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Biomass Production and Antibacterial Activity of *Justicia Gendarussa*: A Valuable Medicinal Plant

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ABSTRACT

Rooting and biomass production of *Justicia gendarussa* has been achieved through a hydroponic system of cultivation. The obtained biomass of leaves, stem and root were examined for antibacterial activity against various human pathogenic organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Shigella sp.*, *Pseudomonas sp.* and *Klebsiella pneumoniae*. Methanolic extract of *J. gendarussa* root responded against *E. coli*. The growth of *Shigella sp.*, *Pseudomonas sp.* and *K. pneumonia* were inhibited by leaf extract. The maximum inhibition zone against *S. aureus* was observed in stem extract treatment.

Keywords: *Justicia, antibacterial, hydroponic, rooting, medicinal plant*

BACKGROUND

Justicia gendarussa Burm. f. (Acanthaceae) commonly known as Black adusa is an evergreen shrub growing in moist environment in India, China, Malaysia, Indonesia, Sri Lanka, Philippines and Bangladesh. Traditionally, *J. gendarussa* has been utilized to treat chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases, muscle pain, lumbago, headache, earache, hemiplegia, hair growth promotion, leucoderma, asthma, antiseptic, haemostatic, nasal bleeding, bone fracture, injuries and fever [1-7]. Various researchers have been reported that *J. gendarussa* possess antiangiogenic effect [8], antioxidant and hepatoprotecti-ve potential [9], antifungal activity[10], anti-bacterial activity [11] anti-arthritic potential [12], antiinflammatory and analgesic activities [13], antinociceptive activity [14], antisickling activity [15], anthelmintic activities [16], Larvi-cidal and adulticidal activity [17] and in vitro HIV type 1 reverse transcriptase inhibitory activity [18]. *J. gendarussa* has been found to contain alkaloids, triterpenoids, tannins, justicin, steroids, and flavonoids, gendarusin A and B [19, 20]. *J. gendarussa* has been considered as rare and endangered medicinal plant due to its unscientific collection from natural habitat and over exploitation for commercial purposes to meet the requirements of various pharmaceutical applications [21-23]. Hence, it is most important to develop a viable method for production of *J. gendarussa* biomass.

Hydroponics, is a plant culture technique which enables plant growth in a nutrient solution with the mechanical support of inert substrate. Largely applied both in laboratory experiments and in commercial crop production [24,25]. Hydroponic system provides numerous advantages: no need for soil sterilization, high yields, good quality, precise and complete control of nutrition and diseases, shorter length of cultivation time, safety for the environment and special utility in non-arable regions. Application of this culture technique can be considered as an alternative approach for large-scale production of some desired and valuable crops [26]. Though hydroponic system has been developed for growing several plants [27-30]. Growing plants in a hydroponic system can overcome cultivation difficulties and this could be a means to manipulate phenotypic variation in bioactive properties. The acceptance of traditional medicine as an alternative form of healthcare and the development of microbial resistance to the available antibiotics has led to investigate the antimicrobial activity of medicinal plants. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activity. Ethanolic

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extracts of *J. gendarussa* was found to have broad spectrum of antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia* coli, *Staphylococcus aureus*, *Staphylococcus faecalis* and *Vibrio cholerae* [11]. The present study was aimed at developing an alternative simple, rapid and economical production of *J. gendarussa* biomass through a hydroponic system of cultivation and evaluates the same for antimicrobial activity, forms a very attractive novel concept to conserve the valuable medicinal plant in its natural habitat.

MATERIALS AND METHODS

Rooting and cultivation of J. gendarussa in hydroponic system

Young stem cuttings with an actively growing side branch obtained from 12 months old *J. gendarussa* plants were used as explants in this study. Explants were placed in thermocole sheets (10mm breadth) with an internal spacing of 5 × 5cm. The cut ends of the planting materials along with the thermocole sheet were dipped to a depth 1.0 cm in 20 litre of nutrient solution containing 1/10 Murashige and Skoog (MS) salts with different concentrations of IBA (0.0, 0.5 and 1.0 mg/l) in plastic tubs (4×2 feet). The tubs were tightly closed with polyethylene sheet. The pH and electrical conductivity of the solution were 5.9 and 859µS. The first fifteen days 1/10 Murashige and Skoog salts with IBA was used to initiate the roots and shoots, next first fifteen days 1/10 Murashige and Skoog [31] salts were used for shoots growth and last 20 days Hoaglands solution was used to increase the biomass production. The diurnal temperature fluctuated between 28°C to 34°C during the study period. Each treatment had 12 replicates. Number of leaves or plant, number of nodes or plant, number of root or plant, root length and chlorophyll were recorded during the growth period and biomass (g plant^{-1}fw. and dw.) were harvested.

Antibacterial activity

Shade dried leaf, stem and root were powdered mechanically and extracted with MeOH and water. The extracts obtained were concentrated to dryness in a rotary evaporator under reduced pressure (11 kpa) at a temperature of 40 ℃ and kept at 4°C for experimental purpose.

A total of five pathogenic bacterial strains such as *Staphylococcus aureus, Escherichia coli, Pseudornonas aeruginosa, Klebsiella pneumoniae* and *Shigella sp.* were used for antibacterial activity studies. All the bacterial strains were procured from MCRC Microbial culture collection centre, Taramani, Chennai. Test organisms were maintained on nutrient agar slants and transferred to fresh slant once a week. Then the slants were incubated at 32°C for 24 hours. Organism were then washed with 3ml saline solution or nutrient broth from agar slant onto a large agar surface of medium such as roux bottle containing 250 ml agar and incubated for 24 hours. Using 50 ml saline solution or nutrient broth the growth from the nutrient surface was washed. Then organisms were stored under refrigeration. Inoculum was adjusted at 530 nm leading to a transmission equivalent to 1×10^8 cells/ml. All the ingredients except agar were dissolved in distilled water, gently heated, cooled and pH was adjusted to 7.4. Agar was then added and dissolved by heating. The medium was then dispensed in 5 ml quantities in test tubes. Sterilization was done by autoclaving at 121°C for 15 minutes. The medium in the tubes was then allowed to solidify as slants.

Leaf, stem and roots extracts of *J. gendraussa* grown hydroponically were tested for antibacterial activity using agar disc diffusion assay [35]. The microorganisms were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37°C and were referred to as seed broth. The bacterial inocula (20 h broth) were uniformly spread on Muller-Hinton agar in sterile Petri plates. The wells with a holding capacity of 10µl were made using a cork borer and the plant extract was poured into each well. The extract containing plates were incubated at 37°C for 24h and the diameter of growth inhibition zone was recorded.

Statistical analysis

A completely randomized block design was followed for all experiments. Each treatment consisted of 12 replications and each experiment was repeated twice, in order to confirm the results. All the results were subjected to analysis of variance (ANOVA) using SPSS version 14.0. Wherever necessary, means were separated using Duncan's Multiple Range Test [34]

RESULT AND DISCUSSION

Results on rooting of stem cutting and growth of *J. gendarussa* in a hydroponic system of cultivation are presented in Table 1. The results clearly indicate that the explants are well adapted to hydroponic system of cultivation and showed

an increase in the number of leaves, number of root and number of node in a 50 days of experiment. Among the different concentrations of IBA studied for the rooting ability of stem cuttings, the optimum root induction was observed in 0.5, mg/l of IBA supplemented 1/10 MS medium followed by 1.0 mg/l than the control plants with in fifteen days. After rooting, IBA was removed for next fifteen days. Then, instead of 1/10 MS medium, the Hogland solution was used for next twenty days to increase biomass production. The plant showed vigorous growth and produced sufficient biomass that was achieved in 50 days of experimentation (table 1,2, fig 2). The attempt was successful in obtain sufficient biomass of *J. gendarussa* in hydroponic system. The study also illustrates that the concentration of IBA play a major role in the induction of roots and biomass production. The results are comparable to those obtained by Karthic and Seshadri [30] reported in *Gymnema sylvestre*. It is a well know fact that in some parts of the world, plant life does not grow in the available soil, climate and other factors that threatens the growth of plants. While consumption of herbal medicines is widespread and increasing harvesting of plants from the wild, the main source of raw materials is causing loss of genetic diversity and habitat destruction. These are some of the main reasons behind the drive to develop hydroponics in a way to produce fresh products in non-arable areas of the world. The use of controlled environments can overcome cultivation difficulties and could be a means to manipulate phenotypic variation in bioactive compounds and biological activities also

Table 1. Rooting and growth of *J. gendarussa* in a hydroponic system of cultivation

IBA treatment	Growth parameter	Growth period (Days)									
		5th	10th	15th	20th	25th	30th	35th	40th	45th	50th
0.0 mg/l	Leaves (no.)	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	11.0
	Nodes (no.)	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	2.0
	Roots (no.)	1.0	2.0	3.0	4.0	5.0	7.0	8.0	10.0	12.0	13.0
	Root length (cm)	0.2	0.8	1.5	2.6	4.7	6.5	8.4	10.6	12.4	13.1
	Chlorophyll (%)	-	-	-	2.8	4.3	7.7	10.5	16.4	21.4	27.5
0.5 mg/l	Leaves (no.)	4.0	5.0	8.0	10.0	12.0	13.0	15.0	17.0	22.0	23.0
	Nodes (no.)	0.0	0.0	1.0	1.0	1.0	2.0	2.0	3.0	3.0	4.0
	Roots (no.)	3.0	6.0	9.0	10.0	13.0	14.0	16.0	18.0	21.0	22.0
	Root length (cm)	0.9	5.2	7.8	8.4	10.7	13.6	19.4	22.8	27.5	30.2
	Chlorophyll (%)	-	-	-	12.4	19.3	22.2	25.8	29.0	38.1	40.8
1.0 mg/l	Leaves (no.)	3.0	4.0	5.0	7.0	8.0	9.0	13.0	16.0	17.0	21.0
	Nodes (no.)	0.0	0.0	1.0	1.0	1.0	1.0	2.0	2.0	2.0	3.0
	Roots (no.)	2.0	5.0	6.0	8.0	10.0	14.0	16.0	17.0	18.0	19.0
	Root length (cm)	0.5	1.0	2.2	3.5	5.5	9.6	12.8	19.4	22.2	25.4
	Chlorophyll (%)	-	-	-	17.8	18.2	21.1	23.4	28.9	35.6	37.2

The first 15days 1/10 MS salts with different concentrations of IBA was used to initiate the roots, next 15days 1/10 MS salts were used for shoots growth and last 20 days Hoaglands solution was used grow the plants under hydroponic system. All the results are average values of 12 plants.

Table 2. Yield of *J. gendarussa* biomass obtained from hydroponic system of cultivation

IBA treatment	Biomass (g/plant)					
	Leaf		Stem		Root	
	fw.	dw.	fw.	dw.	fw.	dw.
0.0	3.12c	0.68c	2.74c	0.53c	3.83b	0.38c
0.5	12.88a	3.41a	4.99a	1.44a	3.91a	0.63a
1.0	10.02b	2.83b	4.61b	1.21b	3.84ab	0.59b

Biomass were harvested after 50 days of growth. In each column, mean value followed by the same letter were not significantly different ($p \leq 0.5$) according to DMRT

The results of antibacterial activity of aqueous and methanol extracts of leaves, stem and roots of hydroponically grown *J. gendraussa* are presented in Fig 1. Totally five bacterial strains viz. *S. aureus, E. coli, Shigella sp., K. pneumoniae* and *Pseudomonas sp.* were screened in this study. The antibacterial activity of methanolic extract of *J. gendraussa* was better than aqueous extract. This could be due to the extraction of phenolic compounds in methanolic extract of *J. gendraussa* and less saponins in the water extracts. *J. gendraussa* plants rooted in 1/10 MS supplemented various concentration of IBA taken for antibacterial activity. Among the

various concentration of IBA rooted plants, IBA (0.5 mg/l) showed maximum inhibition zone in all the pathogenic bacteria tested. Therefore, *J. gendraussa* can be propagated with the use of 0.5 mg/l of IBA and that do not affect the antibacterial activity.

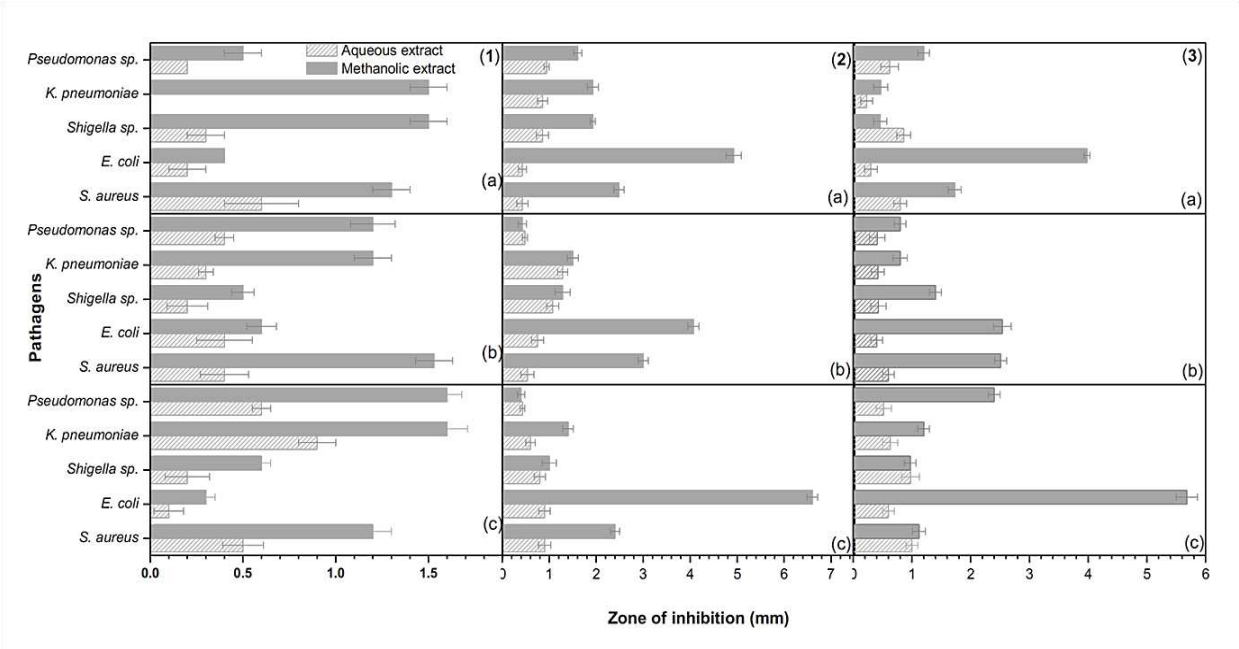

Figure 1. Antibacterial activity of *J. gendarussa* leaf obtained from hydroponic system of cultivation. (1) plants rooted without IBA treatment, (2) plants rooted with 0.5 mg/l of IBA, (3) plants rooted with 1.0 mg/l of IBA. (a) leaf extract (b)stem extract (c) root extract.

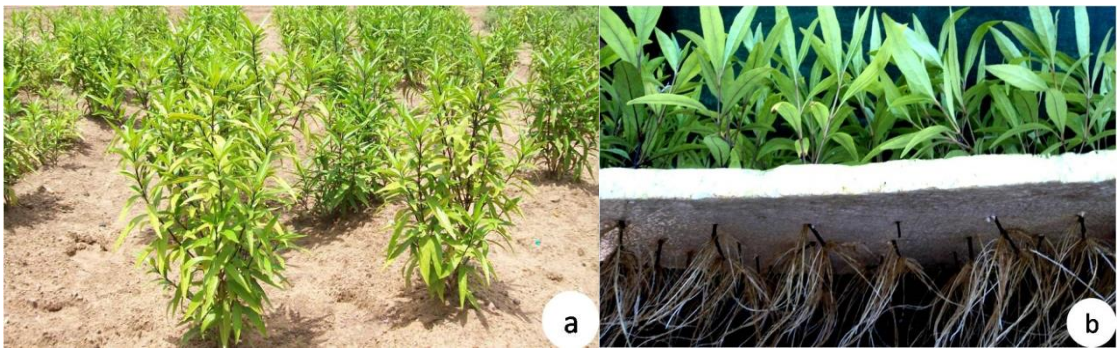

Figure 2. (a) Field grown *J. gendarussa*, (b) Hydroponically grown *J. gendarussa*

Plants rooted in IBA with the concentrations of below or above 0.5 mg/l affected antibacterial potential. Though the use of growth regulators during the rooting may affect the secondary metabolite synthesis and simultaneous biological activity of plants [32,33]. The Leaf, stem and root extracts of *J. gendraussa* were screened for antibacterial activity against various pathogenic bacteria, among these root extract responded against *E. coli*. The growth of *Shigella sp., Pseudomonas sp.* and *K. pneumonia* were inhibited by leaf extract. The maximum inhibition zone against *S. aureus* was observed in stem extract treatment.

CONCLUSION

The study revealed that the rooting and biomass production of *J. gendraussa* through hydroponic system is feasible. In the present study, *J. gendraussa* stem cuttings were rooted through hydroponic system treated with IBA (0.0, 0.5 and 1.0 mg/l) supplemented 1/10 MS basal salts medium. The results showed that the lowest concentration of IBA (0.5 mg/l) to increase the plant growth and yield considerably than other concentrations and control. The methanolic extract of *J. gendraussa* plant parts viz. leaves, stem and root showed potent antibacterial

activity against different tested bacterial pathogens. This study encourages further research on growth of this plant in different hydroponic media followed by extraction of bioactive compounds and carryout clinical evaluation for confirming the therapeutic values.

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