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ANTIBACTERIAL ACTIVITIES OF PHYTOCHEMICAL EXTRACTS FROM THE LEAVES OF *JUSTICIA GENDARUSSA* BURM.F

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ABSTRACT

Phytochemicals are chemical compounds that occur naturally in plants and protect plants against bacteria, viruses, and fungi. They are non-nutritive plant chemicals that have protective and disease preventive properties.The aim of the present study was to investigate the antibacterial activities of phytochemical extracts from the leaves of *Justicia gendarussa*. The antibacterial activities of *Justicia gendarussa* phytochemical extracts were determined by agar disc diffusion and minimum inhibitory concentration (MIC) against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Vibrio cholerae.*The highest zone of inhibitory activity was observed with alkaloid extract of the plant against the test bacteria while less antibacterial activity was observed in terpenoid, flavanoid and glycoside extracts. Therefore, it was confirmed that alkaloid extract of Justicia gendarussa containss a high level of antibactericidal compound which can be used as a novel chemotherapeutic drug for bacterial diseases.

KEYWORDS: ***Justicia gendarussa*, Antibacterial activities, Alkaloid extract and novel chemotherapeutic drug.**

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INTRODUCTION

India is an emporium of medicinal plants. It is one of the richest countries in the world with regard to its genetic resources of medicinal plants. In recent years, the secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents[1]. Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern[2]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens[3].There is a continuous need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action[4]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections[5]. *Justicia gendarussa* belongs to the family *Acanthaceae.* The family *Acanthaceae* is a taxon of dicotyledonous flowering plants containing almost 240 genera and 2200 species[6]. Most are tropical herbs, shrubs or twining vines, some are epiphytes. Only a few species are distributed in temperate region. It is commonly called as 'Neernotchi' in Tamil. Leaves are linear lanceolate, glabrous; flower small, white with pink or purple spots inside. The plant is used in traditional medicinal practice for chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases, fever, hemiplegia, headache, earache, muscle pain, respiratory disorder and digestive trouble. The roots of this plant are also used to treat cough, jaundice, arthritis, cephalgia, facial paralysis, otalgia, hemicranias and liver disorders[7]. The dried leaves are used to repel insects from clothing.

MATERIALS AND METHODS

Plant material

Justicia gendarussa leaves were collected from Medavakkam near Tambaram, Tamilnadu, India. The plant was identified and authenticated by Dr. S.Sankaranarayanan, Head of the department,Department of Medicinal Botany, Sri Sairam Siddha Medical College, Tambaram, Chennai. The plant material was air-dried under shade at room temperature, ground with an electric grinder into fine powder and stored in airtight containers for analysis.

Bacterial strains

The bacterial strains used were Gram positive: *Staphylococcus aureus* MTCC 29213; and Gram negative: *Escherichia coli* MTCC 25922, *Proteus mirabilis* MTCC 13315 and *Vibrio cholerae* MTCC 12657. All bacterial strains were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh – 160036, India.

Phytochemical analysis of the plant extract

The screening of plant secondary metabolites such as alkaloids, terpenoids, flavonoids, glycosides, anthroquinones, tannins and saponnins was performed using the standard procedure given by Trease and Evans[8] and Harborne[9].

Extraction of phytochemicals from the leaves of J.gendarussa

Extraction of Alkaloid

Finely powdered leaf material was extracted with cold distilled methanol with occasional swirling. After filtration, the solvent was removed under reduced pressure at 40 ^{o}C, to minimise any thermal degradation of the alkaloids. The crude alkaloid mixture was then separated from neutral and acidic materials, and from water solubles, by initial extraction with aqueous acetic acid followed by dichloromethane. The basification was done on the aqueous solution and the organic layer of dichloromethane contained crude alkaloid extract[10].

Extraction of Terpenoids

Ground leaves were extracted with hot (60 ^{o}C) 95% ethanol. After filtration, the dark green solvent was evaporated to dryness under reduced pressure at 40 ^{o}C. The residue was partitioned between water and chloroform. The organic layer was separated and condensed to yield dark green syrup. The chloroform extract was then partitioned between hexane and 10% aqueous methanol. The aqueous methanol extract was then used for antibacterial activity as terpenoid extract[11].

Extraction of Flavonoids

The dried leaf powder of *J.gendarussa* was defatted with petroleum ether (40-60 °C). The extract was then percolated with methanol until exhaustion at 40 ^{o}C by rotary evaporator. The condensed material was partition using ethyl acetate. The ethyl acetate extract contains crude flavonoids[12].

Extraction of Glycosides

The powderd leaf material of the plant was extracted three times with methanol at 25 ^{o}C for 24 hours and then concentrated in vaccum. The extract was washed with n-hexane and then the methanol layer was further concentrated to a gummy mass. The later was suspended with water and extracted with equal volume of ethyl acetate to obtain glycoside extract of the plant[13].

Antibacterial activities of four different phytochemical extracts of J.gendarussa tested against pathogenic bacteria

Agar disc diffusion assay

The antibacterial activity was studied using the disc-diffusion method[14]. Bacteria were grown overnight on Muller Hinton (MH) agar plates. Five young colonies were suspended with 5ml of sterile saline (0.9%) and the density of the suspension adjusted to approximately 3×10^{8} colony forming units (CFU). The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90 ° to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile paper disc of 5 mm diameter. Each disc was tapped gently down onto the agar to provide uniform contact. Phytochemical extracts (50µg) were weighed and dissolved in 1ml of 7% Methanol. 5, 10, 15 and 20 microlitres of the compounds were introduced on each disc (five replicates) and 7% methanol alone served as a negative control. The plates were incubated at 37 °C for 24 h; inhibition zones were measured and calculated.

Minimum inhibitory concentration (MIC)

The MIC of the isolated compounds was determined by dilution method[15]. The strains were grown in MH broth to exponential phase with an *A*560 of 0.8, representing 3×10^{8} CFU/ml. Different dilutions of the *J.gendarussa* phytochemical extracts were prepared to give concentrations at 5, 10, 15 and 20 µg/ml respectively. A 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 10^{8} CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% methanol were used as bacterial controls, 4.5 ml of uninoculated MH broth and 0.5 ml plain broth solution served as a blank. The tubes were incubated at 37 °C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at *A*560 nm.

RESULTS AND DISCUSSION

The phytochemical screening of the *J.gendarussa* showed the presence of alkaloids, terpenoids, flavanoids, glycosides, tannins and saponnins (Table-1). The presence of starch, glycosides, saponins, tannins, phenolic compounds, terpenoids, steroids and flavonoids in J.gendarussa was already recorded.[16]

Table 1
Phytochemical screening of leaf extract of J.gendarussa

SL NO.	PHYTO CONSTITUENTS	TEST PERFORMED	OBSERVATION	PRESENT/ABSENT
1	Alkaloids	Dragendorff's test	Orange / red precipitate	++
		Mayers test	Yellowish precipitation	
2	Flavonoids	Alkali reagent test	Intense yellow colour	++
3	Glycosides	Keller-killani test	Formation of brown ring interface	++
4	Tannin	$Fecl_3$ test	Brownish green coloration	++
5	Saponins	Frothing test	Foam	++
6	Terpenoids	Salkowski test	Reddish brown colouration of the interface	++
7	Anthraquinones	Benzene Ammonia Test	Pink colour	--

-- = Negative (absent); ++= Positive (present)

Table 2
Antibacterial activities of phytochemical extracts of J.gendarussa against bacterial species[Diameter of the inhibition zone (mm)*]

Type of extract	*Staphylococcus aureus*				*Vibrio cholerae*			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Alkaloid extract	12.33±0.58	13.9±0.5	14±0	15.33±0.58	9±0	9.33±0.58	10.58±0.41	11.9±0.58
Terpenoid extract	9±0	9.33±0.58	10.03±0.37	11.6±0.55	8.3±0.81	9.3±0.40	10.03±0.37	11.6±0.55
Flavonoid extract	8.3±0.81	9±0	10.9±0.28	11.5±0.5	7.3±0.58	8.38±0.81	9.33±0.58	9.97±0.37
Glycoside extract	7±0.5	8.1±.0.5	9.33±0.58	10.4±0.61	6.9±0.65	8.5±0.40	8.9±0.9	9.32±0.27

Type of extract	*Escherichia coli*				*P.aeroginosa*			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Alkaloid extract	10.33±0.58	11.9±0.5	12±0	13.33±0.58	8.03±0.55	9.6±0.32	10.2±0.60	11.06±0.60
Terpenoid extract	7.5±0.81	8.3±1.17	9.1±0.28	10.9±0.36	8±0	9.31±0.58	10.03±0.37	10.6±0.55
Flavonoid extract	6.6±0.57	7.5±0.86	8.8±0.76	9.5±0.5	5.7±0.64	6.6±0.79	7.4±0.40	8.05±0.45
Glycoside extract	5.7±0.64	6.6±0.79	7.4±0.40	8.05±0.45	5.6±0.57	6.5±0.86	6.83±0.76	7.58±0.5

****The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD of three replicates.***

Phytochemical extracts thus obtained were further subjected for determination of minimal inhibitory concentration by two-fold micro broth dilution method against the bacteria studied. Table-3 indicates that the alkaloid extract was found to be most significant inhibitor than the other extracts. MIC of this extract showed gradient value against the concentration used to inhibit the bacteria. Furthermore, gram positive bacterial species was found most sensitive as compared to gram negative species.

Table 3
Minimal Inhibitory Concentration (MIC) of different phytochemical extracts against bacteria [Optical density values at (560nm)*]

Type of extract	*Staphylococcus aureus*				*Vibrio cholerae*			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Alkaloid extract	0.326±0.05	0.225±0.01	0.142±0.01	0.106±0.03	0.514±0.02	0.425±0.01	0.315±0.01	0.213±0.01
Terpenoid extract	0.544±0.02	0.425±0.01	0.315±0.01	0.213±0.01	0.579±0.05	0.402±0.07	0.396±0.01	0.233±0.04
Flavonoid extract	0.579±0.05	0.462±0.07	0.296±0.01	0.233±0.04	0.660±0.04	0.569±0.09	0.469±0.04	0.409±0.06
Glycoside extract	0.678±0.05	0.462±0.07	0.396±0.01	0.233±0.04	0.647±0.03	0.557±0.02	0.424±0.01	0.332±0.01

Type of extract	*Escherichia coli*				*P.aeroginosa*			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Alkaloid extract	0.559±0.05	0.462±0.07	0.396±0.01	0.233±0.04	0.524±0.02	0.425±0.01	0.335±0.01	0.213±0.01
Terpenoid extract	0.614±0.02	0.425±0.01	0.315±0.01	0.313±0.01	0.679±0.05	0.402±0.07	0.396±0.01	0.233±0.04
Flavonoid extract	0.660±0.04	0.569±0.09	0.469±0.04	0.409±0.06	0.660±0.04	0.569±0.09	0.469±0.04	0.409±0.06
Glycoside extract	0.647±0.03	0.557±0.02	0.424±0.01	0.332±0.01	0.747±0.03	0.557±0.02	0.424±0.01	0.332±0.01

The Minimal Inhibitory Concentration was determined by measuring the turbidity of the bacterial culture that is the mean of triplicates ± SD of three replicates.

CONCLUSION

The present study clearly proved that the alkaloid compound of *J.gendarussa* posses greater antibacterial activity on both gram positive and gram negative bacteria. Development or synthesis of novel antibacterial drugs from this medicinal plant for treating bacterial diseases may be further researched for the benefit of mankind.

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