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Research Article

ANTIMICROBIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL SCREENING OF *JUSTICIA GENDARUSSA* (BURM. f.) AGAINST HUMAN PATHOGENS

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ABSTRACT

To evaluate the phytochemical and antimicrobial properties of ethanolic and aqueous extracts of stem and leaves (*Justicia gendarussa*) against 12 human pathogens. Antimicrobial activity was evaluated by the disc diffusion and broth dilution methods and standard procedure were followed for the identification of phytoconstituents. The aqueous extracts of stem (*J. gendarussa*) showed maximum inhibitory activity against *Shigella flexneri* (26.20mm), *Proteus mirabilis* (24.50 mm), *Escherichia coli* (21.40mm) and *Bacillus subtilis* (20.25mm) respectively and ethanolic extract showed less inhibitory activity. The aqueous extract of leaves showed significant antimicrobial activity against only *Staphylococcus aureus* (26.33mm) while the ethanolic extract of leaves showed slight inhibitory activity against a few organisms. Based on the present result, stem extracts (aqueous and ethanolic) showed significant antimicrobial activity against most of the human pathogens in both methods. The result revealed that the antimicrobial properties of stem and leaves of *J. gendarussa* moreover associated with the presence of phenolic compounds, flavonoids, terpenoids, glycosides and tannins.

Keywords: *Justicia gendarussa*, Phytoconstituents, MIC, Disc-diffusion method, Antimicrobial activity

INTRODUCTION

Medicinal plants are important substance for the study of their traditional use through the verification of pharmacological effects and can be a natural composite source that acts as new anti-infectious agents [1]. A number of medicinal plants have been screened for antimicrobial activity in recent years [2] and efforts have been done to identify their active constituents [3]. Inspite of recent development in the synthetic drug, chemistry and production of antibiotics, plant still occupy an important role in the modern a traditional system in all over the world [4]. Due to the indiscriminate use of antibacterial drugs, the microorganisms have developed resistance to many commercial antibiotics. Therefore investigation of chemical compounds within medicinal plants has become desirable [5]. Many efforts have been made to discover new antimicrobials compounds from various sources such as microorganisms, animals and medicinal plants. Systemic investigation of folk medicine may result in the discovery of novel effective compounds [6, 7]. Therefore several medicinal plants have been evaluated for possible antimicrobial activity and get remedy from variety of antimicrobial origin [8].

Justicia gendarussa (Burm. f.) (Family: Acanthaceae) is a shade-loving, quick growing evergreen scented shrub. It is mostly found throughout the greater part of India and Andaman Islands [9]. It is commonly called as 'karunotchi' in Tamil. It is an erect, branched and smooth under shrub. Leaves are linear lanceolate, glabrous; flower small, white with pink or purple spots inside [10]. 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 [11]. *Justicia* found to contain lignans, naturally occurring phenolic dimmers [12, 13]. Lignans have been used as a lead compounds for the development of antirheumatic agents [12]. The roots of this plant are also used to treat cough, jaundice, thrust, arthritis, cephalgia, facial paralysis, otalgia, hemicranias and liver disorders.

The increasing failure 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 [14]. Plants are known to produce generally good for combination therapy which as multidrug resistance modifiers [15]. Therefore the present study has been extensively evaluated inhibitory activity of *J. gendarussa* stem and leaves extracts (aqueous and ethanol) on human pathogenic microorganisms.

MATERIALS AND METHODS

Collection of plant materials

The fresh aerial parts of the plants of (leaves and stem) *Justicia gendarussa* were collected from natural habitat of Kuttralam which is 450kms away from Tiruchengode. The plant species was initially identified in Department of Botany, Vivekanandha college of Arts and Sciences for Women, Elayampalayam, and Tiruchengode. Further it was authentified by the Scientists of the Botanical Survey of India (BSI) in Coimbatore. The aerial parts of the plants were allowed to dry in shade for two weeks. Dried leaves and stem were powdered separately.

Preparation of extracts

The aerial parts of *J. gendarussa* were shade dried and pulverized. 250g of powdered material was packed in Soxhlet apparatus and subjected to continuous hot percolation for 8h using 450ml ethanol (75% V/V) as solvent. The ethanolic extract was concentrated under vacuum and dried in a dessicator. Aqueous extract made by cold maceration method. About 50g of powdered material mixed with 300ml of distilled water and kept for 7 days at room temperature. The extract obtained from water was filtered through Whatmann filter paper No.1 and residue water content was evaporated (40˚C) with heating mantle. The obtained extracts were stored in refrigerator and were dissociated in dimethyl sulfoxide for prior to use.

Preliminary phytochemical screening

The phytochemical studies were performed as described by [16]. The presence of starch, glycosides, saponins, tannins, phenolic compounds, terpenoids, steroids and flavonoids were analysed.

Microorganism tested

A total of 12 microorganisms were used to assess the antimicrobial activities, it includes, three gram- positive bacteria, *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435), and *Bacillus subtilis* (MTCC 121); seven gram- negative bacteria, *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 432), *Proteus mirabilis* (MTCC 1429), *Salmonella paratyphi* A (MTCC 735), *Salmonella typhimurium* (MTCC 98), *Shigella flexneri* (MTCC 1457) and *Pseudomonas aeruginosa* (MTCC 424); two fungus, *Candida albicans* (MTCC 183) and *Cryptococcus neoformans* (clinical isolate).

The microorganisms were originally obtained from Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh, India.

Minimum Inhibitory Concentration- Disc diffusion method

Antimicrobial activities of ethanolic and aqueous extracts of stem and leaves of *J. gendarussa* were determined by both disc-diffusion [17] and broth dilution methods. Sterile Hi-sensitivity agar (Himedia-M 486) (PH-7.2) was prepared and poured into the plates. The depth of the medium should be ~ 4 mm. Three to four similar colonies of pure cultures were inoculated with tryptone soy broth (Himedia- M 323), incubated at 37˚C for 2-8h and the inoculums size was adjusted to yield uniform suspension containing 10^5-10^6 cells/ml (McFarland's standard). The agar surfaces of the plates were swabbed with test culture in three directions turning the plates to 60˚ between each swabbing. Confluent growth is desirable for accurate result. The sterile discs were (6 mm; Himedia) used for the loading crude plant extracts (ethanol and aqueous). Five different concentrations were prepared (250, 500, 750, 1000 and 1250µg) and loaded in appropriate disc. The impregnated discs were incubated at 37˚C for an hour. The dried discs were placed over the surface of swabbed medium with equal distance to avoid the overlapping of the zone of inhibition. Pre-diffusion time was given to the swabbed plates in refrigerator condition for 15min. The plates were incubated at 37˚C for 16-18h during which the activity was evidenced by the presence of zone of inhibition surrounding the discs. Each experiment was done in triplicate. A panel of antibiotics was used against each microbial strains and which antibiotic given sensitive with particular organism used as a control.

Minimum Inhibitory Concentration- Broth dilution Method

Tube dilution method was used to determine the minimum inhibitory concentration (MIC) of the extracts in Muller Hinton broth (Himedia-M 391) and Sabouraud Dextrose Broth (Himedia-M 033) as specified by National Committee for Clinical Laboratory Standard [18]. A total of 10ml of each broth was dispensed into separate test tube and was sterilized at 121˚C for 15min and then allowed to cool. Two-fold serial dilutions of the extracts in the broth were made from the stock concentration of the extracts to obtain 8 - 4096µg/ml for ethanol and aqueous extracts. About 0.1ml of the standardized inoculums of the microbes was inoculated into the different concentration of the extracts in the broth. The test tubes of the broth were incubated at 37˚C for 24h and 30˚C for 1-7 days for bacteria and fungi respectively and observed for turbidity. The lowest concentration that showed no turbidity in the test tube was recorded as the MIC.

Determination of Activity Index

The activity index of the crude plant extract was calculated as;

$$\text{Activity Index (AI)} = \frac{\text{Zone of inhibition of the extract}}{\text{Zone of inhibition obtained for standard antibiotic Drug}}$$

RESULTS

The preliminary phytochemical screening of *J. gendarussa* obtained successively using ethanolic and aqueous extracts of stem and leaves was performed. Each extracts of this plant were subjected to various qualitative tests to identify the phytoconstituents such as tannins, saponins, terpenoids, glycosides, steroids, phenolics, flavonoids and starch and the results are given in table- 1.

Table 1: Preliminary Phytochemical analysis of *Justicia gendarussa* (Stem and Leaves).

Name of the Phytoconstituents	Stem		Leaves	
	Ethanol	Aqueous	Ethanol	Aqueous
Starch	-	-	-	-
Glycosides	+	+	+	+
Saponins	-	-	-	-
Tannins	+	+	+	+
Phenolic compounds	+	-	+	-
Terpenoids	-	+	+	+
Steroids	-	-	-	-
Flavonoids	-	+	-	+

Ethanolic and aqueous extract of leaves and stem (*J. gendarussa*) were evaluated for the antimicrobial activity against the 12 human pathogens and the results were given in tables- 2, 3 and figure- 1a, 1b, 2a, 2b. Aqueous extract of stem showed maximum zone of inhibition *i.e.* 26.20 ± 2.43mm and AI- 0.72 against *S. flexneri*, 24.50 ± 2.16mm and AI- 0.98 (*P. mirabilis*), 21.40 ± 1.77mm and AI- 0.71(*E. coli*) and 20.25 ± 1.24mm and AI- 0.63 (*B. subtilis*) were observed. The results of aqueous extract moreover nearer to a few control antibiotics. Ethanolic extract of stem showed less antimicrobial activity when compared as *J. gendarussa* stem aqueous extract.The aqueous extract of leaves (*J. gendarussa*) showed the maximum antibacterial activity against *S. aureus* (26.33 ± 4.74mm and AI- 0.65) and *S. flexneri* (11 ± 0.0mm and AI- 0.30), while the ethanolic extract of leaves. Fail to inhibit the most of the organisms and less effective with few organisms. Both the stem and leaves extracts of *J. gendarussa* could not exhibits antifungal activity.

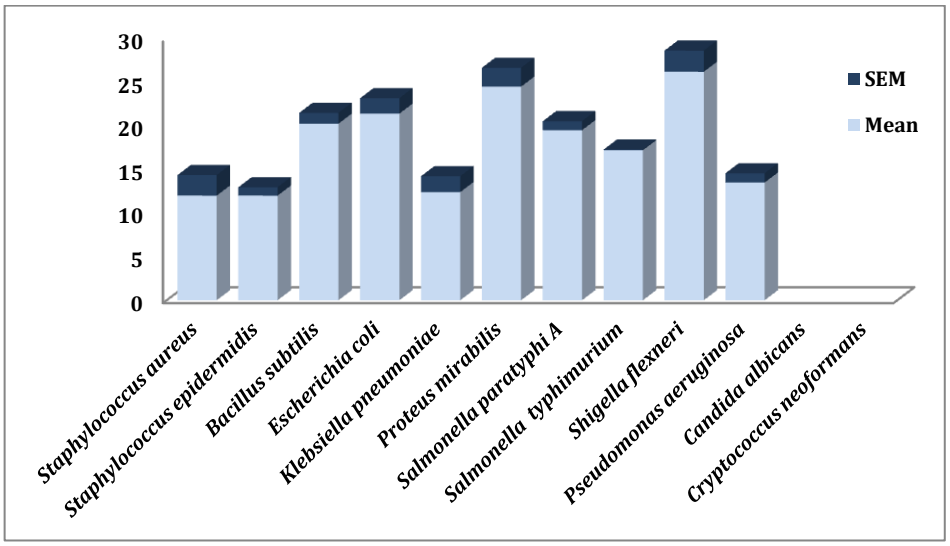

Figure-1a Mean and Standard Error of Aqueous extract of *Justicia gendarussa* (stem)

SEM- Standard Error of Mean

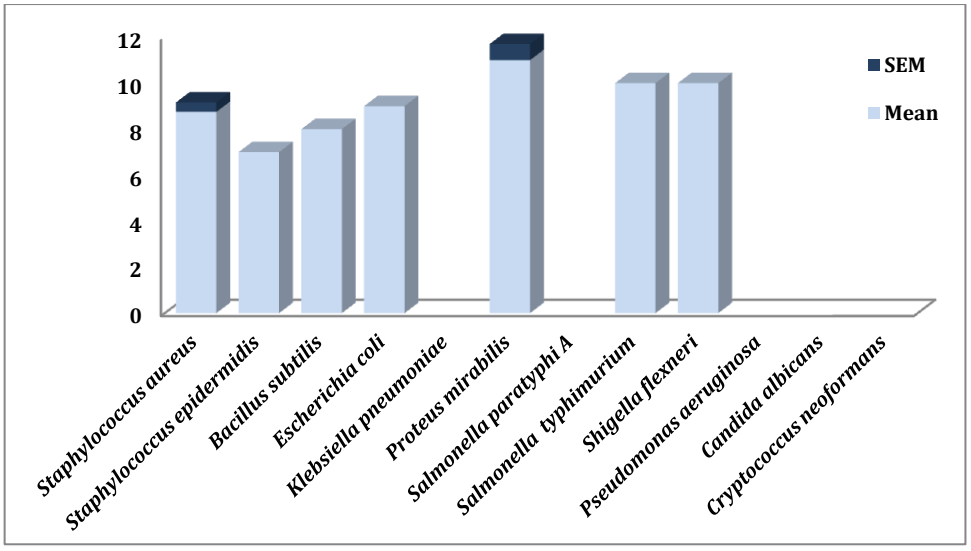

Figure-1b Mean and Standard Error of Ethanolic extract of *Justicia gendarussa* (stem)

SEM- Standard Error of Mean

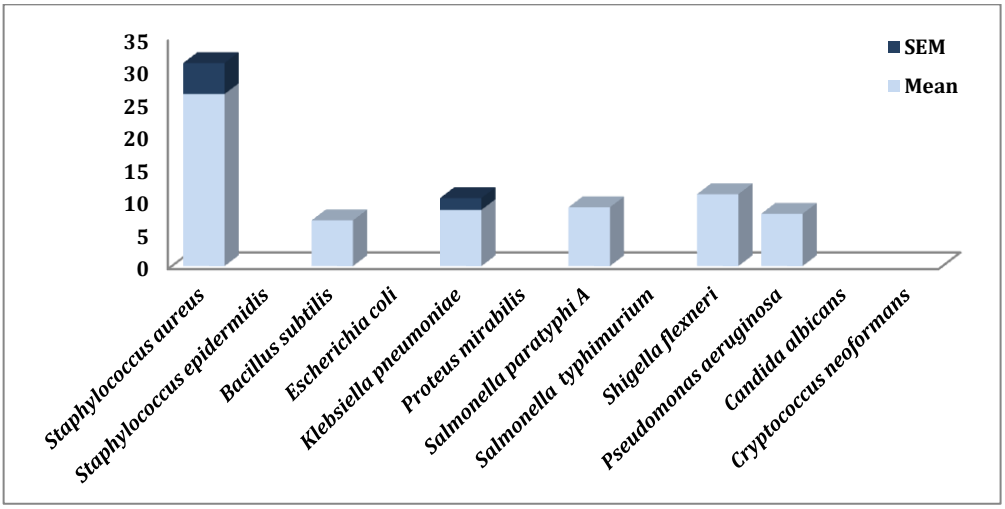

Figure-2b Mean and Standard Error of Aqueous extract of *Justicia gendarussa* (leaves)

SEM- Standard Error of Mean

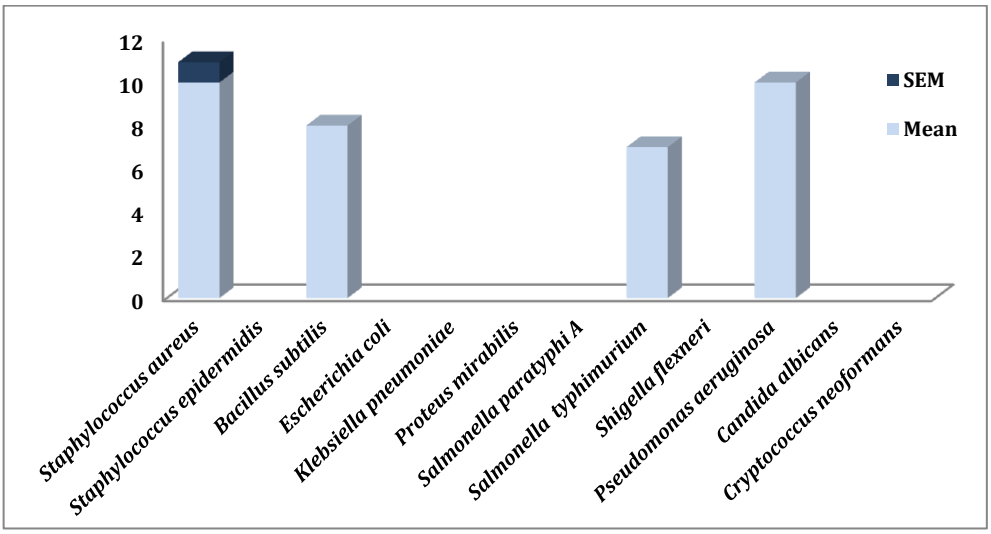

Figure-2b Mean and Standard Error of Ethanolic extract of *Justicia gendarussa* (leaves)

SEM- Standard Error of Mean

Table 2: Antimicrobial activity of stem extract of *Justicia gendarussa* (aqueous and ethanol).

Name of the microorganisms	Zone of inhibition in mm		Stem extracts			
			Aqueous		Ethanol	
	Standard antibiotics (µg/disc)	Zone in mm	Mean ± SEM	Activity Index (AI)	Mean ± SEM	Activity Index (AI)
Staphylococcus aureus	Amoxycillin/Clavulanic acid (30)	40	12.00 ± 2.35	0.30	8.75 ± 0.41	0.21
Staphylococcus epidermidis	Amoxycillin/Clavulanic acid (10)	40	12.00 ± 0.96	0.30	7.00 ± 0.00	0.17
Bacillus subtilis	Ciprofloxacin (5)	32	20.25 ± 1.24	0.63	8.00 ± 0.00	0.25
Escherichia coli	Ciprofloxacin (5)	30	21.40 ± 1.77	0.71	9.00 ± 0.00	0.30
Klebsiella pneumoniae	Nalidixic acid (30)	29	12.40 ± 1.82	0.42	-	-
Proteus mirabilis	Lomefloxacin (10)	25	24.50 ± 2.16	0.98	11.00 ± 0.70	0.44
Salmonella paratyphi A	Chloramphenicol (30)	27	19.50 ± 1.03	0.72	-	-
Salmonella typhimurium	Chloramphenicol (30)	29	17.20 ± 0.08	0.59	10.00 ± 0.00	0.34
Shigella flexneri	Lomefloxacin (10)	36	26.20 ± 2.43	0.72	10.00 ± 0.00	0.27
Pseudomonas aeruginosa	Lomefloxacin (10)	30	13.50 ± 1.06	0.45	-	-
Candida albicans	Nystatin (100)	24	-	-	-	-
Cryptococcus neoformans	Ketoconazole (10)	35	-	-	-	-

SEM- Standard Error of Mean

Table 3: Antimicrobial activity of leaves extract of *Justicia gendarussa* (aqueous and ethanol)

Name of the microorganisms	Zone of inhibition in mm		Leaves extracts			
			Aqueous		Ethanol	
	Standard antibiotics (µg/disc)	Zone in mm	Mean ± SEM	Activity Index (AI)	Mean ± SEM	Activity Index (AI)
Staphylococcus aureus	Amoxycillin/Clavulanic acid (30)	40	26.33 ± 4.74	0.65	10 ± 0.94	0.25
Staphylococcus epidermidis	Amoxycillin/Clavulanic acid (10)	40	-	-	-	-
Bacillus subtilis	Ciprofloxacin (5)	32	7.00 ± 0.00	0.21	8.00 ± 0.00	0.25
Escherichia coli	Ciprofloxacin (5)	30	-	-	-	-
Klebsiella pneumoniae	Nalidixic acid (30)	29	8.60 ± 1.81	0.29	-	-
Proteus mirabilis	Lomefloxacin (10)	25	-	-	-	-
Salmonella paratyphi A	Chloramphenicol (30)	27	9.00 ± 0.00	0.33	-	-
Salmonella typhimurium	Chloramphenicol (30)	29	-	-	7.00 ± 0.00	0.24
Shigella flexneri	Lomefloxacin (10)	36	11.0 ± 0.00	0.30	-	-
Pseudomonas aeruginosa	Lomefloxacin (10)	30	8.0 ± 0.00	0.26	10 ± 0.00	0.33
Candida albicans	Nystatin (100)	24	-	-	-	-
Cryptococcus neoformans	Ketoconazole (10)	35	-	-	-	-

SEM- Standard Error of Mean

Table 4: Minimum inhibitory concentration of aqueous extracts of *Justicia gendarussa* (Stem and Leaves)

Name of the microorganism	Concentration of extracts (in µg/ml)											MIC in (µg /ml)
	4096	2048	1024	512	256	128	64	32	16	8	Control	
Stem - Aqueous extract												
Bacillus subtilis	-	-	-	-	+	+	+	+	+	+	+	512
Escherichia coli	-	-	-	-	+	+	+	+	+	+	+	512
Proteus mirabilis	-	-	-	-	-	+	+	+	+	+	+	256
Salmonella paratyphi A	-	-	-	-	+	+	+	+	+	+	+	512
Shigella flexneri	-	-	-	-	-	-	+	+	+	+	+	128
Leaves - Aqueous extract												
Staphylococcus aureus	-	-	-	-	-	+	+	+	+	+	+	256
Shigella flexneri	-	-	+	+	+	+	+	+	+	+	+	2048

In disc –diffusion method, the extracts showed more than 15mm zone against human pathogens were further tested for MIC by broth dilution technique. *J. gendarussa* stem aqueous extract showed considerable inhibitory activity against *B. Subtilis*, *E. coli* and *S. paratyphi* A (512µg/ml), *P. mirabilis* (256µg/ml) and *S. flexneri* (128µg/ml), whereas aqueous extract of leaves shown significant antibacterial activity against only *S. aureus* (256µg/ml).

These results indicated that the stem and leaves extracts (aqueous and ethanol) possess the inhibitory activity against the tested microorganisms. But stem extracts showed significant MIC values in contrast to leaves extracts (table 4).

DISCUSSION

Plants are the important sources of potential compounds for the development of new natural therapeutic agents. There are several reports available on the antibacterial, antiviral and antifungal properties of plants. Many phytoconstituents of plant origin has been shown to be certainly targeted against resistant pathogenic bacteria [19]. In recent days, there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive components and their ability to treat various diseases [20]. Moreover the current cost of the chemotherapeutic agents is unbearable to the public, particularly in developing countries like India [19]. Therefore

attempts must be directed towards the development of effective natural, non-toxic drugs for treatments. The present investigation was an attempt to explore the antimicrobial activity and phytochemical analysis of *J. gendarussa*. Preliminary phytochemical screening of stem and leaves were analysed, tannins and glycosides were present in both the extracts (stem and leaves), phenolic compounds present in ethanolic extract and absent in aqueous, terpenoids present in leaves extract and absent in stem, flavonoids present in aqueous extracts and saponins, steroids, starch were absent in both the extracts (stem and leaves). The similar results is reported [21] and the present result helpful in assessing chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds. The antimicrobial activity of *J. gendarussa* aqueous stem extract shown significant antibacterial activity against *S. flexneri* (26.20mm), *P. mirabilis* (24.50mm), *E. Coli* (21.40mm), *B. Subtilis* (20.25mm), *S. paratyphi* A (19.50mm) and *S. typhimurium* (17.20mm) and aqueous leaves extracts shown significant inhibitory activity against *S. aureus* (26.33mm) only and all other extracts were less effective with tested organisms. A research report from Bangladesh also stated the similar results [22]. In the present study it was interestingly noted that aqueous extract of stem and leaves of *J. gendarussa* shown considerable antibacterial activity when compared as ethanolic extract and this result indicated that the polarity of water. Further, these extracts could not exhibit antifungal property. In overall assessment it was concluded that the antimicrobial properties of *J. gendarussa* utmost related with the presence of phenolic compounds, tannins, terpenoids and flavonoids. However, further studies are obligatory to isolate, characterize the phyto- constituents and which will be accounted for the antimicrobial properties against human pathogens.

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REFERENCES

1. Ponmurugan Karuppiah and Shyamkumar Rajaram. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian J Tro Biomed 2012;597-601.
2. Premanath R, Sudisha J, Lakshmi Devi N, Aradhya SM. Antibacterial and antioxidant activities of Fenugreek (Trigonella foenum graecum L.) leaves. Res J Med Plants 2011;5 :695-705.
3. Tijjani MB, Bello IA, Aliyu AB, Olurishe T, Maidawa SM, Habila JD, et al. Phytochemical and antibacterial studies of root extract of *Cochlospermum tinctorium* A. rich. (Cochlospermacease). Res J Med Plants 2009;3 :16-22.
4. Constabel F Medicinal plant biotechnology. PlantaMed 1990;5(6) :421-425.
5. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethonopharmacol 1998;62 :183-193.
6. Tomoko N, Takashi A, Hiromu T, Yuka I, Munekazu I, Totshiyuki T, Tetsuro I, Fijio A, Iriya I, Tsumotu N and Kazuhito W. Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin resistant *Staphylococcus aureus*. J Health Sci 2002;48 :273-276.
7. Roja G and Rao PS. Anticancer compounds from tissue cultures of medicinal plants. J Herbs Spices Med Plants 2000;7 :71-102.
8. Subramani SP and Goraya GS. Some folklore medicinal plants of Kolli Hills: Record of a Watti Vaidyas Sammelan. J Econ Taxon Bot 2003;27(3) :665-678.
9. Paval J, Kaitheri SK, Potu BK, Govindan S, Kumar RS, Narayanan SN, *et al.,* Anti-arthritic potential of plant *Justicia gendarussa* Burm. F. Clinics 2009;64(4) :357-62.
10. Bachheti RK, Pandey DP, Archana joshi and Vikas Rana. Chemical analysis of aerial parts of *Justicia gendarussa.* Int J ChemTech Research 2011;3(1) :244-247.
11. Periyanayagam K, Umamaheswari B, Suseela L, Padmini M, Ismail M. Evaluation of antiangiogenic effect of the leaves of *Justicia gendarussa* (Burm. f) (Acanthaceae) by Chrio Allontoic Membrane method. Ame J Inf Dis 2009;5(3) :180-2.
12. Mrunthunjaya K and Hukkeri VI. Antioxidant and free radical scavenging potential of *J. gendarussa Burm*, leaves *invitro.* Natural Prod Sci 2007;3 :199-206.
13. Rantnasooriya WD, Derianyagala SA. and Dehigas DC. Antinociceptive activity and toxicological study of aqueous leaf extract of *J. gendarussa*. Phcog Mag 2007;3 :145-155.
14. Elizabeth KM. Antimicrobial activity of *Terminalia bellerica*. Indian J Clin Biochem 2005;20 :150-153
15. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomed 2008;15(8) :639-652.
16. Wagner H, Bladt S and Zgainski EM. Plant drug analysis 1984. Springer-Verlag:B erlin;1984 :298-334.
17. Bauer AW, Kirby MDK, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc diffusion method. Am J Clin Pathol 1966;45 :493-96.
18. NCCLS (National Committee for Clinical Laboratory Standards), Performance standard for antimicrobial susceptibility testing. 8th Informational Supplement. National Committee for Clinical Laboratory Standards, Villanova, Pa 2002.
19. Gopalakrishnan Sarala, George Shibumon and Benny PJ. Antimicrobial effect of *Punica granatum* on pyogenic bacteria. J of Pharma and Biomed Sci 2010;3(06).
20. Lobo R, Prabhu KS, Shirwaikar A, Shirwaikar A. Curcuma zedoaria Rosc. (white turmeric): a review of its chemical, pharmacological and ethnomedicinal properties. J Pharm Pharmacol 2009;61 :13-21.
21. Kumar J, Nino, Amala lourthuraj A. In Vitro regeneration and phytochemical analysis of *Justicia gendarussa*. Indian J. Innovations Dev 2012;1(2).
22. Sikder MAA, Nawshad Hossian AKM, Siddique AB, Ahmed M, Kaisar MA, Rashid MA. In Vitro antimicrobial screening of four reputed Bangladeshi medicinal plants. Phcog J 2011;3.