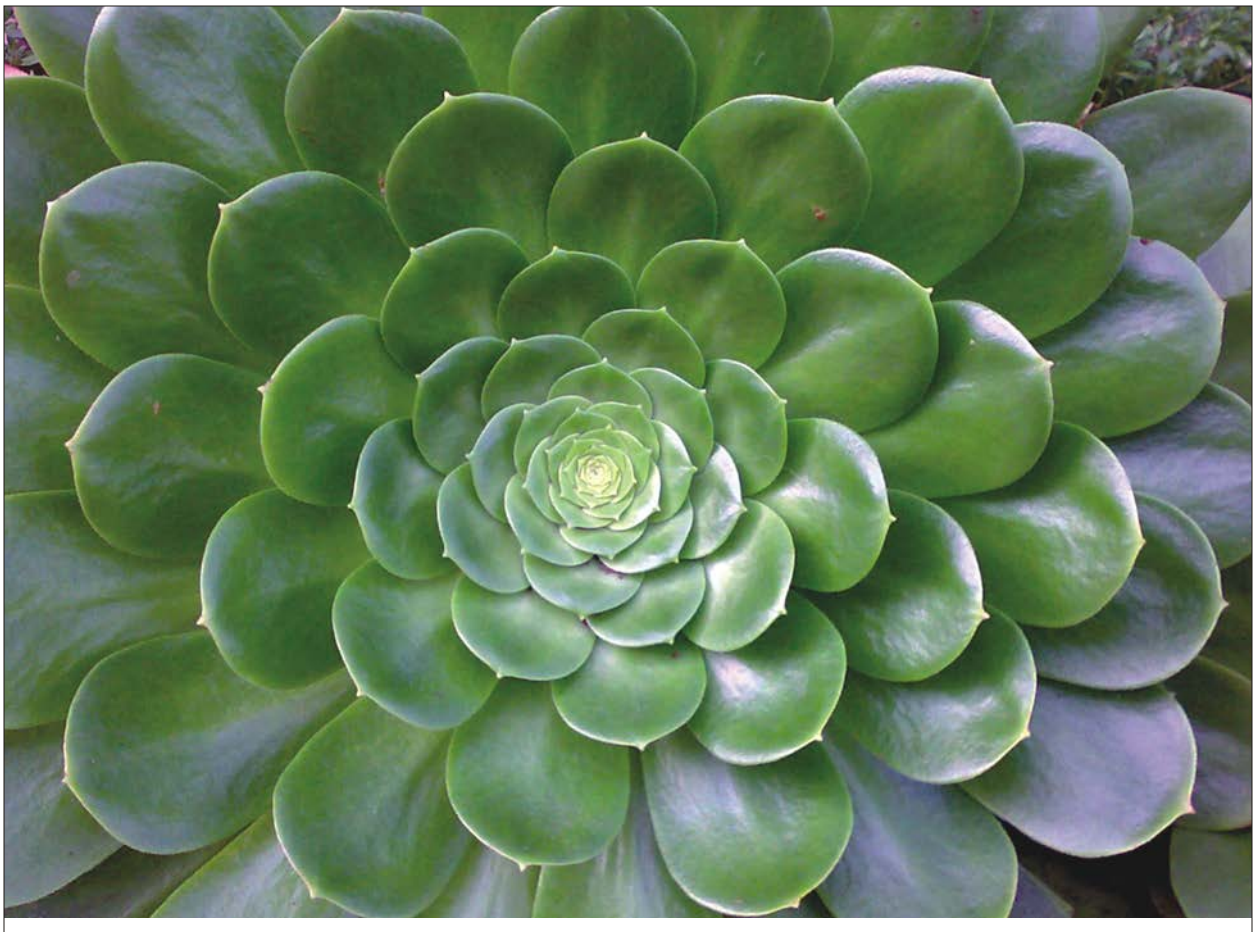

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Antibacterial and Anti-Inflammatory Activity of *Justicia gendarussa* Burm. F. Leaves

S. Nirmalraj, M. Ravikumar, M. Mahendrakumar, B. Bharath and K. Perinbam
P.G. and Research Department of Botany, Government Arts College, Nandanam, Chennai, 600035, Tamil Nadu, India

Corresponding Author: K. Perinbam, P.G. and Research Department of Botany, Government Arts College, Nandanam, Chennai, 600035, Tamil Nadu, India

ABSTRACT

The leaves of *Justicia gendarussa* are used in the folk medicine for the treatment of various ailments like rheumatic pain, jaundice, cephalgia, hemiplegia and eczema, etc. The leaves of the plant consists of important phytoconstituents including O-di-substituted aromatic amines, 2-aminobenzyl alcohol, O methyl ethers, friedelin, lupeol and β-sistosterol. In the present investigation, different solvent extracts of *J. gendarussa* leaves were screened and compared for membrane stability and antibacterial activities. Even at lower concentrations, methanolic extract of *J. gendarussa* shown good antibacterial and anti-inflammatory activity. At higher concentrations, methanolic extracts gave relatively more projecting antibacterial activity when compared with gentamycin. The anti-inflammatory effects of the crude drug were compared with Diclofenac as positive control. The methanolic extracts of *J. gendarussa* leaves possessed a broad spectrum antibacterial activity against both gram-negative and gram positive organisms like *Staphylococcus aureus* (MTCC 96), *Staphylococcus mutans* (MTCC 497), *Bacillus subtilis* (MTCC 441), *Micrococcus luteus* (MTCC 1538), gram negative organisms; *Proteus vulgaris* (MTCC 426), *Klebsiella pneumoniae* (MTCC 109), *Escherichia coli* (MTCC 443) and *Shigella flexneri* (MTCC 1457) with a zone of inhibition from 7-12 mm. The extract also showed good membrane stability to be considered to have significant anti-inflammatory action.

Key words: *Justicia gendarussa*, anti-inflammation, human red blood cell, diclofenac

INTRODUCTION

Indian system of medicine is mainly depends on plant derivatives. Plants offer a large variety of secondary metabolites which play a significant role in their metabolic pathways. Some species of plants are used for particular disease in a specific region, the same plant is used in different ailment in some other region. Inflammation is injury of living tissues incurred by the result of various enzymes activities. *Justicia gendarussa* Burm. F. is an ever green shrub belongs to Acanthaceae family which grows up to 1.5-2 m. *Justicia gendarussa* is frequently grown in Indian gardens as hedge plant, which is propagated by cuttings. It hardly withstands heavy rain and thrives in shade. It is used as a tribal medicine for various ailments such as bronchitis, inflammations, vaginal discharges, dyspepsia, eye diseases and fevers, etc. The leaves were used in the form of decoction, infusions and paste (Anonymous, 1959; Kirtikar and Basu, 1935). The leaves of *J. gendarussa* contains phytoconstituents such as alkaloids, flavonoids, saturated steroidal saponins or triterpinoidal saponins, amino acids and aromatic amines are rich in potassium salts (Ratnasooriya *et al*., 2007). The leaves also contains 2-amino benzyl alcohol, 2

(2'-amino benzyl amino) benzyl alcohol and their respective 0-methyl ethers, friedelin, lupeol and β-sitosterol (Arokiyaraj *et al*., 2007; Herrera-Mata *et al*., 2002). Long-chain 2-amino alcohols display interesting biological effects like immunosuppresive, anti-inflammatory and cytotoxic activities, as well as inducing apoptosis (Constantinou-Kokotou, 2002). The detection of traditional medicine as an alternative form of healthcare and the development of microbial conflict to the available antibiotics has led to the investigation of the antimicrobial activity of medicinal plants. The increasing disappointment if chemotherapeutics and antibiotics resistance exhibited by pathogenic microbial infectious agents have lead to the screening of several medicinal plants for their potential antimicrobial activity (Elizabeth, 2005). Plant drugs are known to be good for combination therapy which as multidrug resistance modifiers (Hemaiswarya *et al*., 2008). In the present study, the antibacterial and anti-inflammatory activity of different solvent extracts of *Justicia gendarussa* were studied.

MATERIALS AND METHODS

Plant material: The leaf samples of *J. gendarussa* were collected from Semmedu hamlet, Kolli hills, Tamil Nadu, South India. The plant was authenticated by Botanical survey of India, Coimbatore.

Solvent extraction: *Justicia gendarussa* leaves were dried at room temperature and ground into fine powder. Hundred grams (100 g) of powdered plant material was extracted with 500 mL (1:5) of different solvents of increasing polarity such as hexane, diethyl ether, dichloromethane, ethyl acetate and methanol. The soxhlet extraction method was followed for 6 h with 10-12 cycles under laboratory conditions. The extracts were then concentrated under reduced pressure in a rotary vacuum evaporator (Superfit Rotavap Pub-6, India). All the obtained extracts were weighed, packed and stored at 4°C (Nabere *et al*., 2013).

Antibacterial study: Eight bacteria were used in this study consisted of both gram positive and gram negative such as, *Staphylococcus aureus* (MTCC 96), *Staphylococcus mutans* (MTCC 497), *Bacillus subtilis* (MTCC 441), *Micrococcus luteus* (MTCC 1538), *Proteus vulgaris* (MTCC 426), *Klebsiella pneumoniae* (MTCC 109), *Escherichia coli* (MTCC 443) and *Shigella flexneri* (MTCC 1457). Sensitivity of different bacterial strains to various solvent extracts was measured using disc diffusion method (Bauer *et al*., 1966; Chew *et al*., 2011). The plates containing Mueller- Hinton agar were spread with 0.2 mL of the overnight grown bacterial inoculum. About 6 mm diameter of sterile Whatman filter paper (No. 1) were soaked with 10 μL of extract (20 mg mL^{-1}) and deposited in plates. The plates were incubated at 37°C up to 24 h and diameter of the resultant zone of inhibition was measured. For each combination of extract and the bacterial strain, the experiment was performed in triplicate. The bacteria with a clear zone of inhibition of more than 12 mm were considered to be sensitive. Sensitivity of different bacterial strains to DMSO (Dimethyl sulfoxide) was measured to evaluate this solvent toxicity. Antibacterial activity of different solvent extracts was compared with commonly employed antibiotic gentamycin (10 μg $disc^{-1}$).

Anti-inflammatory (membrane stability) activity assay

HRBC membrane stabilization method: The anti-inflammatory activity of different solvent extracts of *Justicia gendarussa* leaves were assessed *in vitro* by Human Red Blood Cell (HRBC)

membrane stabilization method. Blood was collected from healthy volunteers and was mixed with equal volume of Alsever's solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05% and sodium chloride 0.42% in distilled water) and centrifuged with isosaline. To 1 mL of HRBC suspension, equal volume of test drug in three different concentrations, 1000, 500 and 250 g mL^{-1} was added. All the assay mixtures were incubated at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated spectrophotometrically at 560 nm (James *et al*., 2009). The percentage of haemolysis was calculated then by the equation:

$$\text{Hemolysis (\%)} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

The percentage of membrane protection can be hence calculated using the equation:

$$\text{Protection (\%)} = \frac{\text{OD of test}}{\text{OD of control}} \times 100$$

Here "OD of test" is optical density or the test sample's absorbance and "OD of control" is optical density or absorbance of the negative control. Here, the negative control used was Alsever's solution with blood without plant extract in it.

RESULTS AND DISCUSSION

Antibacterial activity: Table 1 depicted the antibacterial activity of different solvent extract of *J. gendarussa* via. hexane, diethylether, dichloromethane, ethyl acetate and methanol extract. Among the tested extracts, the better zone of inhibition was observed with methanol fractions of *J. gendarussa* (12 mm) against *E. coli* (MTCC 443) and *Bacillus subtilis* (MTCC 441). All the extracts of *J. gendarussa* exhibited inhibition effect on the eight bacteria tested in the present study. The maximum inhibition against *Bacillus subtilis*, *Escherichia coli* and this antibacterial activity could be endorsed to different compounds identified in the plant. Antibacterial activity is accredited to several phytochemicals such as flavonoids, saponins, tannins and terpenoids (Sonal *et al*., 2011), O-disubstituted aromatic amines, 2-aminobenzyl alcohol, O methyl ethers, friedelin, lupeol and β-sistosterol (Arokiyaraj *et al*., 2007). These various potentialities related to the compounds found in the extracts of our plant could explain their high use in South Indian traditional medicine in the treatment of infectious diseases.

Table 1: Anti bacterial activity of *Justicia gendarussa* Burm. F

	Zone of inhibition (mm)						
Test microorganisms	Positive control	Solvent control	Hexane	DEE	DCM	Ethyl acetate	Methanol
Staphylococcus aureus (MTCC 96)	12	-	6	5	4	5	7
Staphylococcus mutans (MTCC497)	13	-	7	6	4	7	9
Bacillus subtilis (MTCC 441)	15	-	6	5	3	5	12
Micrococcus luteus (MTCC1538)	18	-	4	6	5	7	11
Proteus vulgaris (MTCC426)	13	-	6	5	4	6	8
Klebsiella pneumoniae (MTCC109)	17	-	6	5	6	8	9
Escherichia coli (MTCC443)	12	-	5	6	7	6	12
Shigella flexneri (MTCC1457)	15	-	6	7	5	3	9

DEE: Diethyl ether, DCM: Dichloromethane, Positive control: Gentamycin

Table 2: HRBC membrane stabilization activity *Justicia gendarussa (*Burm. F.)

Treatment and concentration (mg mL^{-1})	Absorbance (560 nm)	Hemolysis (%)	Inhibition (%)
Control	-	0.345	-
Hexane			
1000	0.162	46.96±0.012	53.03
500	0.152	44.06±0.015	55.94
250	0.148	42.90±0.051	57.10
Diethyl either			
1000	0.156	45.22±0.05	54.78
500	0.149	43.19±0.004	56.81
250	0.135	39.13±0.002	60.87
Dichloromethane			
1000	0.131	37.97±0.005	62.03
500	0.121	35.07±0.023	64.93
250	0.117	33.91±0.011	66.09
Ethyl acetate			
1000	0.097	28.12±0.032	71.88
500	0.132	38.26±0.041	61.74
250	0.119	34.49±0.081	65.51
Methanol			
1000	0.082	23.80±0.05	76.20
500	0.095	27.54±0.032	72.46
250	0.091	26.38±0.081	73.62
Diclofenac sodium			
50	0.089	25.85±0.05	74.14

HRBC membrane stabilization assay: The lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since, Human Red Blood Cell (HRBC) membrane is similar to lysosomal membrane, their study was undertaken to check the stability of HRBC membrane by the extracts to predict the anti-inflammatory activity *in vitro* (Varadarasu *et al*., 2007). Table 2 revealed that the Human Red Blood Cell (HRBC) membrane stability of *J. gendarussa* via. anti-inflammatory activity of different extracts. *Justicia gendarussa* showed significant anti-inflammatory activity in a concentration dependent manner. *Justicia gendarussa* methanolic extract was determined the concentration of 1000, 500 and 250 mg mL^{-1}. The maximum concentration was observed the methanolic extract (1000 mg mL^{-1}) 76.20, 72.46 and 73.62% protection of HRBC in hypotonic solution respectively. All the results were compared with standard Diclofenac at 50 mg mL^{-1}, which showed 74.14% protection of HRBC in hypotonic solution.

CONCLUSION

The methanolic leaf extract of *Justicia gendarussa* possesses potent anti-inflammatory activity by inhibiting the release of prostaglandins or other inflammatory mediators from cell membrane by stabilizing membrane and anti bacterial activity by zone of inhibition. In further studies, it can be possible to formulate natural anti inflammatory, anti-bacterial drugs of *Justicia gendarussa* leaf extract.

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REFERENCES

Anonymous, 1959. The Wealth of India-Raw Materials. 1st Edn., CSIR, New Delhi, India, Pages: 312.

Arokiyaraj, S., K. Perinbam, P. Agastian and K. Balaraju, 2007. Immunosuppressive effect of medicinal plants of Kolli hills on mitogen-stimulated proliferation of the human peripheral blood mononuclear cells *in vitro*. Indian J. Pharmacol., 39: 180-183.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Chew, Y.L., E.W.L. Chan, P.L. Tan, Y.Y. Lim, J. Stanslas and J.K. Goh, 2011. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. Biol. Med. Chem. Complement. Altern. Med., Vol. 11.

Constantinou-Kokotou, V., 2002. Synthesis and biological activities of long chain 2-amino alcohols. Lett. Peptide Sci., 9: 143-152.

Elizabeth, K.M., 2005. Antimicrobial activity of *Terminalia bellerica*. Indian J. Clin. Biochem., 20: 150-153.

Hemaiswarya, S., A.K. Kruthiventi and M. Doble, 2008. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine, 15: 639-652.

Herrera-Mata, H., A. Rosas-Romero and C.V. Oscar, 2002. Biological activity of sanguinaria (Justicia secunda) extracts. Pharmaceut. Biol., 40: 206-212.

James, O., O.P. Nnacheta, H.S. Wara and U.R. Aliyu, 2009. *In vitro* and *in vivo* studies studies on the anti oxidative activities, membrane stabilization and cytotoxicity of water spinach from ipogi ponds (Nigeria). Int. J. PharmTech. Res., 1: 474-482.

Kirtikar, K.R. and B.D. Basu, 1935. Indian Medicinal Plants. Lalit Mohan Basu Publication, Allahabad, India, pp: 1896-1898.

Nabere, O., H. Adama, G. Samson, K. Kiessoum and Z. Patrice *et al*., 2013. Antibacterial and phytochemical studies of three *Acanthaceae* species used in burkina faso traditional medicine. J. Applied Pharmaceut. Sci., 3: 49-55.

Ratnasooriya, W.D., S.A. Deraniyagala and D.C. Dehigaspitiya, 2007. Antinociceptive activity and toxicological study of aqueous leaf extract of *Justicia gendarussa* Burm. F. in rats. Pharmacog. Mag., 3: 145-155.

Sonal, P., K. Nayana, S. Bakula and S. Mamta, 2011. Botanical identification and physicochemical investigation of leaf of *Nili-Nirgundi* (*Justicia gendarussa*). Int. J. Pharmaceut. Sci. Rev. Res., 10: 116-121.

Varadarasu, M., S. Mounisamy, V. Kavimani, S. Balu and D. Quine, 2007. Evaluation of anti-inflammatory and membrane stabilizing property of ethanol extract of *Cansjera rheedii* J.Gmelin (Opiliaceae). Iran. J. Pharmacol. Therapeut., 6: 235-237.