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Research Article

Phytochemical Evaluation, GC-MS Analysis of Bioactive Compounds and Antibacterial Activity Studies from *Justicia gendarussa* Burm.F. Leaf

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ABSTRACT

Justicia gendarussa Burm F. (family Acanthaceae) known as Willow-leaved justicia in English, it is native to china. It is commonly found throughout the greater part of India and Andaman islands. *J. gendarussa* is one of the important herbal being used in Ayurvedic system of medicine. Most herbal medicines and their derivative products were often prepared from crude plant extracts, which comprise a complex mixture of different extracts. The aim of this study was to carry out for analysed phytochemical constituents such as flavonoids, alkaloids, steroids, terpenoids, saponins, phenolic compounds and carbohydrates from methanol, chloroform and petroleum ether extract of *Justicia gendarussa* and identification of bioactive compounds from different extracts of *Justicia gendarussa* by Gas chromatography and Mass spectroscopy (GC-MS). The bioactive principles were described with their molecular formula, retention time, molecular Weight, peak area (%). Physico – chemical values were analysed such as foreign organic matter, Moisture content, Total ash. Florescence analysed in leaf for Visible light condition under the UV rays (254nm, 366nm). The antimicrobial study was also carried out against two micro organisms such as Pseudomonas vulgaris and Pseudomonas pneumonia. Our results confer the utility of this plant extract in developing a novel broad spectrum antimicrobial agent.

Keywords: Phytochemical, GC-MS analysis, Pharmacognosy, antimicrobial components, *J. gendarussa*.

INTRODUCTION

WHO (2001) estimated that 80% of world population rely on medicinal plants for their primary health care needs. Out of the 3,50,000 plant species known so far, about 35,000 (some estimate up to 70,000) are used worldwide for medicinal purposes and less than about 0.5% of these have been investigated for their phytochemical and pharmacological potential[1]. Nearly, 95 % of plants used in traditional medicines are collected from forests and other natural sources. The plants collected from different sources show wide disparity in therapeutic values and also much variation in market rates. In the recent years, there has been greater expansion of indigenous drug industry in India. Consequently, the demand for the new material (medicinal plants) has enormously increased[2]. Medicinal plants are the best source for the production of new herbal drugs. The efficacies of the plant extracts are due to the presence of phytochemical. Systematic screening of the plant extract and phytochemicals result in the discovery of novel effective compounds[3]. Tribal generally obtained the medicine from a single species of plants. The indigenous traditional knowledge of medicinal plants of various ethnic communities, Ethno medicine these tribal groups living in biodiversity rich areas possess a wealth of knowledge on the utilization and conservation of food and medicinal plants. Tribal in India which are widely used by all section of peoples either directly as folk remedies or different indigenous system of medicine or indirectly in the pharmaceutical preparations of modern medicines[4]. Tribes are mostly mingled with the forest ecosystem assisting the native societies to "live in harmony with nature"[5]. The Phytochemical form medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action may too very likely differ. The existence of this great and partially unexplored source of bioactive

Figure 1: *Justicia gendarussa*

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Table 1: Preliminary Phytochemical Screening of *Justicia gendarussa* leaves.

S.NO	Class of Chemical Compounds	Methanol extract	Chloroform extract	Petroleum ether extract
1.	Alkaloids			
	Mayer's test	+	-	-
	Wagner's	+	-	-
2.	Flavonoids			
	Lead acetate test	-	+	+
	H_2SO_4	-	+	+
3.	Steroids			
	Liebermann- Burchard test	+	+	-
4.	Terpenoids			
	Salkowski test	-	-	-
5.	Anthroquinone			
	Borntrager's	-	-	-
6.	Phenols			
	Ferric chloride test	+	-	-
	Lead acetate test	+	-	-
7.	Saponin	-	+	-
8.	Tannin	-	+	-
9.	Carbohydrates	+	+	+
10.	Oils & Resins	-	+	+

+ = Present; - = Absent

Table 2: Identification of chemical constituents from Methanol extract of the leaves by GC-MS.

S.NO	Retention time	Peak Name	Molecular Formula	Molecular weight	% peak area
1	3.49	3-Amino-2-oxazolidinone	$C_3H_6N_2O_2$	102	1.73
2	8.61	2,5-Dimethyl-4-hydroxy-3(2H)-furan one	$C_6H_8O_3$	128	2.29
3	13.82	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	5.27
4	14.54	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154	0.52
5	17.07	Sucrose	$C_{12}H_{22}O_{11}$	342	11.49
6	22.88	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	21.34

Table 3: Identification of chemical constituents from chloroform extract of the leaves by GC-MS.

S.NO	Retention time	Peak Name	Molecular Formula	Molecular weight	% peak area
1	4.59	1,6;2,3-Dianhydro-4-O-acetyl-a-d-allopyranose	$C_8H_{10}O_5$	186	1.77
2	6.72	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144	8.10
3	9.25	3-Hexen-2-one, 3,4-dimethyl-	$C_8H_{14}O$	126	0.89
4	10.51	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	6.78
5	14.69	a-D-lucopyranosiduronamide, methyl 2,3-di-O-methyl-	$C_9H_{17}NO_6$	235	0.29
6	18.07	1,6-Anhydro-á-D-glucopyranose (levoglucosan)	$C_6H_{10}O_5$	162	3.44
7	19.47	Megastigmatrienone	$C_{13}H_{18}O$	190	5.93
8	20.03	4,4,5,8-Tetramethylchroman-2-ol	$C_{13}H_{18}O_2$	206	2.78

compounds fits well with the increasing demand of new drugs, especially in the field of antibiotics.

Justicia gendarussa Burm. F. Description

Justicia gendarussa Burm. f. Syn: *Gendarussa vulgaris* (figure.1) is an erect undershrub, 0.6 to 1.2 m in height with subterete branches. Leaves are simple, lanceolate or linear – lanceolate, 7.5 to 12.5 cm long, glabrous, short-petioled, pale green beneath and dark violet green above, 8 pairs of main nerves, mid rib and main nerves prominent on the under surface. Stems and branches are dark violet. Flowers are 5-12.5 cm long from the uppermost leaf -axils; white coloured, spotted with purple

Table 4: Identification of chemical constituents from petroleum ether extract of the leaves by GC-MS.

S.NO	Retention time	Peak Name	Molecular Formula	Molecular weight	% peak area
1	5.84	2-Cyclopenten-1-one, 2-hydroxy-	$C_5H_6O_2$	98	0.97
2	12.10	1-Hexadecyne	$C_{16}H_{30}$	222	0.75
3	12.42	Benzofuran, 2,3-dihydro-	C_8H_8O	120	3.12
4	11.61	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	230	2.82
5	15.0	Phytol	$C_{20}H_{40}O$	296	0.88
6	17.27	2(1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro- 4,8a-dimethyl-6-(1-methylethenyl)-	$C_{15}H_{22}O$	218	14.17
7	18.42	3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180	1.45
8	18.52	Ethanol, 2-[4-(1,1-dimethylethyl)-2-methylphenoxy]-	$C_{13}H_{20}O_2$	208	0.01
9	24.77	Squalene	$C_{30}H_{50}$	410	8.06

Table 5: Physico - Chemical Parameters of *Justicia gendarussa* leaves.

S.No	Parameters	Leaf powder (%)
1	Foreign organic matter	0.34 ± 0.59
2	Moisture content	06.58 ± 0.28
3	Total ash	11.63 ± 0.72
4	Acid Insoluble ash	7.05 ± 0.18
5	Water soluble ash	7.23 ± 0.76
6	Water insoluble ash	7.36 ± 0.18
7	Sulphated ash	4.51 ± 0.78

and clustered in the interrupted spikes. Fruits glabrous capsules. Calyx 3.8-5mm; with nearly glabrous linear segments. The leaves and roots are acrid, febrifuge, thermogenic, emetic, anodyne, emmenagogue, diaphoretic, insecticidal and antipyretic[6,7].

1) Scientific Classification

Kingdom:Plantae
Division :Tracheophyta
Class :Magnoliopsida
Order :Lamiales
Family :Acanthaceae
Genus :*Justicia*
Species : *gendarussa*

2) Local names of the plant

English : Black vasa, Black Malabar nut
Sanskrit : Nilanirgundi, Indrani
Hindi : Nilinargandi, Udasanbhalu
Bengal : Jagatmadan, Jogmodon
Marathi :Bakas,Kalaadulsa
Kannada:Karinekki
Telugu : Addasarambu, Nallavavili,
Malayalam :Vatankolli, Vatankutti, Karinochil
Tamil :Vadaikkutti, Karunochi

MATERIALS AND METHODS

Collection of plant samples

Plant was collected locally from Irulappatti (Vill) in Pappireddipatti (Po), Dharmapuri (Dt), Tamil Nadu, India, and got identified by Assistant Prof. Dr. S.Murugesan, Department of Botany, Periyar University, Salem, Tamil Nadu and India.

Extraction of Plant Material

The fresh plant samples (Plant leaves) were collected and washed under running tap water and dried an oven at 40^0c for 3 days. The dried plant materials were ground into powder. About 10 g of dry powered plant material was extracted by soxhlate apparatus using methanol, Chloroform and Petroleum Ether solvent. Extracts was then concentrated using a rotary evaporator and the concentrated residual extracts were stored at 4^0c in a dry airtight container until further use.

Preliminary Phytochemical Screening

Preliminary Phytochemical analysis was carried out for all the extracts as per standard methods described by Brain and Turner 1975 and Evans 1966.

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

Mayer's test

Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner's test

Filtrates were treated with wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

H_2SO_4 test

Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

Detection of Steroids

Liebermann- Burchard test

2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The color changed from violet to blue or green in some samples indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test

0.2g of the extract of the whole sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was

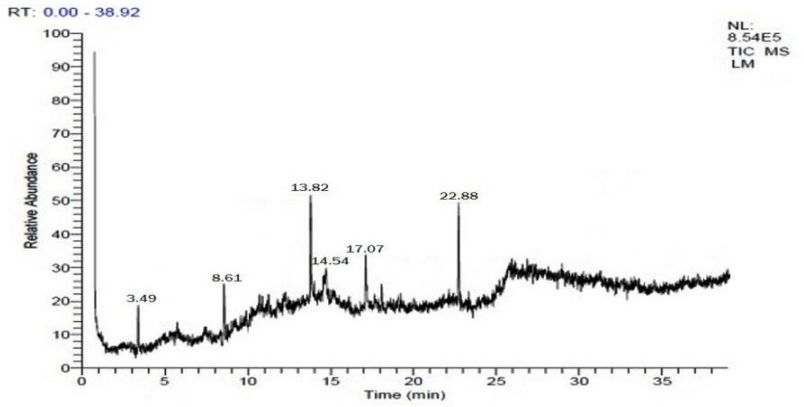


Figure 2: GC-MS Analysis of *Justicia gendarussa* of leaves methonal extract.

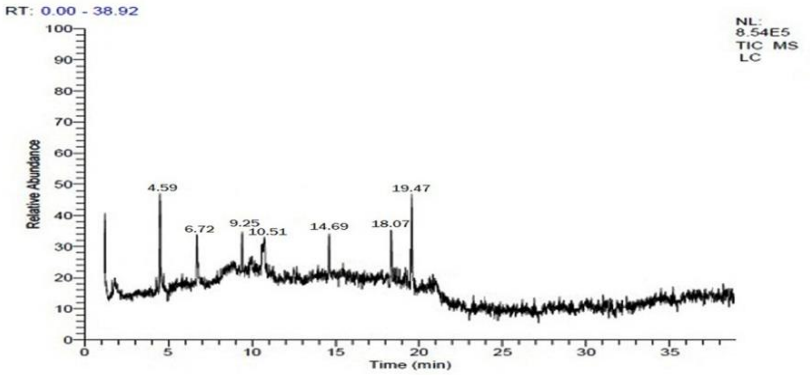


Figure 3: GC-MS Analysis of *Justicia gendarussa* of leaves chloroform Extract.

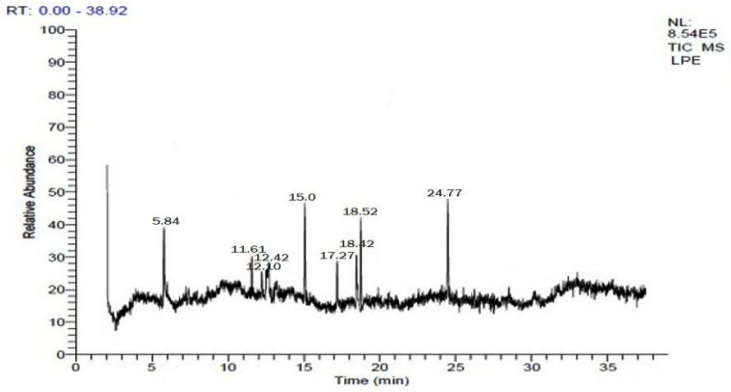


Figure 4: GC-MS Analysis of *Justicia gendarussa* of leaves petroleum ether Extract.

carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

Detection of Anthroquinones

Borntrager's test

About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink color indicates the presence anthraquinones.

Detection of Phenols

Ferric chloride test

Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

Lead acetate test

Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of phenol.

Detection of Saponins

Froth test

About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

Detection of Tannins

Table 6: Florescence analyses of leaves on *Justicia gendarussa* in various solvents.

S.NO	Solvent treatment	Visible light	Short UV (254nm)	Long UV (366nm)
1	Drug + Distilled water	Light yellow	Dark brown	Dark black
2	Drug + H_2SO_4	Greenish brown	Black	Light yellow
3	Drug + NaOH in water	Ceramic yellow	Light green	Blackish green
4	Drug + NaOH in methanol	Yellowish green	Greenish black	Dark green
5	Drug + Hcl	Light green	Light yellow	Dark yellow

Table 7: Antibacterial activity of *Justicia gendarussa* Stem in Methanol, Chloroform and Petroleum Ether Extracts (The Concentration of control, 25µl, 50µl, 75µl, 100µl).

Name of the Extracts	Name of Organism	Zone of inhibitor (mm)				
		Control	25µl	50µl	75µl	100µl
Methanol Extract	*P. vulgaris*	18	-	14	19	25
	P. pneumonia	19	20	24	27	33
Chloroform Extracts	*P. vulgaris*	18	-	11	13	18
	P. pneumonia	19	-	13	18	26
Petroleum Ether Extracts	*P. vulgaris*	18	-	14	18	22
	P. pneumonia	19	-	12	15	17

Ferric chloride test

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

Detection of Carbohydrates

Fehling's test

0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Detection of Oils and Resins

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Gas Chromatographic–Mass Spectrophotometric (GC-MS) Analysis

GC-MS analysis was carried out for all the three extracts (Methanol, chloroform, petroleum ether) of the leaves and stem of *Justicia gendarussa* Burm.F. Alpha Omega Hi-tech Bioresearch Centre, 16, Anbu Nagar, Gorimedu, Salem – 636 008, Tamil Nadu, India. Pharmacognosy Analysis of Physico chemical constant soft the powder bark has been done to evaluate the quality and purity of the drug. Various physicochemical parameters like Moisture contents, foreign organic matters and Ash values were calculated as per WHO guidelines. Many herbs show fluorescence when the cut surface or powder is exposed to UV light and this can be useful in their identification.

Antibacterial activity

The disc diffusion Method (Bauer et al. 1996) was used to screen the antimicrobial activity. In vitro antimicrobial avitivity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media in to sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts (Control, 25µl, 50µl, 75µl and 100 µl) are 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^0C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

RESULT

Preliminary Phytochemical Screening of Justicia gendarussa leaves

Phytochemical analysis is very useful in the evaluation of some active biological components of medicinal plants. The phytochemical screening carried on the leaves extract of *Justicia gendarussa* revealed the presence of some active ingredients such as Carbohydrates were present in all extracts. Flavonoids and Oils & Resins were present in Chloroform, Petroleum ether extract but absent in methanol extract (Table-1).

Identification of chemical constituents from different extract of the leaves by GC-MS

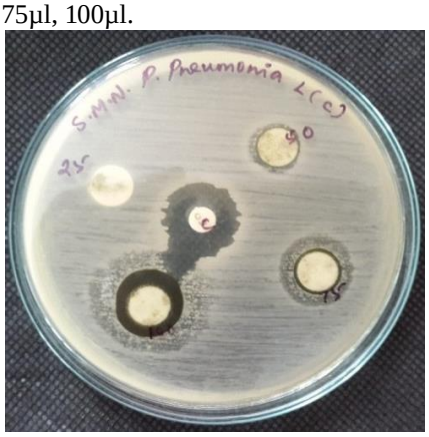
The chemical constituents identified by the GC – MS analysis in various extracts of methanol, chloroform and petroleum ether extract of the leaves of *Justicia gendarussa* were enumerated along with molecular formula, Retention time, molecular weight, peak area and given in Tables 2 – 3, which shows the presence of 23 bioactive phytochemical compounds in the methanolic, chloroform and petroleum ether extract of the leaves of *Justicia gendarussa*. The mass spectra of identified compounds from *Justicia gendarussa* were presented in Fig. 1-3.

Physico – Chemical values and Florescence analyses from Justicia gendarussa leaves

The results of Physico – Chemical values and Florescence analysis of *Justicia gendarussa* leaves recorded were given. In leaves Physico – chemical values such as Foreign organic matter recorded for as 0.34, Moisture content recorded for as 06.58, Total ash recorded for as 11.63, Acid Insoluble ash recorded 7.05, Water soluble ash recorded for as 7.23, Water insoluble recorded for as

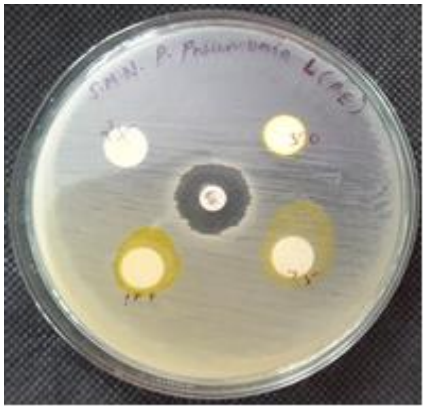
P. vulgaris *P. pneumonia*

Figure 5: Antibacterial activity of *Justicia gendarussa* leaves in methanol extract
The Concentration of 25µl, 50µl, 75µl, 100µl.



P. vulgaris *P. pneumonia*

Figure 6: Antibacterial activities of *Justicia gendarussa* leaves in chloroform extracts
The Concentration of 25µl, 50µl, 75µl, 100µl.



P. vulgaris *P. pneumonia*

Figure 7: Antibacterial activity of *Justicia gendarussa* leaves in petroleum ether extracts, The Concentration of 25µl, 50µl, 75µl, 100µl.

7.36 and Sulphated ash recorded for as 4.51 respectively. (Tables – 5). Florescence analysed by different solvents such as Distilled water, H_2SO_4, NaOH in water, NaOH in methanol and HCl. *Justicia gendarussa* leaves of florescence showed in short UV (254nm) and long UV (366nm) (Table 6).

Antibacterial activity of Justicia gendarussa leaves in different extract

The results of antibacterial activity carried out of methanol, Chloroform and Petroleum Ether extracts from the leaves of *J. gendraussa*. The methanolic and chloroform extracts expressed remarkable antibacterial activity against tested *P.pneumonia* and *P.vulgaris*. Methanol and chloroform extracts exhibited the highest inhibition zone was observed against *P. pneumonia*. Petroleum Ether Extracts were showed highest antibacterial activity against *P. vulgaris* Fig. 5,6,7.

DISCUSSION

The color reactions for the qualitative detection of phenolics, flavonoids, alkaloids, saponins and steroids showed the presence of all the compounds in all the samples except saponins[8]. The leaf showed better result for preliminary phytochemical screening than the stem[9]. GC-MS chromatogram of the methanolic extract of Justicia wynaadensis showed 30 peaks and have been identified after comparison of the mass spectra[10]. Similarity groups of different solvent extracts in their antibacterial action were also revealed by cluster analysis as different solvents have various degrees of solubility for different phytoconstituents[11]. The number of muti-drug resistant strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing due to the indiscriminate use of commercial antimicrobial treatment of infectious diseases[11,12].

CONCLUSION

The present results revealed that the methanol, chloroform and petroleum ether extracts of *Justicia gendarussa* leaves exhibited potent in different types of phytochemical constituents. In the present study 23 chemical constituents have been identified from various extract of methanol, chloroform and petroleum ether extracts with different concentration of *Justicia gendarussa* leaves by Gas chromatogram Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of whole plant for various ailments by traditional practitioners. In Identification of Chemical constituents and Physico - Chemical characters which may be useful for Pharmacognostical studies. The different extracts of *J. gendraussa* leaves showed potent antibacterial activity against different tested bacterial pathogens of *P.pneumonia and P. vulgaris*. The result of present investigation clearly indicates the antibacterial activity of *J. gendraussa* leaves and the present work should inspire additional study of the *Justicia gendarussa* for their use in therapeutics.

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