

**Antimicrobial, Antifungal and HRBC Membrane Hemolysis and Membrane Stabilization Properties of Various Extracts of *Justicia gendarussa***Amin M. Mir¹, Muhammad W. Ashraf², Himani Himani³, Bilal A. Mir³¹Assistant Research Professor, Department of Mathematics & Natural Sciences, Prince Mohammad Bin Fahd University, AlKhobar, Saudi Arabia²Dean Department of Mathematics & Natural Sciences, Prince Mohammad Bin Fahd University, AlKhobar, Saudi Arabia³Assistant Professor, Department of Microbiology UCBMSH Dehradun Uttarakhand India

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ABSTRACT

Diseases of microbial origin are of important and utmost public health concern. This heightened concern is due to the increasing use of antimicrobial drugs and increasing resistance to antimicrobial agents. Presently, scientists have shown increased interest in the use of plant products against microbial diseases. Inflammation due to injury or age-related natural inflammation is due to denaturation of proteins. The plant *Justicia gendarussa* is used as antimicrobial and anti-inflammatory medicine. Consequently, this study evaluated *Justicia gendarussa* leaf extracts as antibacterial, antifungal and anti-inflammatory agents. The study evaluated the growth inhibition of many bacterial and fungal strains as well as the membrane stabilizing effect by various extracts of this plant. The extracts inhibited the growth of *Escherichia coli*, *Micrococcus luteus*, *Bacillus pumilus*, *B. cereus*, *B. lecheniformis*, *Salmonella typhi*, *Streptococcus mutans* bacterial strains, with the ethanol extract showing the highest inhibition as compared to water extract, whereas the petroleum ether extract inhibited the growth of only *Micrococcus luteus*, *Bacillus pumilus*, *B. lecheniformis* and *J. gendarussa*. Petroleum ether, ethanol and water extracts also inhibited the growth of many fungi species, viz, *Aspergillus niger*, *Fusarium*, *Nigrospora oryza*, and *Aspergillus flavus*. Among the extracts, the ethanol extracts showed the greatest inhibition against the growth of *Aspergillus niger* and *Nigrospora oryza*. All the extracts of *Justicia gendarussa* showed marked membrane stabilizing effect, among which the ethanol extract showed more membrane stabilization followed by water and petroleum ether extracts.

Keywords: Bacteria, Fungi, Inflammation, Phytochemicals, *Justicia gendarussa*.

Introduction

Justicia gendarussa also known as Gendarussa is an evergreen febrifuge, emetic, emmenagogue and diaphoretic shrub, found in all parts of India and Andaman Islands and is used for the treatment of lunacy, debility, snakebite, and amenorrhoea and stomach troubles. *Justicia gendarussa* belongs to the family Acanthaceae, and is prominent in Asian countries such as Sri Lanka, India, and Malaysia.¹ *J. gendarussa* had been used as folk medicines in India for the treatment of many diseases like rheumatism, bronchitis, fever, eczema, and jaundice.² *J. gendarussa* is a well-known traditional male anti-fertility drug in Papua, Indonesia.³ Thus, *J. gendarussa* herbal drug possess a good potential to develop a phyto-pharmaceutical product as a non-hormonal male contraceptive.⁴ Leaves and tender shoots are used as diaphoretic and a recipe of the leaves is given against cephalalgia, hemiplegia and facial paralysis, and the juice of the leaves are used to manage hemorrhages. The roots also have several medicinal uses and the bark has emetic properties. However, there are no published scientific studies on the anti-inflammatory, antifungal and specific anti-bacterial activities of the

Leaves of *J. gendarussa*.

Despite the continuous efforts and progresses in modern medicine, it has been found that more than 70% of the World's developing population still depend on complementary and alternative systems of medicine, also known as traditional medicine.⁵ Also, the continuous use of allopathic medicines leads to the development of microbial resistance to a particular type of medicine.

Inflammation is a response by living tissue to any kind of injury and is characterized by pain, redness, heat and swelling.⁶ Vasoactive chemicals increase the permeability of the arterioles which then allows blood cells, chemical substances, blood proteins and fluids to accumulate in that region, and that fluid accumulation causes swelling.⁶ The study investigated the HRBC membrane hemolysis and membrane stabilization, antifungal and antibacterial potentials of various extracts of the leaves of *Justicia gendarussa*.

Materials and Methods

Collection and identification of plant material:

The fresh aerial parts of the plant *Justicia gendarussa* were collected from a local herbal garden in Dehradun in June 2019. The plant sample was authenticated by Botanical Survey of India, Northern Regional Centre, and Dehradun. The voucher specimen was submitted at B.S.I herbarium with voucher no. 113521. The plant leaves were separated from the stems and dried in the shade at room temperature in the Laboratory and then crushed to coarse powder.

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Extraction procedure

The dried leaf powder (125 g) was extracted with 500 mL of solvent in a Soxhlet extractor containing thimble, fitted with condenser for 24 h. Various extraction solvents were used successively as per their polarity index; petroleum ether, ethanol and water. The corresponding extract of each solvent was then boiled to one fourth of its volume, and finally the extract was made into a powdered form, by keeping the extract in a desiccator.

Antimicrobial assay

The different extracts were evaluated for antibacterial and antifungal activities. All the microbial strains were procured from the Department of microbiology UCBMSH, Dehradun.

Six gram positive and two gram negative bacteria viz. *E. coli*, *Bacillus pumilus*, *Bacillus cereus*, *Bacillus licheniformis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Streptococcus mutans* were used to evaluate the antibacterial potential of the different extracts as per the standard method.⁷

Evaluation of the antifungal potential of plant extracts

Antifungal activity of the extracts was evaluated against four pathogenic fungi namely *Aspergillus flavus*, *Aspergillus Niger*, *Fusarium* and *Nigrospora oryza*, using standard method.⁸

Effects of *Justicia gendarussa* extracts on HRBC membrane haemolysis and membrane stabilization**Preparation of Human Red Blood Cells (HRBC) Suspension**

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.^{9,10}

Heat Induced Haemolysis

The principle involved here is stabilization of human red blood cell membrane by hypotonicity-induced membrane lysis. The assay mixture contains 1 mL phosphate buffer [pH 7.4, 0.15 M], 2 mL hypo saline [0.36%], 0.5 mL HRBC suspension [10% v/v] with various concentrations of plant extracts and standard drug; Diclofenac sodium at various concentrations (0.5, 1.0, 1.5, 2.0, 2.5 µg/mL) and control (distilled water instead of hyposaline to produce 100% haemolysis) were incubated at 37°C for 30 min and centrifuged at 8000 rpm for 5 min. The haemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.¹¹

The percentage of haemolysis of HRBC membrane was calculated as follows:

$$\% \text{ Haemolysis} = (\text{Absorbance of Test Sample} / \text{Absorbance of Control}) \times 100$$

The percentage of HRBC membrane stabilization was calculated as follows:

$$\% \text{ Protection} = 100 - [(\text{Absorbance by Test sample} / \text{Absorbance by Control}) \times 100]$$

Results and Discussion

The extracts were screened for antibacterial activity against both gram (+ve) and gram (-ve) bacteria. The results indicated that the ethanol extract was found to be the most effective as it inhibited the growth of all the test bacteria, i.e., *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *B. pumilus*, *B. cereus*, *B. lecheniformis*, *Salmonella typhi*, and *Streptococcus mutans* (Table 1). The maximum zone of inhibition of 16 mm was caused by the ethanol extract against *Bacillus pumilus*. The petroleum ether extract also inhibited the growth of *Micrococcus luteus*, *Bacillus pumilus*, and *B. lecheniformis*, with maximum inhibition against *Bacillus pumilus* (12 mm). The aqueous extract inhibited the growth of all the bacteria, with maximum inhibition against *Bacillus pumilus* followed by *Streptococcus mutans*. These results indicated that the plant *Justicia gendarussa* have good antibacterial activity (Table 1).

The results of the antifungal activity indicated that the ethanol extract was most effective against all the selected fungi with maximum zone of inhibition of 16 mm against *Aspergillus niger*. The petroleum ether extract was effective against *Aspergillus niger*, *Aspergillus flavus* with the zone of inhibition of 10 and 08 mm, respectively. The aqueous extract showed activity against *Aspergillus niger*, *Aspergillus flavus*, *Nigrospora oryza*, *Fusarium* with maximum inhibition potential against *Aspergillus niger* (Table 2).

The ethanol extract showed the maximum anti-inflammatory activity followed by the water extract and then Petroleum ether extract (Figures 1, 2 and 3).

In recent years, a magnificent change has taken place and attraction has re-focused towards traditional medicines, mainly because of side effects of modern drugs, higher cost, drug resistance development against various microbial strains, and drug addiction.¹² So, scientific communities nowadays are looking into the utilisation of traditional medicine preparations, which could be derived from plants, fungi and from some microorganisms. Scientists are also working on anti-inflammatory plants, as inflammatory diseases are very common especially in the elderly both in developed and in developing countries.¹³ Several chemical constituents of plant origin, like curcumin, resveratrol, baicalein, boswellic acid, betulinic acid, ursolic acid and oleanolic acid are now used as possible drugs against inflammatory diseases.¹⁴ Various extracts of *J. gendarussa* have been investigated for anti-inflammatory activity, with promising results.

Table 1: Antibacterial activity of *Justicia gendarussa* Extract against different bacteria

Bacterial Species	Plant Extracts (100 µg/mL)			
	Petroleum ether extract (ZOI) (mm)	Ethanol Extract (ZOI) (mm)	Water Extract (ZOI) (mm)	Standard antibiotic (Ampicillin) (ZOI) (mm)
<i>Escherichia coli</i>	-	14	10	15
<i>Micrococcus luteus</i>	11	12	08	16
<i>Bacillus pumilus</i>	12	16	12	18
<i>B. cereus</i>	--	12	07	17
<i>B. lecheniformis</i>	10	11	13	19
<i>Salmonella typhi</i>	--	12	05	16
<i>Streptococcus mutans</i>	--	13	11	15

Table 2: Antifungal activity of *Justicia gendarussa* extract against different fungi

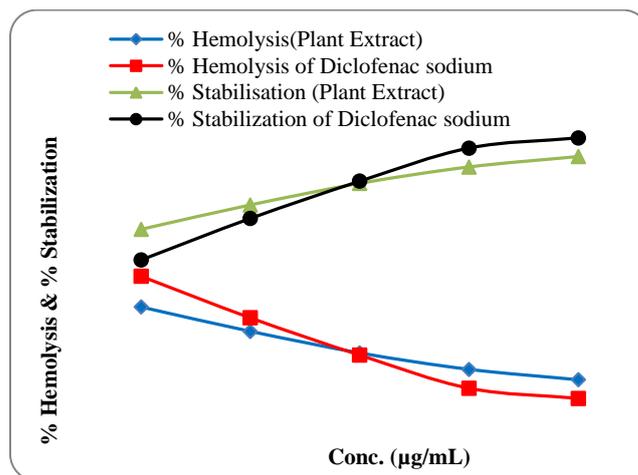
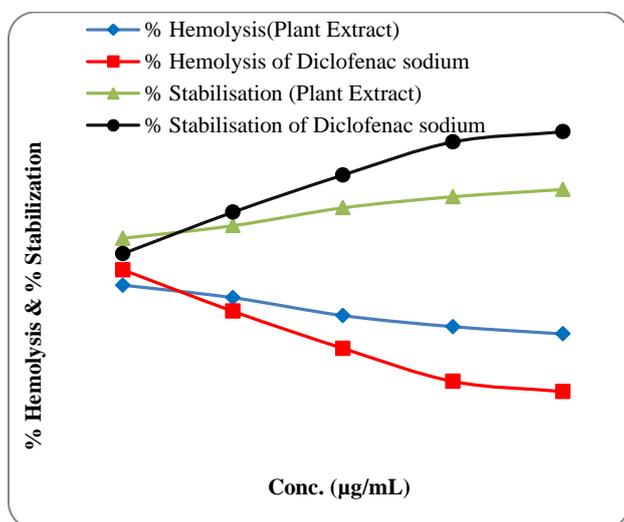
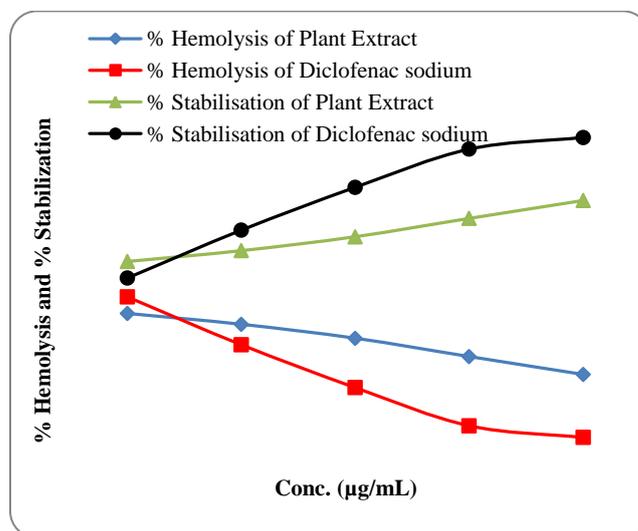
Fungi Species	Plant Extracts (100µg/mL)			
	Petroleum ether extract (ZOI) (mm)	Ethanol Extract (ZOI) (mm)	Water Extract (ZOI) (mm)	Standard antibiotic (Ketoconazole) (ZOI) (mm)
<i>Aspergillus niger</i>	10	16	12	24
<i>Aspergillus flavus</i>	08	12	10	21
<i>Nigrospora oryza</i>	-	14	09	19
<i>Fusarium</i>	-	12	08	22

Among the plant extracts, the ethanol extract of the plant showed the highest effects on the HRBC membrane haemolysis and membrane stabilization activity followed by the water and petroleum ether extracts. The results are in line with previous report.¹⁵

The ethanol and the water extract of the plant inhibit the growth of *Escherichia coli*, *Micrococcus luteus*, *Bacillus pumilus*, *B. cereus*, *B. lecheniformis*, *Salmonella typhi*, and *Streptococcus mutans*, although the ethanol extract showed highest inhibition as compared to the water extract, whereas the petroleum ether extract inhibited the growth of only *Micrococcus luteus*, *Bacillus pumilus*, *B. lecheniformis*. These antibacterial results collaborate with previous findings.¹⁶

The plant extracts of the *J. gendarussa* caused the inhibition of *Aspergillus niger*, *Fusarium*, *Nigrospora oryza*, and *Aspergillus flavus*. The ethanol extract showed the highest inhibition as compared to water and petroleum ether extracts. Although the antifungal study of *J. gendarussa* have not been reported previously but the constituents present in the *J. gendarussa* showed varied number of properties.¹⁷

Altogether, it had been found that the ethanol extracts of the plant leaves possess antibacterial, antifungal activities and effects on the HRBC membrane haemolysis and membrane stabilization.

**Figure 2:** HRBC Membrane Hemolysis and Percentage Stabilization by ethanol extract of *Justicia gendarussa* and Standard.**Figure 1:** HRBC Membrane Hemolysis and standardization by Petroleum ether extract of *Justicia gendarussa* and standard**Figure 3:** HRBC Membrane Hemolysis and Stabilization by Water extract of *Justicia gendarussa* and Standard

Conclusion

The study has revealed that the ethanol, aqueous and petroleum ether extracts of *Justicia gendarussa leaves* possess antibacterial, antifungal and effects on the HRBC membrane hemolysis and membrane stabilization. The plant extracts possesses a measurable amount of phytochemicals which is directly or indirectly related to the antibacterial, antifungal and the effects on the HRBC membrane haemolysis and membrane stabilization.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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