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Antimicrobial activity of ethanolic extracts of *Justicia neesii*

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Abstract

Agar well diffusion method was used to evaluate the antimicrobial potential of *Justicia neesii* extract. The maximum activity index (AI) values are observed against *Klebsiella pneumonia* (1.208) and low AI value for *Streptococcus faecalis* (0.963) compared to other bacterial species. The maximum AI values are observed against *Saccharomyces cerevisiae* (1.147) and low AI value for *Fusarium axisporum* (0.986) compared to other fungal species. The MIC and MBC/MFC values indicated the bacteriostatic/ fungistatic nature of the extract and also having good correlation with the zone of inhibition values. The total activity values indicated that *J. neesii* extract can show antimicrobial activity even at higher dilutions, except for Gram negative bacteria.

Introduction

Wide range of plants from tropical and sub-tropical regions of India were reported to posses antimicrobial properties (Prasannabalaji et al., 2012). *J. neesii* is one of such plant belongs to the family Acanthaceae grows in tropical regions of India as a small tropical herb. In the previous studies on this plant reported the presence of various types of lignans. Three β-apolignans including 1,4-dihydotaiwanin C, jusneesiin, jusneesiinol (Rajasekhar, 1998) and two arylnaphthalide lignans including jusmicranthin and justirumalin are found to be present (Rajasekhar, 1999; Gopalaiah, 2001). The plant was also found to contain diphyllin glycosides like neesiinoside A and neesiinoside B (Subbaraju, 2001). But, the review of scientific literature reviled that there is no significant pharmacological work done on *J. neesii*. So it was consider worthwhile to elucidate the antimicrobial properties of *J. neesii* plant extracts.

Materials and Methods

Collection and identification of plant: Plant material free from infection was collected from different areas of East Godavari district, Andhra Pradesh during the month of February 2014 on day time. The plant was taxonomically identified by the experts of Botanical Survey of India, Hyderabad (BSI/DRC/2013-14/Tech./915-A).

Extraction of plant material: Whole plant parts including leaves, stem, twigs, flowers, seeds, roots were separated and made free from soil matter. They were dried and powdered by using hand pulveriser to a course powder. Then the powder was extracted with ethanol by using sohxlet apparatus at a temperature of 50-55°C for 8 hours. The extracts were concentrated using vacuum evaporator and the semisolid mass was dried in vacuum desiccators. The yield of plant extract was found to be 10.58 % (w/w).

Preparation of nutrient agar: The weighed amount of NaCl (5 g), peptone (10 g), beef extract (10 g) are dissolved in 1,000 mL of the water, then agar (20 g) is added slowly on heating with continues stirring until agar is completely dissolved and pH is adjusted to 7.2 to 7.4. This nutrient agar medium is then sterilized by moist heat sterilization method using autoclave at temperature of 120°C at 15 lb pressure maintained for 15 min.

Preparation of potato dextrose agar (PDA): A potato was peeled and 100 g was measured, finely chopped and boiled to a mash in distilled water. The dextrose was measured (12.5 g) and placed in a 1L measuring cylinder. Agar was measured (12.5 g) and added to the measuring cylinder (with the dextrose). The potato

mash was stirred and strained into the cylinder. Hot distilled water was added to make up 500 mL. The contents was continuously poured and stirred until consistency was achieved. The content was then poured into a conical flask, plugged with cotton wool, over which aluminum foil was tightly wrapped. The flask was then autoclaved at 121°C for 24 hours (Murray, 1995).

Microbial cultures: Bacterial cultures of Gram positive bacteria- *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3160), *S. faecalis* (MTCC 459), Gram negative bacteria- *Escherichia coli* (MTCC 46), *Pseudomonas aeruginosa* (MTCC 1688), *Klebsiella pneumonia* (MTCC 4032) and fungal cultures of *Aspergillus flavus* (MTCC 277), *Aspergillus niger* (MTCC 2723), *Candida albicans* (MTCC 183), *Fusarium axisporum* (MTCC 1755), *Saccharomyces cerevisiae* (MTCC 4742) were procured from Institute of Microbial Technology, Chandigarh, Punjab, India. All the test organisms were maintained on nutrient/potato dextrose agar slopes and sub-cultured once in every two weeks.

Preparation of inoculums: The test inoculums are prepared as per the McFarland method. McFarland standard was prepared by adding 0.5 mL of 1.175%w/v barium chloride to 85 mL of 1%v/v sulfuric acid and mixed. Then the volume was made up to 100 mL with 1% sulfuric acid. The optical density of the prepared solution was checked at 625 nm range gives an absorbance ranging from 0.08 to 0.10. The bacterial inoculums are prepared from 24 hour old cultures by taking 3-5 morphologically similar colonies of respective micro organisms and transferred into 5 mL sterile saline solution and adjusted to 0.5 McFarland turbidity standards equivalent to the cell density of 1-5 x 10^8 CFU/mL. The fungal inoculums are prepared from seven days old culture plates by taking 3-5 morphologically similar colonies of respective micro organisms and transferred into 5 mL sterile saline solution and adjusted to 0.5 McFarland turbidity standards. From this solution 1:10 dilution are preformed three times with growth medium to get inoculums density of 1-5 x 10^3 CFU/mL (Mcfarland, 1907).

Antimicrobial assay: The anti-microbial assay was carried out by agar well diffusion assay (Perez, 1990). The sterilized microbial medium was cooled to 50°C. 20 mL of the microbial media was taken into sterile universal bottles and seeded with 0.2 mL of respective cultures of standard inoculums size aseptically. Then the seeded media was transferred to the sterile petri dishes under aseptic conditions. The wells are created by using sterile cork borer of 6 mm diameter at equidistance points. The respective wells are supplied with 200, 400 and 800 µg of test drug in 25 µL volume. The petri plates were then kept in refrigerator for diffusion and then transferred to biological incubator and bacterial culture plates are incubated at 37°C for 24 hours and fungal culture plates at 27°C for 48 hours. Ciprofloxacin and nystatin are used as positive controls at 10 µg/well concentration to compare the antibacterial and antifungal affects respectively. Each experiment was performed in triplicate and the zone of inhibition values are noted after incubation period by using Antibiotic zone reader. Activity index for each extract was calculated by using following formula.

Activity index (AI) = Inhibition Zone of the sample/ Inhibition Zone of the standard.

Minimal inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) is the concentration required to inhibit growth of a specific isolate in vitro under standardized conditions. The MIC of test extract on each organism was determined by using serial dilution method. For this purpose 10 mg/mL concentration of test solution was prepared and two fold serial dilutions are made to get 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.019 mg/mL concentrations of solutions. 1 mL of test solution is then taken into each test tube and diluted with 1 mL of sterile nutrient agar media (for bacteria) or potato dextrose agar media (for fungi). The test tubes were then inoculated with 0.1 mL of microbial suspension of standard size. The tubes were incubated at 37°C for 24 hours for bacteria and 28° C for 48 hours for fungi in a biological incubator and observed for change in turbidity and compared with the growth in control tubes which contains 75% ethanol in water (Pelczer et al., 2004).

Minimum bactericidal/ fungicidal concentration (MBC/ MFC): The MBC/MFC is the lowest concentration at which the culture has been completely sterilized. 50 µL of the media from each test tube showing no visible growth was taken and inoculated into 2 mL of sterile broth media and incubated as done in MIC determination.

Total activity (TA) determination: Total activity is the volume at which the test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in mL/g (Eloff, 2004).

Total activity = Extract per gram dried plant part/MIC of extract

Results

The antimicrobial nature of ethanolic extract of *J. neesii* was estimated by the zone of inhibition and activity index values. The plant extracts at all the concentrations showed significant antibacterial activity compared standard drug ciprofloxacin in dose-dependent manner (Table I, II).

The maximum AI values are observed against *K. pneumonia* (1.208) and low AI value for *S. faecalis* (0.963) compared to other bacterial species. However, the maximum zone of inhibition values are observed for Gram positive bacteria compared to Gram negative bacteria. The minimal inhibitory concentration values have shown good correlation with IZ values. The Gram positive bacteria showed lowest MIC of 0.039 mg/mL compared to the other species. Higher MBC values are observed for most of the bacteria compared to their MIC. The plant having higher antibacterial potential

Table I

Antibacterial activity of *J. neesii* against Gram positive bacteria

Treatment	Test organism					
	Bacillus subtilis		*Staphylococcus aureus*		*Streptococcus faecalis*	
	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
Ciprofloxacin (10 µg/well)	23.3 ± 0.7	-	27.0 ± 0.6	-	27.3 ± 0.3	-
J. neesii (200 µg/well)	12.7 ± 0.7	0.543	13.7 ± 0.3	0.506	12.7 ± 0.9	0.464
J. neesii (400 µg/well)	18.0 ± 0.6	0.771	19.3 ± 0.7	0.716	17.3 ± 0.3	0.634
J. neesii (800 µg/well)	26.0 ± 0.6	1.114	28.0 ± 0.6	1.037	26.3 ± 0.9	0.963

Values are expressed in mean ± SEM; n = 3

Table II

Antibacterial activity of *J. neesii* against Gram negative bacteria

Treatment	Test organism					
	Escherichia coli		*Pseudomonas aeruginosa*		*Klebsiella pneumonia*	
	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
Ciprofloxacin (10 µg/well)	17.7 ± 0.3	-	18.0 ± 0.6	-	16.0 ± 0.6	-
J. neesii (200 µg/well)	09.0 ± 0.6	0.509	10.7 ± 0.3	0.593	10.0 ± 0.6	0.625
J. neesii (400 µg/well)	14.7 ± 0.3	0.830	16.0 ± 0.6	0.889	15.7 ± 0.9	0.979
J. neesii (800 µg/well)	20.3 ± 0.3	1.150	20.7 ± 0.3	1.148	19.3 ± 0.9	1.208

Values are expressed in mean ± SEM; n = 3

Table III

Antifungal activity of *J. neesii* against pathogenic fungi

Treatment	Test organism									
	Aspergillus flavus		*Aspergillus niger*		*Candida albicans*		*Fusarium axisporum*		*Saccharomyces cerevisiae*	
	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI	IZ (mm)	AI
Nystatin (10 µg/well)	23.7 ± 0.3	-	24.3 ± 0.3	-	27.0 ± 0.6	-	24.3 ± 0.9	-	25.0 ± 0.6	-
J. neesii (200 µg/well)	13.0 ± 0.6	0.549	14.7 ± 0.9	0.603	13.0 ± 0.6	0.481	11.7 ± 0.3	0.480	13.3 ± 0.7	0.533
J. neesii (400 µg/well)	18.7 ± 0.3	0.789	18.0 ± 0.6	0.740	18.7 ± 0.7	0.691	15.7 ± 0.9	0.644	19.3 ± 0.9	0.773
J. neesii (800 µg/well)	25.0 ± 0.6	1.056	25.0 ± 0.6	1.027	28.7 ± 0.3	1.062	24.0 ± 0.6	0.986	28.7 ± 0.9	1.147

Values are expressed in mean ± SEM; n = 3

against Gram positive bacteria (TA: 2712.82 mL/g).

The plant extracts at all the concentrations showed significant antifungal activity compared standard drug nystatin in dose-dependent manor (Table III).

The maximum AI values are observed against *S. cerevisiae* (1.147) and low AI value for *F. axisporum* (0.986) compared to other fungal species. Low MIC values are observed for *Candida albicans* and *S. cerevisiae* (0.039 mg/mL) compared to other fungi. The plant having higher antifungal potential against Gram positive bacteria *Candida albicans* and *S. cerevisiae* (TA: 2712.82 mL/g). The plant has shown good MIC and MBC/MFC values against all microorganisms (Table IV).

Discussion

The activity index values are helpful in estimating the potential of antimicrobial activity quantitatively compared to the respective standards. The plant extract has shown higher AI values against Gram negative bacteria which means that the extracts are having good activity against the Gram negative bacteria compared to the standard. However, when we observe the IZ values the highest inhibition was observed against Gram positive

bacteria and fungi. So we can say that the plant extract is having good antibacterial activity against both the Gram strains and showing higher activity against Gram negative bacteria compared to the standard ciprofloxacin. The low IZ values of Gram negative bacteria may be due to its resistant cell wall composition. The higher values of MBC/MFC than that of MIC indicated that bacteriostatic/fungistatic nature of the extracts, which were observed for the active extracts. Total activity indicates the volume at which extract can be diluted which still having ability to kill microorganism. From the results we can say that the plant extract can show antimicrobial activity even at higher dilutions, except for Gram negative bacteria like *E. coli.*

Our preliminary phytochemical investigation shows the presence of flavonoids, glycosides, lactones, lignins, phenols, phytosterols, quinins, reducing sugars, saponins and terpinoids. The ability of flavonoids in forming complexes with cell walls of micro organisms and changing the structural integrity, positioned them in class of antibiotics. The antibiotic nature of flavonoids also increases with their lipophilicity (Jeyaseelan, 2012). The terpenes and phenols also exhibit antimicrobial properties (Shabir, 2011). The antimicrobial potential of triterpinoid saponins and lignans are also found in recent studies (Khan, 2011; Vasilev, 2005). The significant antimicrobial properties observed in this screening may be due to the presence of one of these phytochemicals in the ethanolic extract of the *J. neesii*.

This experiment concluded that *J. neesii* is having potential antimicrobial activity. However, elucidating the exact phytochemicals responsible for this activity of *J. neesii* can be helpful in developing lead compounds and to overcome the limitations of current work.

Table IV

MIC, MBC/MFC and TA values of *J. neesii*

Microorganism	MIC	MBC/ MFC	Total activity (TA)
Bacillus subtilis	0.039	0.156	2712.82
Staphylococcus aureus	0.039	0.078	2712.82
Streptococcus faecalis	0.039	0.156	2712.82
Escherichia coli	0.312	1.250	339.10
Pseudomonas aeruginosa	0.156	0.650	678.20
Klebsiella pneumonia	0.156	0.312	678.20
Aspergillus flavus	0.078	0.312	1356.47
Aspergillus niger	0.078	0.312	1356.47
Candida albicans	0.039	0.156	2712.82
Fusarium axisporum	0.156	0.625	678.20
Saccharomyces cerevisiae	0.039	0.312	2712.82

TA was calculated by taking the yield: 105.8 mg/g dried plant and values are expressed in mL/g

References

Eloff JN. Quantifying the bioactivity of the plant extracts during screening and bioassay guided fractionation. Phytomedicine 2004; 11: 370-71.

Gopalaiah K, Kavitha J, Kanumuri RV, Rajasekhar D, Subbaraju GV. *Justicia* lignans: Part 9- Two new lignans from *Justicia neesii* Ramamoorthy. (White flower variety). Indian J Chem. 2001; 40B: 596-600.

Jeyaseelan EC, Jashothan PTJ. *In vitro* control of *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) by Ricinus communis L. Asian Pac J Trop Biomed. 2012; 2: 717–21.

Khan NA. *In vitro* antimicrobial activity of triterpenoid saponin from *Tephrosia purpurea* seeds extract. Eur J Chem. 2011; 2: 189-92.

Mcfarland J, Jama MD. The nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. JAMA 1907; 14: 1176.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH. Manual of clinical microbiology. 6th ed. London, Mosby Year Book, 1995.

Pelczer MJ, Chan ECS, Noel RK. Microbiology. 4th ed. India, Tata McGraw Hill, 1998.

Perez C, Paul M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Acta Biol Med Exp. 1990; 15: 113-15.

Prasannabalaji N, Muralitharan G, Sivanandan RN, Kumaran S, Pugazhvendan SR. Antibacterial activities of some Indian traditional plant extracts. Asian Pac J Trop Dis. 2012; S291-95.

Rajasekhar D, Subbaraju GV. Justicia lignans- V. Three new beta-apolignans from *Justicia neesii* Ramamoorthy. Tetrahedron 1998; 54: 13227-36.

Rajasekhar D, Vanisree M, Subbaraju GV. Justicia lignans: Part 4- Two new arylnaphthalide lignans from *Justicia neesii* Ramamoorthy. Indian J Chem. 1999; 38B: 713-17.

Shabir G, Anwar F, Sultana B, Khalid ZM, Afzal M, Khan QM, Ashrafuzzaman M. Anti-oxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of gold mohar [*Delonix regia* (Bojer ex Hook.) Raf]. Molecules 2011; 16: 7302-19.

Subbaraju GV, Rajasekhar D, Kavitha J, Jimenez JI. Justicia lignans: Part 7-Two new diphyllin glycosides from *Justicia neesii* ramamoorthy. Indian J Chem. 2001; 40B: 313-19.

Vasilev NP, Ionkova I. Cytotoxic activity of extracts from Linum cell cultures. Fitoterapia 2005; 76: 50-53.

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