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INHIBITORY POTENTIAL OF *AVICENNIA MARINA* AGAINST BACTERIAL PATHOGENS OF URINARY TRACT INFECTION (UTI) FROM INFECTED PATIENTS FOR HEALTH AND SANITATION

A. SHEELA DEVI[a,*], JOHANNA RAJKUMAR[b], JOSEPH[a] and SUNITHA JAIN[a]

[a]Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, CHINNA KOLAMBAKKAM (T.N.) INDIA
[b]Department of Biotechnology, Rajalakshmi Engineering College, CHENNAI (T.N.) INDIA

ABSTRACT

Urinary tract is the most common site of bacterial infection in humans. The infectious diseases remain one of the greatest challenges to global health. Urinary tract infection (UTI) is the second most common infectious disease and possesses a significant healthcare burden. Increased use of antibiotics, the pathogens become resistant to it quickly. Hence screening of antibacterial activity of medicinal plants is significant. Owing to the widespread applications of mangrove plants, *Avicennia marina* was tested for antibacterial activity against UTI pathogens isolated from infected patients. The results showed that *E. coli* was more effective than *S. aureus*. Further MIC and MBC results revealed that the extract showed highest activity against both the pathogens. Followed by time kill studies was done where a considerable decrease in a number of bacterial cells were observed. Further comparative study of wild and treated pathogens in biochemical aspects was also studied by performing assay of total protein, protease activity, alkaline posphatase (ALPase) activity, superoxide dismutase (SOD) activity and β-galactosidase (β-Gal) release. All the results of assay showed effective activity of *A. marina*. Finally peptidoglycan (murein) hydrolase and catalase genes expression studies was done using real time reverse transcriptase polymererization chain reaction (RT-PCR).

Key words*: Aviccenia marina*, Bacterial pathogens, Genes expression, Urinary tract infection.

INTRODUCTION

Traditional and indigenous system of medicines persists in all over the world. Importance of medicinal plants of other ecosystems like marine ecosystem (mangrove plants)

[*]Author for correspondence; E-mail: sheeladevi.kvcet@gmail.com

is a rich source of medicinal plants having potential for miracle drugs. The unique traditional system of healthcare progressed from generation to generation within the society is still prevalent within the remote rural areas of the country[1]. Resistance to antimicrobial compounds in bacteria results from either evolution of "New Drug" or from variation in existing drug. The multidrug resistance of the bacteria contributes to their survival and virulence. The other virulent factors of serum resistance could be used as index of pathogenicity. These virulence factors may act independently or their actions may be complementary to each other[2].

EXPERIMENTAL

Sample collection and extract preparation

A healthy and fresh *A. marina* leaves were collected from Pichavaram, Cuddalore district, TamilNadu, India. The powder of this plant leaves was subjected to successive soxhlet extraction with methanol[3]. Clinical isolates of *E. coli and S. aureus* were obtained from urinary tract infected patients.

Screening of antibacterial activity of of *A. marina*

The methanol extract of *A. marina* was subjected to antibacterial activity by performing Kirby- Bauer disc diffusion method for *E. coli* and *S. aureus*[4].

Determination of MIC and MBC of methanol extract of *A. marina*

Five different concentrations of the extracts, from 2.5 mg/mL to 0.15 mg/mL were used against the pathogens by broth dilution method. The result was calculated by comparing the growth of wild bacteria[3].

Determination of time dependent reduction rate of bacterial population by methanol extract of *A. marina* (Time kill studies)

Two different concentrations of the extract (5.0 and 10 mg/mL) were used. The test were analysed at four different time intervals (0, 4, 8 and 24 hrs) and compared to controls. The experiment was repeated thrice and the results are expressed as CFU/mL[5].

Comparative studies of wild and treated UTI pathogens in biochemical aspects

Assay of total protein of bacterial cultures (wild and treated by the extract) were carried out by Bradford's test[6]. Azocasein method was used for protease activity measurement[7]. The ALP activity was determined using *p*-nitrophenyl phosphate as the substrate. Supernatant of each sample was taken and the activity of the enzyme was

measured[8]. The β-Gal release assay was used to identify the ability of extract that induces autolysis in pathogens. The action of β-Gal on MUG was measured[9]. The SOD activity was estimated by nitro butyl tetrazonium method[10].

Gene expression study

Analysis of peptidoglycan (murein) hydrolase and catalase genes expression using real time (Semi-quantitative) RT-PCR

The assay was used to identify either the extract induces up regulation or down regulation of the genes expression. For preparation of total RNA, the phenol-guanidinium thiocyanate based tri reagent (GeNei™, Bangalore) was used[11]. Complementary DNA was synthesized by RT-PCR, peptidoglycan hydrolase and catalase genes were amplified using primers (GeNei™, Bangalore).

RESULTS AND DISCUSSION

Determination of antibacterial activity and MIC and MBC of the extract

Maximum antibacterial activity was found at 1000 µg/mL on both the pathogen. The MIC of the extracts *on E. coli* and *S. aureus* was found to be 2.50 and 2.70 mg/mL, respectively, and for MBC 4.77 and 4.90 mg/mL, respectively. It showed that the inhibitory activity of the extract is increased when the concentration get increased.

Time kill studies

There was apparent decrease in number of colonies, observed for each time period interval for treated organisms. But there is considerable increases in number of colonies were seen in wild cultures. Also the extract showed more inhibitory activity on *E. coli* followed by *S. aureus*.

Comparative studies of wild and treated UTI pathogens in biochemical aspects

The results showed that the amount of total protein present in supernatant of both wild organisms was slightly lower when compared with treated samples. Hence the protein level was increased in supernatant due to its liberation from the cells. The activity of protease in wild pathogens was slightly higher when compared with treated samples. The protease activity was gradually decreased due to the activity of the extract. Hence there was a decrease in protease activity when concentration increases, which revealed that the extract may act as a protease inhibitor and thus inhibits enzyme activity.

Table 1: Effect of antibacterial activity and results for biochemical analysis

Conc of extract (mg/mL)	Antibacterial activity (mm)		Total protein analysis (µg/mL)				Protease activity (Units/mL)			
	E. coli	*S. aureus*	*E. coli*		*S. aureus*		*E. coli*		*S. aureus*	
			S	P	S	P	S	P	S	P
Wild	-	-	10.90 ± 0.05	26.60 ± 0.30	10.25 ± 0.50	26.87 ± 0.02	17.70 ± 0.36	16.50 ± 0.40	15.87 ± 0.31	14.30 ± 0.20
2.5	17.10 ± 0.17	17.10 ± 0.17	11.88 ± 0.06	25.57 ± 0.16	12.62 ± 0.13	25.32 ± 0.16	16.50 ± 0.20	15.40 ± 0.20	14.63 ± 0.25	13.30 ± 0.17
5	18.20 ± 0.20	18.20 ± 0.20	12.20 ± 0.05	24.13 ± 0.13	14.38 ± 0.10	24.60 ± 0.05	15.50 ± 0.26	13.53 ± 0.15	13.93 ± 0.15	12.50 ± 0.52
10	20.07 ± 0.12	20.07 ± 0.12	13.15 ± 0.30	23.30 ± 0.18	18.30 ± 0.05	23.50 ± 0.05	14.23 ± 0.25	12.77 ± 0.15	12.50 ± 0.53	11.43 ± 0.50

Conc of extract (mg/mL)	ALP activity (Units/m L)		SOD assay (Units/mL)			
	E. coli	*S. aureus*	*E. coli*		*S. aureus*	
	S	S	S	P	S	P
Wild	2249.90 ± 5.53	2097.88 ± 38.40	41.12 ± 1.44	125.04 ± 2.73	39.73 ± 1.87	96.63 ± 1.55
2.5	1156.27 ± 4.22	1030.05 ± 6.38	35.82 ± 1.24	91.32 ± 2.10	31.98 ± 2.6 6	76.09 ± 1.74
5	358.40 ± 5.75	231.25 ± 13.06	21.71 ± 2.11	76.41± 2.26	24.49 ± 0.46	59.72 ± 1.41
10	234.94 ± 7.31	174.13 ± 9.97	19.46 ± 1.85	54.17 ± 3.69	17.63 ± 1.41	28.15 ± 1.06

S: Supernatant, P: Pellet

Bacterial ALP is located in the periplasmic space, since this space is much more subject to environmental variation. The ALP is comparatively resistant to inactivation, denaturation, and degradation, and also has a higher rate of activity[12]. This shows that the activity of ALP in both wild pathogens was slightly higher when compared with treated samples. The ALP activity was gradually decreased due to the activity of the extract. Hence there was a decrease in ALP activity when concentration increases. The release of β - Gal in treated pathogens was gradually increased when incubation time increases due to the ability to induce autolysis, in terms of the incipient release of β-Gal from the periplasmic space of the pathogens leads to loss of integrity and permeability of the cell wall. The activity of wild *E.coli* was higher compared with *S.aureus*. The obtained results indicated that the extract was a potent source to suppress the activity of SOD on both the pathogens and the stress tolerance levels of the organisms were reduced.

Table 2: Results of time kill study and β-galactosidase assay

Time (hrs)	Time Kill Study						B-galactosidase assay		
	E. coli			*S. aureus*			Time (hrs)	*E. coli*	*S. aureus*
	Control	T1	T2	Control	T1	T2			
0	54.57 ± 0.78	51.60 ± 0.35	50.73 ± 0.25	54.47 ± 0.95	53.70 ± 0.92	52.43 ± 0.81	0	0.08 ± 0.12	0.03 ± 0.00
4	61.50 ± 1.30	41.47 ± 0.55	38.23 ± 1.11	60.70 ± 1.21	45.53 ± 0.60	41.07 ± 0.25	12	0.72 ± 0.01	0.72 ± 0.01
8	85.03 ± 1.85	35.00 ± 0.80	28.57 ± 1.16	72.20 ± 0.66	41.07 ± 0.55	32.77 ± 1.40	24	0.83 ± 0.02	0.83 ± 0.02
10	122.50 ± 0.66	32.50 ± 1.18	12.43 ± 1.00	118.57 ± 1.35	38.30 ± 0.82	18.90 ± 0.90	36	1.29 ± 0.04	1.22 ± 0.03

Genes expression study

The 16S rRNA gene, peptidoglycan hydrolase gene and catalase genes present in wild and treated cells of both pathogens were shown in Fig. 1, 2 and 3, respectively. In this study, the extract showed greatest effect on peptidoglycan hydrolase gene expression and moderate effect on catalase gene expression. The fold expression of peptidoglycan hydrolase gene was activated (up regulated) and catalase gene was suppressed (down regulated) by the extract.

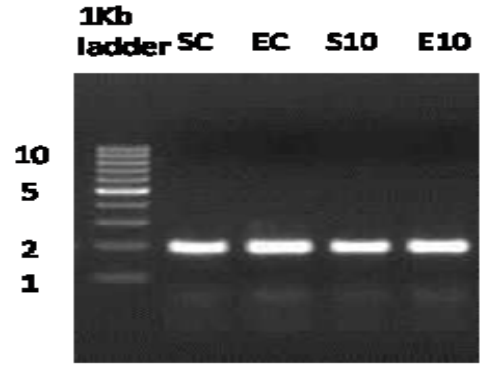

Fig. 1: Visualization of 16S rRNA in agarose gel gene in agarose gel

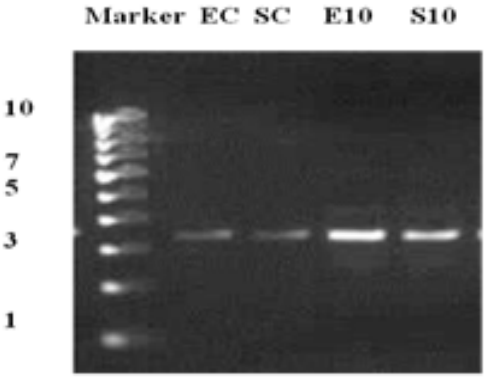

Fig. 2: Visualization of peptidoglycan hydrolase gel

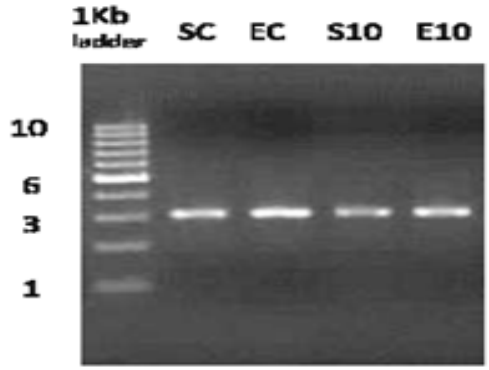

Fig. 3: Visualization of catalase gene in agarose

C: control, S: *S.aureus*, E: *E.coli*, 10: mg/mL concentration of the extract used for treatment

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