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Efficacies of bactericidal Justicia gendarussa extract inhibiting protein synthesis against methicilin resistant staphylococcus *aureus*

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Abstract: *Bacterial infection is one of the most serious global health issues in 21st century .The rise in antibiotic-resistant microorganisms in recent years has led to an increasing search for new antibiotics. Plant secondary metabolites have been used for centuries in traditional medicines and therefore represent a source of potentially active compounds. Justicia gendarussa is reputed for its beneficial effects in Respiratory disorders like cough, cold, bronchitis, throat infections, pulmonary infections and allergic disorders like bronchial asthma.The bacterial diseases can be attributed to Staphylococcus Aureus can be controlled by the extract of Justicia gendarussa extract.*

Key Words: *Bacterial infection,Justicia gendarussa,secondary metabolites,Traditional medicine*

I. Introduction

Bacterial infection is one of the most serious global health issues in 21st century (Morris and Masterton, 2002). Burn patients are at high risk for nosocomial infections due to multi-resistant bacterial species and a high proportion of which was due to Gram-negative organisms (Moore, 1999). Bacterial colonization of burned and devitalized tissue is inevitable and invasive bacterial infection is still one of the major problems in the treatment of burn victims. Antimicrobial resistance settings have failed to address this essential aspect of drug usage (Monnet et al., 1998). There are a number of clinically efficacious antibiotics becoming less effective due to the development of resistance. The emergence of bacterial resistance to antibiotic is a major health problem and therefore, it is critical to develop new antibiotics with novel mechanism of action to overcome these problems (Wang et al., 2003).

The great interest in the use and importance of Indian medicinal plants by the World Health Organisation in many developing countries has led to intensified efforts on the documentation of ethnomedical data of medicinal plants (Dhar et al., 1968; Waller, 1993; Perumal Samy and Patricraja, 1996). It is a necessity from the scientific point of view, to establish a rational relationship between chemical, biological and therapeutical activities of folklore medicine (Gentry, 1993).

Natural products have been used for thousands of years in folk medicine and recommended for a wide range of ailments. Plants have been the traditional source of raw materials for medicines. A rich heritage of knowledge on preventive and curative medicines was available in ancient scholastic work included in the Atharva veda, Charaka, Sushruta, etc. An estimate suggests that about 13,000 plant species worldwide are known to have use as drugs. These constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access and ancestral experience (Martin-Bettolo, 1980). Biologically active compounds from natural sources has always been of great interest to scientists working on infectious diseases. In recent years there has been a growing interest to evaluate plants possessing antibacterial activity for various diseases (Clark and Hufford, 1993). A number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants (Malcom and Sofowora, 1969; Bhakuni et al., 1974; Taniguchi et al., 1978; Moskalenko, 1986; Brantner and Grein, 1994; Grosnenor et al., 1995; Perumal Samy and Ignacimuthu, 1997).

The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials (Das .S, et al 1999). They have been reported to have antibacterial properties against many of microbial organisms (Nadkarni, 1976; Rotimi et al., 1988). For example, a few recent studies have demonstrated the antibacterial activity of traditionally used plants against some selected pathogens from clinical sources (Navarro et al., 1996). The plant kingdom represents a largely unexplored reservoir of biologically active compounds not only as drugs, but also as unique templates that could serve as a starting point for synthetic analogs and an interesting tool that can be applied for a better understanding of biological processes. Folkloric uses are supported by a long history of human experience. Numerous biologically active plants are discovered by evaluation of ethnopharmacological data, and these plants may offer the local population immediately accessible therapeutic products (Aquino et al., 1995). In this

study, we evaluated the in vitro antibacterial activity of different extract from the leaves of Justicia gendarussa against methicilin resistant Staphylococcus aureus

II. Materials And Methods

2.1 STERILIZATION OF GLASSWARE

Glassware were soaked overnight in cleaning solution and washed thoroughly with running tap water. They were then cleaned with detergent solution and rinsed several times with tap water and finally in distilled water and air dried. The glassware and media were sterilized in an autoclave at 15psi for 20 minutes, at 120 ^{o} C

2.2 MEDIA USED

2.2.1. NUTRIENT AGAR PER LITTER/GRAM (NA)

Peptone-5.0g
Yeast extract-2.0g
NaCl-5.0g
Agar-18.0g
Distilled water-1000ml
pH-7.0

2.2.2. NUTRIENT BROTH PER LITTER/GRAM (NB)

Peptone-5.0g
Yeast extract-2.0g
NaCl-5.0g
Distilled water-1000ml
pH-7.0

2.2.3. MULLER HILTON AGAR

Approximate Formula* Per Liter
Beef Extract Powder-2.0 g
Acid Digest of Casein-17.5 g
Starch-1.5 g
Agar-17.0 g
pH-7.0

2.3 COLLECTION OF PLANT MATERIAL

Leaves of *Justicia gendarussa* were collected from Medicinal Plant Garden at Sri Sairam Siddha Medical College and Research Centre, West Tambaram , Chennai 600 044.

2.4 PHYTOCHEMICAL SCREENING TEST OF *Justicia gendarussa* petal

I. Test for Alkaloids

(A) Dragendroffs reagent:

8g of bismuth nitrates Bi $(No_3)_3$ 5 H_2o was dissolve in 20ml of HNo_3 and 2.72g of Potassium iodide in 50ml of H_2o. These were mixed and allowed to stand for deposition of KNo_3 Crystals. The Supernatant was decanted off and made up to 100ml with distilled water.

Procedure: To 0.05ml of *Punica granatum* leaf extract 2ml of HCl was added. To this acidic medium 1ml of dragendroffs reagent was added on, orange or red precipitate produced immediately indicate the presence of alkaloids.

II. Test of Flavonoids

A. Alkaline reagent test

To 1.0 ml of *Punica granatum* leaf extract a few drops of dilute sodium hydroxide was added. A intense yellow color if produced indicates the presence of flavonoids.

III. Test for Glycosides

The *Punica granatum* leaf extract was hydrolyzed with HCl for few hours on a water bath and the hydrolysate was subjected to Bornbagers test to the presence of different glycosides.

A. Bornbager's test

Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. If the ammonia layer acquired the pink color it indicates the presence of glycosides.

IV. Test for Saponins

(a) The *Punica granatum* leaf extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formations of 1cm layer of foam shows the presence of saponins

(b) 1ml of the *Punica granatum* leaf extract was treated with 1% lead acetate solutions. Formation of white precipitate indicate the presence of saponins

(c)

V. Test for Tanins

(a) Ferric chloride test

To 1.2 ml 0f *Punica granatum* leaf extract few drops of 5% aqueous $FeCl_3$ solution was added. A violet colour formation indicates the presence of tannins.

VI. Test for Triterpenoids

(A) Nollers test

5mg of *Punica granatum* leaf extract was dissolved in 2ml of 0.01 % anhydrous tannic chloride in pure thionyl chloride. A purple color formed then changed to deep red after few minutes and this indicated the presence of Triterpenoids

2.5 PARTIAL PURIFICATION OF COMPOUND USING TLC

The crude dichloromethane extract was spotted on to the pre-coated TLC plates (60 F_2 54) and developed with a solvent system of hexane, chloroform and methanol in the ratio of 1:0.5:0.1. The developed plate was viewed under UV(260 nm). The spots were eluted with chloroform as solvent and used to study its antibacterarial activity.

2.6 CULTURE COLLECTION AND MAINTENANCE

The studies of organisms were obtained from CAS in Botany University of Madras Guindy Campus Chennai 600 025 *Staphylococcus aureus,*

2.6.1 PREPARATION OF CULTURE FOR ANTIBACTERIAL STUDIES

Twelve hour old bacterial suspension was adjusted to 0.5 OD and 1.0ml from the above was inoculated into 50 ml of nutrient broth and incubated at 37 ^{0} C in an orbital shaker at 150 rpm. To determine the growth rate, culture was removed at 4h interval and the growth was monitored by measuring the optical density at 540nm in spectrophotometer (Deepvision model 1371 VU/Vis Spectrophotometer, India). The growth curve was drawn by plotting OD value against the incubation time.

2.7 ANTIBACTERIAL ACTIVITY OF CRUDE AND ISOLATED COMPOUND

The antibacterial activity of the crude and purified compound was assayed using the disc diffusion method. Bacteria were grown overnight on Mueller Hinton agar plates, five colonies were suspended in 5 ml of sterile saline (0.9%) and the bacterial population in the suspension was adjusted to ~$3x10^8$ CFU/ml. A sterile cotton swab was dipped into the suspension and the swab rotated several times with firm pressure on the inside wall of the tube to remove the excess fluid. The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90º to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile disc of 9 mm diameter. Each disc was placed firmly on to the agar to provide uniform contact with the bacteria. Bioactive compound (50 µg) was weighed and dissolved in 1 ml of 7% ethanol. The different concentration of bioactive compound was introduced on to each disc and the control disc received only 7% ethanol. The plates were incubated at 37°C for 24 h and the inhibition zone was measured and calculated. The experiments were carried out in duplicate three times. The results (mean value, *n*=3) were recorded by measuring the zones of growth inhibition surrounding the discs.

2.8 MINIMAL INHIBITION CONCENTRATIONS (MIC)

MIC determination was performed using serial broth dilution method. The Crude and isolated compound solution (100 µlg ml^{-1}, pH 6.1) was serially diluted to different concentrations from 25, 50, 75 and 100 µlg $ml^{-1}$with NB. Each of the compound dilutions (5 ml) was inoculated with 10 µl of the bacterial culture, and then the mixture was incubated at 37°C in a test tube on a shaker for 2 days. OD values were taken under 540nm and dry weight of Bacterial culture was estimated.

Data analysis

Bacterial growth was expressed as OD values the dry weight of Bacteria (g l^{-1}). Inhibitory rate of the *Justicia gendarussa* Crude extract and isolated compound for bacteria was measured as percentage of bacterial growth inhibition, which was calculated by the following formula:

% growth inhibition of a fungus

$$= \frac{\text{BDWof a control bacteria - BDW of a treated sample}}{\text{BDW of a control bacteria}} \times 100$$

Where BDW is the mathematical means of the dry weight of bacteria (g l^{-1}) of the bacterial cultures.

2.9 AGAROSE GEL ELECTROPHORESIS

REAGENT REQUIRED FOR GEL ELECTROPHORESIS

A. 1.4% Agarose gel:

nanopure water..... 392 ml

50X TAE buffer..... 8 ml

electrophoresis grade agarose... 5.5 g

Heat to boiling.

Excess gel can be stored at room temperature and remelted for future use. To expedite gel runs, we make 1.6 l and divide into 200 ml aliquots in covered, 500 ml erlenmeyer flasks. These can quickly be remelted in a microwave oven as needed.

B. 0.5 M EDTA (pH 8.0):

mw= 336.2 g (anhydrous), 354.2 g (H20), 372.24 g (2 H20) For 500 ml, start with approximately 450 ml water and adjust pH initially with NaOH pellets. Autoclave.

C. 50X TAE buffer:

Tris-borate..... 242 g

glacial acetic acid..... 57 ml

0.5 M EDTA, pH 8.0... 100 ml

Dilute to 1 L with nanopure water

D. Running Buffer (1X TAE):

Dilute 50X TAE to 1X and use the amount appropriate for the particular gel apparatus.

E. 1 Kb Ladder (1 µg/ml):

ladder stock..... 5 µl

1 M NaCl..... 44 µl

glycerol loading dye (6X).. 7.5 µl

We use Life Technologies 1 Kb Plus DNA Ladder.

F. Glycerol Loading Dye (6X):

bromophenol blue... 0.26 g

glycerol..... 30 ml

To 100 ml with nanopure water

G. 1 M NaCl

mw= 58.44 g/liter nanopure water

H. Ethidium Bromide:

10 mg/ml, store in a dark bottle at 4° C.

Method

1. Prepare gel solution as outlined under Recipes section.
2. Pour the gel when the agarose has cooled to about 55° C. Insert the proper comb for the particular gel rig. The gel should be allowed to cool until it has set (it will turn whitish and opaque when ready). The amount of agarose depends on the size of the gel rig. Gels should be fairly thin, approximately 1/4 to 1/2 inch.
3. Carefully remove the comb and place the gel in the gel rig with the wells closest to the cathode (black) end. Cover the gel with 1X TAE running buffer.
4. Cut a piece of parafilm and place a 5 µl drop of glycerol loading dye onto the waxy side for each sample to be loaded.
5. Keeping samples on ice, pipette up 5 µl of a sample, wipe the excess oil from the pipette tip with a Kimwipe and add the sample to one of the drops of loading dye.
6. Switch the pipette tip to another pipette set for 10 µl. Mix the sample and loading dye by filling and emptying the pipette a few times then load the mixture into a well.
7. Continue loading the rest of the samples, placing 5 µl of 1 Kb ladder at both ends of the series of samples and between every 10 samples.

8. Place the cover on the gel rig and run the samples towards the anode (red) end. For a small gel, we set the power pack to about 60 ma. For a large gel, we use about 120 ma. Milliamperage increases during the run, so check it periodically. Stop the run before the bromophenol blue loading dye front exits the gel.
9. Turn off the power pack, remove the gel and place it in a stain box with 40 µl ethidium bromide: 200 ml 1X TAE for approximately 45 minutes. NOTE: Ethidium bromide is light sensitive and must be stored in darkness.
10. Visualize with U.V. light (take proper precautions!) and photograph with a polaroid Photo documentation camera.
11. Dispose of the gel properly. (check to see how your facility handles disposal of ethidium bromide).

2.10 PROTEIN INHIBITION ACTIVITY OF *Justicia gendarussa* AGAINST *Staphylococcus aureus*

Protein Inhibition activity was performed using serial broth dilution method. The Crude and isolated compound solution (100 µlg ml^{-1}, pH 6.1) was serially diluted to different concentrations from 25, 50, 75 and 100 µlg $ml^{-1}$with NB. Each of the compound dilutions (5 ml) was inoculated with 10 µl of the bacterial culture, and then the mixture was incubated at 37°C in a test tube on a shaker for 20 hrs. Then Extra cellular proteins were estimated by Bradford Method (1976) and SDS PAGE was performed to analyse the inhibition activity in Malecular Mass level.

2.11 EXTRA CELLULAR PROTEIN ESTIIMATION BY BRADFORD METHOD (1976)

The protein content of the culture filtrate was estimated by the dye binding method of Bradford (1976).

REAGENT

Coomassie Brilliant Blue G-250 (100 mg) was dissolved in 50 ml of ethanol. To this, 100 ml of 85% (v/v) phosphoric acid was added and made up to one liter. The concentration in the reagent was 0.01 % (w/v), CBB G-250, 4.7 % (v/v), ethanol and 8.5 % (v/v) phosphoric acid.

PROCEDURE

To 1.0 ml of culture filtrate, 5 ml of CBB was added, mixed thoroughly and read at 595 nm in a Beckman DU-50 Spectrophotometer. The reagent with sterile uninoculated medium served as blank. The amount of protein was calculated using Bovine Serum Albumin Fraction V (Sigma Chemicals Co., USA) as the standard.

2.12 SODIUM DODECYL SULPHATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

SDS-Poly acrylamide gel electrophoresis was performed on slab gel with separating and stacking gels (10 & 5 % w/v) by the method of (Laemmli, 1970).

REAGENTS

STOCK SOLUTIONS

Solution A	1.5 M Tris HCl buffer (pH 8.8) with 0.4 % (w/v) SDS
Solution B	0.5 M Tris HCl buffer (pH 6.8) with 0.4 % (w/v) SDS
Solution C	30 % (w/v) acrylamide with 0.8 % bisacrylamide
Solution D	1.4 % Ammonium persulphate
Solution E	1 % SDS
Solution F	N,N,N,N' tetramethyl ethylene diamine (TEMED)

PREPARATION OF GEL

Separating gel [10 % (w/v)]		**Stacking gel [5 % (w/v)]**	
Solution A	0.75 ml	Solution B	0.38 ml
Solution C	2.0 ml	Solution C	0.5 ml
Solution D	0.3 ml	Solution D	0.15 ml
Solution E	0.6 ml	Solution E	0.3 ml
Distilled water	2.6 ml	Distilled water	1.98 ml
Solution F	0.005 ml	Solution F	0.005 ml

Tank buffer (pH 8.3)		**Sample Buffer**	
Tris	3.0 g	Glycerol	2.0 ml
Glycine	14.4 g	β-mercaptoethanol	1.0 ml
SDS	1.0 g	10 % SDS (w/v)	4.0 ml
Distilled water	1.0 L	Solution B	1.7 ml

		Bromophenol blue (aqueous)	0.2 ml
		Distilled water	0.6 ml

PROCEDURE

The enzyme solution was mixed with an equal volume of sample buffer, boiled in a water bath for 3 min, cooled and added to the wells then the power supply was connected with cathode in the upper tank and anode in the lower tank. Electrophoresis was carried out at room temperature with constant voltage and 20 mA current supply was maintained until the tracer dye reached 0.5 cm above the lower end.

STAINING OF SEPARATED PROTEINS

At the end of electrophoresis, gel was removed and stained with silver staining method of Blum *et al.* (1987). After staining, the gels were stored in 7 % (v/v) acetic acid.

DETERMINATION OF MOLECULAR MASS

The molecular mass of the purified Tannase was determined on SDS-PAGE. Purified protein samples were run on SDS-PAGE with concurrent run of standard protein ladders consist 100 kDa (sigma chemicals).After separation, the gels were stained with silver nitrate as described by Blum *et al*. (1987).

III. Result

3.1 PHYTOCHEMICAL SCREENING OF METHANOL PETAL EXTRACT OF *Justicia gendarussa*

The phytochemical screening of the *Justicia gendarussa* studied showed the presence of flavonoids terpenoids, saponins and tannins (Table -1, Plate-2).

TABLE 1. PHYTOCHEMICAL SCREENING OF METHANOL PETAL EXTRACT OF *JUSTICIA GENDARUSSA*

S/No.	**Constituents**	**Methanol petal extract of** *Justicia gendarussa*
1.	**Alkaloids**	+
	Mayers reagents	
3.	**Tannin**	+
	$FeCl_3$ test	
4.	**Saponins**	-
	Frothing test	
5.	**Terpenoids**	-
	Nollers test	

- = Negative (absent)
+ = Positive (slightly present)

3.2 SCREENING OF ANTIBACTERIAL COMPOUND FROM THE LEAF OF *Justicia gendarussa* BY TLC

The partially purified dichloromethane extract was loaded on to pre coated TLC (60 F_2 54) and it was developed using solvent system in the ratio of 1:0.5:0.1 (hexane, chloroform and methanol) the spot gave out 3 non visible spot from the top to bottom had an RF value 0.15, 0.31 and 0.40 respectively (Plate-4). The colorless spot with the RF value 0.40 fluorescent with UV light in violent color, inhibited the growth of pathogenic bacteria and fungi when compare to other spots. (Table-2, Plate 3)

Table-2 Partial charecterization of methanol petal extract of *Justicia gendarussa* by TLC

Methanol leaf extract of *Justicia gendarussa*				
Component No.	**UV Light 360nm Rf value**	**UV light 240nm Rf value**	**Iodine Rf value**	**Normal Light Rf value**
1	0.40	0.40	0.38	0.40
2	0.31	0.29	0.19	0.30
3	0.15	0.16	-	-

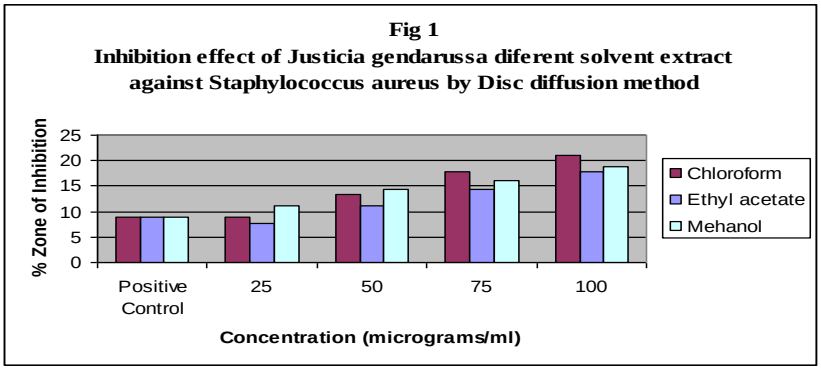
EFFECT OF CRUDE EXTRACT FROM LEAF OF *Justicia gendarussa* ON THE GROWTH OF PATHOGENIC BACTERIA BY DISC DIFFUSION METHOD

The different organic solvent extracts of *Justicia gendarussa* namely Methanol, Ethyle acetate and Chloroform crude extract at different concentration (25, 50, 75 and 100 µl) was tested against *Staphylococcus aureus*. The chloroform extract of *Justicia gendarussa* exhibited more bactericidal action than other with higher inhibition zone was found at 100µl concentration. (Table-3 Fig 1).

TABLE 3. THE ANTIBACTERIAL ACTIVITY OF THE DIFFERENT SOLVENT EXTRACT FROM THE LEAVES OF *Justicia gendarussa* WERE TESTED AGAINST BACTERIAL PATHOGEN AND EFFECTIVE INHIBITION WAS DETERMINED BY DISC DIFFUSION METHOD.

Different organic solvent extracts of *Justicia gendarussa*	**Zone of inhibition (mm)[a] *Staphylococcus aureus***				
	Positive control 10 µl Ampicillin	**Different concentrations Crude extract (µl)**			
		25 µl	**50** µl	**75** µl	**100** µl
Methanol	8mm	10mm	13mm	14.5mm	17mm
Ethyl acetate		07mm	10mm	13mm	16mm
Chloroform		08mm	12mm	16mm	19mm

[a] The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.



EFFECT OF CRUDE EXTRACT FROM LEAF OF *Justicia gendarussa* ON THE GROWTH OF ANTIBACTERIAL ACTIVITY BY MINIMUM INHIBITORY CONCENTRATION.

Minimum Inhibitory Concentration (MIC) assays were also performed to determine the antifungal activities of different solvent extract at different time interval. *Justicia gendarussa* methanolic extract inhibited the growth of *Staphylococcus aureus* at 100 micro grams/ml concentrations. Methanolic inhibited *Staphylococcus aureus* growth greatly than other solvent extract. 100 micro grams/ml *Justicia gendarussa* methanolic extract showed higher percentage ranging from 20-50% of inhibition in *Staphylococcus aureus* growth phases whereas Chloroform and ethyl acetate extract were inhibited at the % range of 18-42% and 18-44% respectively. (Fig 2a, 2b, 3a & 3b).

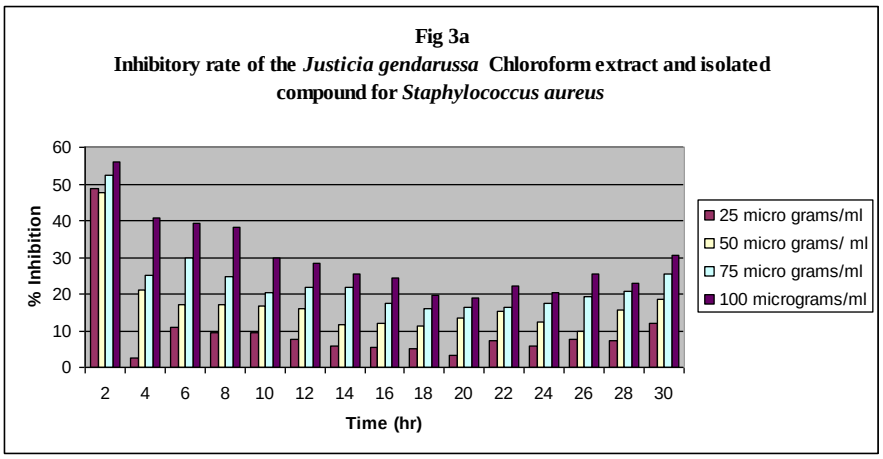


Fig 3b
Inhibitory rate of the *Justicia gendarussa* Ethyl acetate extract and isolated compound for *Staphylococcus aureus*

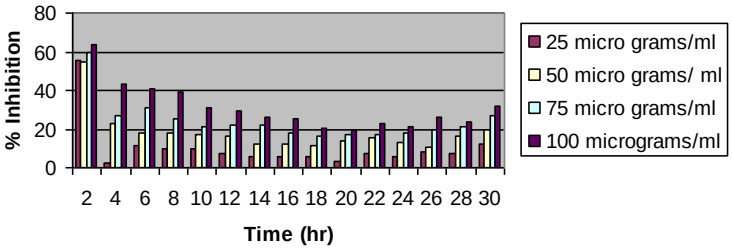
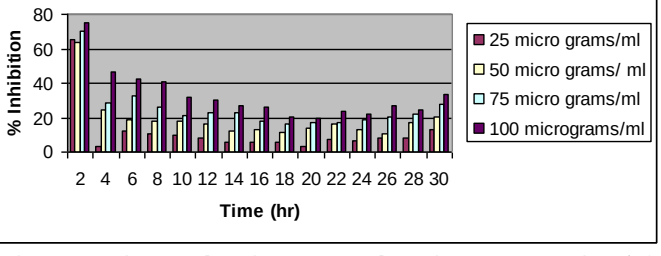


Fig 3c
Inhibitory rate of the *Justicia gendarussa* Methanol extract and isolated compound for *Staphylococcus aureus*



EXTRA CELLULAR PROTEIN ESTIIMATION BY BRADFORD METHOD (1976)

The Protein inhibition activity at different concentration of *Justicia gendarussa* methanolic extract was higher in *Staphylococcus aureus* much higher than other extracts. The highest concentrated *Justicia gendarussa* methanolic extract (100 micrograms/ml) showed maximum inhibitory activity in the stationary phase of the Bacterial growth. (Fig 5a, 5b; Plate 5)

Fig 5a
Amount of Protein presented in *Justicia gendarussa* Methanolic extract of Protein Inhibition assay

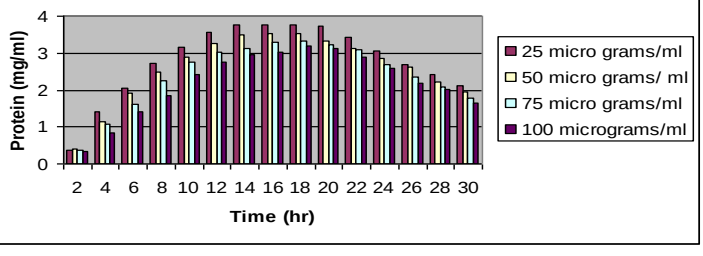
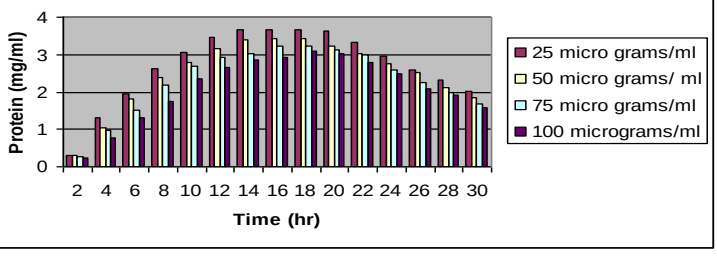
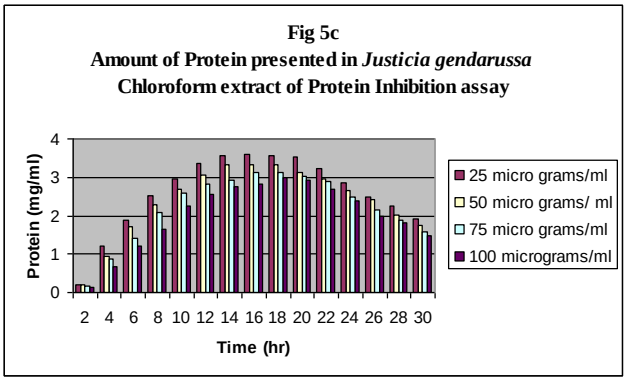


Fig 5b
Amount of Protein presented in *Justicia gendarussa* Ethyl acetate extract of Protein





IV. Discussion

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs, and has necessitated the search for new antimicrobials from alternative sources. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents. One way to prevent antibiotic resistance is by using new compounds which are not based on the existing synthetic antimicrobial agents (Shah 2005). Phytochemicals from 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 (Fabricant and Fansworth 2001).

Although antibacterial effects of some medicinal plants were reported previously (Erasto et al. 2004; Martini et al. 2004; Verdrengh et al. 2004; Suzgec et al. 2005), very limited knowledge about mechanisms of their action and spectrum of sensitive bacterial species or strains. In the present investigation demonstrate, sensitivities of defined, commonly used laboratory strain of methicilin resistant bacteria *Staphylococcus aureus* to flavonoids. To learn about a possible mechanism of antibacterial activity of one of the medicinal plant *Justicia gendarussa.*

Therefore, the present investigation reveals on the synthesis of DNA, and proteins in *Justicia gendarussa* extrats treated bacteria. Interestingly, DNA and syntheses were strongly inhibited in methicilin resistant bacteria *Staphylococcus aureus* relatively shortly (4 h) after the addition of *Justicia gendarussa* extrats, suggesting that at least one of these processes may be a target for antibacterial activity of *Justicia gendarussa* extrats. Protein synthesis inhibition, although significant, was delayed relative to that of DNA, indicating that this might be a secondary effect rather than a direct influence of *Justicia gendarussa* extrats on translation. Snyder and Gillies (2002) has been reported some flavonoids and alkaloids extracts act as inhibitor of DNA topoisomerases type I. The antibacterial activity and acting mechanism of silver nanoparticles (SNPs) on Escherichia coli ATCC 8739 were investigated in this study by analyzing the growth, permeability, and morphology of the bacterial cells following treatment with SNPs. The experimental results indicated 10 μg/ml SNPs could completely inhibit the growth of 107 cfu/ml E. coli cells in liquid Mueller–Hinton medium. Meanwhile, SNPs resulted in the leakage of reducing sugars and proteins and induced the respiratory chain dehydrogenases into inactive state, suggesting that SNPs were able to destroy the permeability of the bacterial membranes (Wen-Ru et al., 2010)

Although most of these studies were performed using eukaryotic enzymes, when considering evolutionary conservation of the crucial parts of their structures, it is likely that prokaryotic topoisomerases may also be sensitive to *Justicia gendarussa*. Therefore, this plant phytocompounds extract might influence DNA topology significantly, which should, in turn, lead to strong negative effects on DNA replication. Moreover, it was demonstrated previously that efficiency of transcription of genes coding for rRNA strongly depends on DNA topology, including supercoiling (Hraiky et al. 2000; Rochman et al. 2004). It was speculated previously that genistein-mediated stabilization of the covalent topoisomerase II–DNA cleavage complex may be responsible for moderate inhibition of growth of *Staphylococcus aureus* strains (Verdrengh et al. 2004).

V. Conclusion

Many bacterial diseases can be attributed to *Staphylococcus aureus.* It appears that *Justicia gendarussa* methanol extract can inhibit protein synthesis invitro , therefore, they may be used to treat or prevent some bacterial disease caused by *Staphlococcus aureus* .The present study may lead to the development or synthesis of the new antibacterial drugs from this medicinal plant that is beneficial for bacterial disorder patients…..

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