH, Nitric oxide and reducing power assay were tested on the whole plant *Justicia Betonica* and the free radical scavenging activity was compared with standard reference drug gallic acid.

Keywords: *Justicia Betonica*, Amikacin, Gallic Acid, Antibacterial and Antioxidant Activity.

INTRODUCTION

The medicinal plant *Justicia Betonica* very commonly known as white shrimp / squirrel tail belongs to family acanthaceae, this is regarded as useful medicinal plant in folkloric medicine and Kenyans use this plant for the treatment of diarrhea², orchitis³, headache and to reduce stomach gas. In our country inflorescence of the flowers is used orally for the treatment of vomiting⁴ and constipation⁵. The Indian and Sri Lankan community apply the crushed leaves of the plant to provide relieve of pain and swelling^{6,7}. Decoction of the whole plant is used by the local tribals in Tanzania to provide relief of stomach ache⁸.

MATERIALS AND METHODS

Collection of Plant

The whole plant of the medicinal plant *Justicia Betonica* was collected from interior parts of Jeddangi forest region of East Godavari District, Andhra Pradesh and the plant was

authenticated by taxonomist Prof. Dr. S.B.Padal.

Preparation of the Extract

The whole plant was dried under the shade coarsely powdered and was subjected to extraction process using soxhlet apparatus using ethyl alcohol for 72 hours. The solvent was evaporated and the crude extract was dried in a dessicator for few days and this extract powder was used for evaluation of antibacterial and antioxidant activities.

Antibacterial Activity

The various bacterial strains like *Klebsiella Pneumonia*, *Pseudomons Aeruginosa*, *Escherichia Coli*, *Bacillus Subtilis*, *Staphylococcus Aereus* were procured from microbes specialty lab in Rajahmundry, East Godavari District, Andhra Pradesh.

The Antibacterial activity is determined according to the standard method using Agar cup plate method¹. 20 ml of sterile nutrient

agar medium was poured in sterile petri dishes and allowed to solidify. The petri dishes were incubated at 37° for 24 hours to check for sterility. The medium was seeded with organisms by Pour plate method using sterile agar broth 1 ml culture. Bores were made on the medium using borer. *Justicia Betonica* whole plant extract was dissolved in water to obtain 50 mg/ml, standard drug Amikacin at 20ug /ml was taken as standard reference. All the plates were kept in a refrigerator at 2° to 8° for a period of 3 hours, later they were incubated at 37° for 24 hours. The diameter zone up inhibitions were measured and recorded and the results are tabulated in table 1. The maximum zone of inhibition is seen with *Pseudomonas Aeruginosa* 13.20mm ± 0.44 as compared to Amikacin 16mm ± 0.52.

Antioxidant Activity

DPPH Radical Scavenging Assay

Justicia Betonica whole plant extract was subjected to DPPH radical scavenging assay, the extract at a concentration of 100ug/ml showed maximum inhibition is 68.60% and the results was compared with standard drug gallic acid 2.5 ug/ml 81.20%. The results are tabulated in table 2.

Nitric Oxide Scavenging Activity

The whole plant of *Justicia Betonica* extract was subjected to nitric oxide scavenging assay and the extract showed maximum inhibition at 100 ug/ml is 42.40% and the result is compared with standard drug gallic acid 2.5 ug/ml 56.40%. The results are tabulated in table 3.

Reducing Power Assay

The concentration of *Justicia Betonica* whole plant extract 100 ug/ml and standard drug gallic acid 2.5 ug.ml were prepared using distilled water, 1% of potassium ferricyanide, 10% trichloro acetic acid, 0.1% ferric chloride and 0.2M phosphate buffer were prepared using distilled water. Gallic acid was taken as the reference standard. Then 1 ml of each concentration of both extract and standard were taken separately and mixed with 1 ml of 0.2M phosphate buffer (p^H 6.6) and 1 ml of

potassium ferricyanide. Incubate all these samples at 50°C for 20 min. Then add 1 ml of 10% trichloro acetic acid and centrifuge at 2000 RPM for 10 min. Now separate the upper layer (2.5ml) and then add (2.5ml) distilled water, 0.5ml of freshly prepared ferric chloride. Finally measure the absorbance at 700nm. The results are tabulated in Table 4.

RESULTS AND DISCUSSION

The *Justicia Betonica* whole plant extract produced significant antibacterial activity against both gram positive and gram negative bacteria and the zone of inhibitions for various organisms are quite encouraging and the maximum zone of inhibition is produced with *Pseudomonas Aeruginosa* 13.20mm as compared to standard drug Amikacin which produced zone of inhibition 16mm. The *Justicia Betonica* whole plant extract exhibited good anti oxidant activity, the scavenging activity of the extract showed maximum inhibition in DPPH radical assay 68.60% against standard drug gallic acid 81.20%, similarly in Nitric oxide and reducing power assay methods the whole plant extract produced significant anti oxidant activity with maximum percentage of inhibition 42.40% against 56.40% and Reducing power response of 0.584 against standard drug gallic acid 0.686 respectively.

CONCLUSION

The results of antibacterial and antioxidant activities are excellent and the whole plant extract of *Justicia Betonica* is considered as traditional folkloric medicine in our country as well in Sri Lanka and African Continent, the local natives were using this medicinal plant for the treatment of many diseases. The antibacterial and antioxidant activities are said to be encouraging and a sincere attempt need to be initiated to prepare possible herbal preparation which can be used as an authentic medicine in Ayurvedic treatment. The researcher is advised to pursue further work on this plant and explore the possible isolation of bioactive phytoconstituents responsible for various biological activities.

Table 1: Antibacterial activity of Justicia Betonica whole plant extract

S.No.	Organism used	Extract of <i>Justicia Betonica</i> Zone of inhibition in mm	Standard Drug Amikacin 20 ug /ml Zone of inhibition in mm
1	<i>Klebsiella Pneumonia</i> (ATCC33495)	12.5±0.40	17±0.50
2	<i>Pseudomonas Aeruginosa</i> (ATCC10662)	13.2±0.44	16± 0.52
3	<i>Escherichia Coli</i> (ATCC10536)	12.8± 0.36	18± 0.24
4	<i>BacillusSubtilis</i> (ATCC11774)	12.2±0.52	19± 0.46
5	<i>Staphylococcus Aereus</i> (ATCCBAA1026)	10.8± 0.30	16+ 0.50

Values are mean ± SEM n= 3, zone of inhibition in mm, Standard drug Amikacin

Table 2: DPPH Radical Scavenging Assay of Justicia Betonica whole plant extract

S.No.	Test	Concentration ug/ml	% of inhibition
1	<i>Justicia Betonica</i> whole plant extract	100	68.60 ± 0.38
2	Gallic Acid (Standard)	2.5	81.20 ± 0.44

Values are Mean + SEM n = 3

Table 3: Nitric Oxide Scavenging Activity of Justicia Betonica whole plant extract

S.No.	Test	Concentration ug/ml	% of inhibition
1	<i>Justicia Betonica</i> whole plant	100	42.40± 1.20
2	Gallic Acid (Standard)	2.5	56.40 ±2.30

Values are Mean + SEM n = 3

Table 4: Results of Absorbance in Reducing power assay

Tested Material	Concentration (ug /ml)	Reducing power Absorbance
<i>Justicia Betonica</i> whole plant	100	0.584± 0.02
Gallic Acid (Standard)	2.5	0.686± 0.04

Values are Mean + SEM n = 3

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