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ANTIBACTERIAL ACTIVITY OF LEAVES AND BARK OF *AEGICERAS CORNICULATUM* L.

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Abstract: The study investigated antibacterial activities in leaves and bark of (*Aegiceras corniculatum* L.) mangrove plants. Mangroves are salt-tolerant plants of tropical and subtropical intertidal regions of the world. Nearly 65 species of mangrove reported from world and approximately 59 species from 29 families are reported in India. The leaves and bark of *Aegiceras corniculatum* were tested against the seven bacterial strains are *Escherichia coli(u), Escherichia coli, Pseudomonas aeruginosa, Pseudomonas aeruginosa, Klebsiella pneumoniae , Salmonella typhi* and *Bacillus subtilis* using Agar well diffusion method. Antibacterial activity of 10 (WB, MB, EB, CB, PB, WL, ML, EL, CL, PL) different extracts were studied. The antibacterial activity studied at range of 50-500 µg / ml concentration of extracts. Antibacterial activity of methanolic (MB, ML) and chloroform extracts (CB, CL) shows more significant than ethyl acetate, water and petroleum ether extracts. The results suggested that these plants could be exploited in the management of various infectious diseases and their extracts might have roles as pharmaceuticals and preservatives.

Keywords: Antibacterial activity, mangrove, methanol, chloroform, ethyl acetate, petroleum ether

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INTRODUCTION

Microbial infections are major public health problems in the developed countries. Antibiotics are used to treat these infections. Due to indiscriminate use of commercial antibiotics, the incidence of multiple antibiotic resistances in human pathogens is increasing (Jeyachandran, R. and Mahesh, A., 2007). Infectious diseases caused by bacteria and fungi affect millions of people worldwide. Throughout the history of mankind, infectious diseases have remained a major cause of death and disability. Today infectious diseases account for one-third of all deaths in the world; the World Health Organization estimates that nearly 50,000 people die each day throughout the world from infectious diseases. The discovery of antibiotics was an essential part in combating bacterial infections that once ravaged humankind (Usha, P.T.A, Jose, S. and Nisha , A.R., 2010). Decreased efficiency of antibiotics and steadily increasing bacterial resistance to existing antibiotics is a serious problem which has necessitated the development of new drug alternative. Natural compounds are a source of numerous therapeutic agents. Recent progress to discover drugs from natural sources has resulted in compounds that are being developed to treat cancer, resistant bacteria, viruses and immunosuppressive disorders (Amghalia et. al., 2009).

Mangroves are rich source of phenolic compounds. Along with phenolic, tannins, saponins, alkaloids, steroids are also reported from mangrove plants which are showing antimicrobial potential (Gupta et. al., 2012). In mangrove species, phenolics are abundant components, which prevent damage from herbivores, but they also exhibit a diversity of other biological activities of historic and potential importance to humans (Wei et al, 2010). Extracts of mangroves are used for treatment of various diseases like hepatitis, asthmas, ulcer diabetes, diarrhea etc., as well as in treatment of skin disorders like leprosy, boils, wounds etc. The mangrove extracts also used as insecticides and pesticides. Mangrove extracts have been used for diverse medicinal purposes and have a variety of antibacterial, antiherpetic and anthelminthic activities. (Bandaranayake, 1998).

Aegiceras corniculatum is one of important species of mangrove. It is commonly known as Black Mangrove or River Mangrove. It is a species of shrub or tree mangrove in the Myrsinaceae family with a distribution in coastal and estuarine areas ranging from India through South East Asia to Southern China, New Guinea and Australia. It grows in mud in estuaries and tidal creeks, often at the seaward edge of the mangrove zone. It has salt glands in the leaves for secretion of excess salt and show tolerance to the changes in salinity gradients. *Aegiceras corniculatum* grows as a shrub or small tree up to 7 meter high. Its leaves are alternate, entire, leathery and minutely dotted. Its flowers produced in umbellate cluster and flowers are small, fragrant and white in colour. The fruit is curved and cylindrical or horn-shaped, light green to pink in colour.

Materials and Methods

Preparation of Extracts:-The leaves and bark of plant separated and washed. The samples were then dried at room temperature and ground into powder. Dried and powdered plant material was extracted with range of solvents from non-polar to polar such as petroleum ether, chloroform, ethyl acetate, methanol and water. 20gm of powdered plant material was mixed with 50ml of solvent and solicited at 33KHz for 40 min. The extraction was carried out repeatedly until solvent get colorless. The extracts were filtered and concentrated in rotary evaporator to dryness. All obtained extracts were dissolved in 0.1% dimethyl sulfoxide (DMSO) and diluted to yield various working concentrations. These extracts were filtered using a 0.45 um cellulose nitrate membrane and stored at -20^{0}C till further analysis.

Antibacterial assay:-The effects of various plant extracts on the several bacterial strains were assayed by Agar well diffusion method. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the plant extracts to inhibit the microorganisms were determined by micro dilution method using plant fractions serially diluted in sterile nutrient broth.

Test microrganisms:-The seven bacterial strains used were *Escherichia coli(u), Escherichia coli(p), Pseudomonas aeruginosa(u), Pseudomonas aeruginosa(p),Klebsiellapneumoniae, , Salmonella typhi*and*Bacillus subtilis.* These are antibiotic resistant bacterial strains causing urine infection in females and remaining found in pus cells. All bacteria cultured on nutrient agar medium at 37^{o}C and stored on nutrient agar medium slant at 4^{o}C till use.

Preparation of inoculums:-The bacterial isolates were first grown in a nutrient broth for 24 hr before use and standardized to 0.5 McFarland standards (10^{6}cfu/ml).

Preparation of extract:-20gm of powdered plant material was mixed with 50ml of solvent and sonicated at 33 KHz for 40 min. The extraction was carried out repeatedly until solvent get colorless. The extracts were filtered and concentrated in rotary evaporator to dryness. All obtained extracts were dissolved in 0.1% dimethyl sulfoxide (DMSO) and diluted to yield various working concentrations.

Statistical Analysis: The results of antimicrobial activities were analyzed statistically by one way ANNOVA using SPSS software. All experiments were performed in triplicates and results were mean along with standard deviation of each experiment. The condition $p<0.05$ was considered to be statistically significant

RESULTS AND DISCUSSION-

In present study the antibacterial activity of 10 (WB, MB, EB, CB, PB, WL, ML, EL, CL, PL) different extracts was studied on clinical isolates of bacteria obtained from urine and pus samples. The bacterial strains *Escherichia coli(u), Escherichia coli(p), Pseudomonas aeruginosa(u), Pseudomonas aeruginosa(p),Klebsiellapneumoniae, Salmonella typhi*and*Bacillus subtilis* were used for assay. The antibacterial activity studied at range of 50-500µg/ml concentration of extracts. Among all extracts methanolic (MB, ML) and chloroform extracts (CB, CL) were shown to be more significant. While ethyl acetate and water extracts showed a moderate antibacterial activity but the petroleum ether extracts showed very low activity. The extracts inhibited growth of all tested bacteria at concentration of 400-500ug/ml concentration with zone of inhibition in a range 10-15mm in diameter. The standard antibiotics streptomycin, kanamycin, ampicillin were used as reference drugs for comparison of antibacterial activity.

The chloroform extracts showed strong antibacterial activity against *Escherichia coli(u), Bacillus subtilis, Salmonella typhi* with zone of inhibition in a range 13-14mm in diameter. While methanolic extracts showed good activity against *Escherichia coli(p), Pseudomonas aeruginosa(u), Pseudomonas aeruginosa(p),* bacterialstrains with zone of inhibition in a range 13-14mm in diameter. The bacterial strain *Klebsiella pneumonia*e strongly inhibited by ethyl acetate extracts (EB,EL) with zone of inhibition 13.56 and 14.66 mm in diameter. Ethyl acetate extracts showed good activity against all bacterial strains with zone of inhibition 13-15mm in diameter. Water extracts showed moderate activity against all bacteria with zone of inhibition 11-13 mm in diameter. While petroleum ether extracts were showed very low antibacterial activity with zone of inhibition 10-11mm in diameter. The tested microbes had developed resistance against many standard antibiotics therefore activity was compared with kanamycin. Among 10 extracts studied, not a single extract was showing activity more than standard reference antibiotic but the activity showed by chloroform, methanol and ethyl acetate extracts was comparable to activity showed by standard reference antibiotics.

The values of minimum inhibitory concentration and minimum bacterial concentration were estimated by using broth micro dilution method. The lowest value of MIC was shown by chloroform extracts of leaves against *Escherichia coli(u), Bacillus subtilis and Salmonella typhi* was 125 µg /ml . Bark extracts of MIC value for same bacteria were 125, 62.50, 125 µg /ml. The ethyl acetate extracts (EB, EL) shows lower MIC value (62.50 and 125ug/ml) against *K. pneumonia*. The methanolic extracts were effective against *Escherichia coli(p), Pseudomonas aeruginosa(u), Pseudomonas aeruginosa(p)* and showed 125 µg /ml of MIC value. Chloroform extracts of bark shows lowest value of MBC against *Escherichia coli(u), Bacillus subtilisand Salmonella typhi* was 250 µg /ml. The MBC value of leaves extracts of same bacteria were 250, 62.50, 250 µg /ml. The ethyl acetate extracts (EB, EL) showed lowest MBC value (125 and 250

µg /ml) against K. pneumonia. The methanolic extracts were effective against bacterial isolates of *Escherichia coli(p), Pseudomonas aeruginosa(u), Pseudomonas aeruginosa(p)* and shows the value of MIC 250ug/ml.

The results of this present study clearly indicated that, the extracts from *A. corniculatum* showed antimicrobial activity against experimental pathogenic bacteria. The active compounds present in plant extracts showed growth inhibition which appeared as clear areas around the well. The antimicrobial activites reported from mangrove plants could be due to presence of alkaloids, tannins, flavonoids, saponins, quinones etc. (Fennel et al,2004, Shabudin et al, 2012, Nuridiani et al, 2012) The marine halophytes were already known for their antibacterial properties because of presence of unique compounds (Rvikumar et al, 2002, Gupta et al, 2012)

Table 1. Antibacterial activity of *A. corniculatum* bark extracts based on agar well diffusion method.

Name of bacteria	Conc. µg/ml	Diameter of Zone of inhibition in mm					
		WB	MB	EB	CB	PB	Std.
E.coli(u)	50	0	0	0	0	0	15.33±0.33
	100	0	11.11±0.19^{c}	0	0	0	
	200	11.22±0.19^{c}	11.33±0.33^{c}	10.22±0.19^{d}	10.77±0.69^{d}	0	
	400	12.00±0.57^{c}	11.66±0.66^{c}	11.00±0.57^{c}	13.33±0.33^{c}	10.22±0.19^{c}	
	500	13.11±1.17^{b}	13.00±1.00^{b}	12.22±0.38^{b}	14.22±0.38^{b}	11.44±0.38^{b}	
E.coli(p)	50	0	0	0	0	0	14.66±0.33
	100	0	0	0	0	0	
	200	10.44±0.19^{c}	0	0	0	0	
	400	10.77±0.19^{c}	11.66±0.33^{c}	10.77±0.38^{c}	10.66±0.33^{c}	10.22±0.19^{c}	
	500	11.88±0.20^{b}	12.86±0.35^{b}	12.77±0.19^{b}	12.22±0.38^{b}	11.11±0.20^{b}	
B.subtilis	50	0	0	0	0	0	16.33±0.33
	100	0	0	0	10.44±0.50^{d}	0	
	200	0	0	10.33±0.33^{d}	11.88±0.69^{c}	0	
	400	10.55±0.38^{c}	10.44±0.50^{c}	11.44±0.50^{c}	13.00±0.66^{b}	10.33±0.33^{c}	
	500	12.55±0.69^{b}	12.88±1.01^{b}	13.11±0.19^{b}	13.77±0.38^{b}	11.55±0.11^{b}	
P.auroginosa (u)	50	0	0	0	0	0	16.88±0.39
	100	0	0	10.11±0.19^{e}	0	0	
	200	0	0	10.55±0.2^{d}	0	0	
	400	10.22±0.19^{c}	10.66±0.33^{c}	11.11±0.19^{c}	0	0	
	500	12.11±0.2^{b}	13.88±0.76^{b}	12.66±0.33^{b}	10.77±0.19^{b}	10.55±0.19^{b}	
P.auroginosa (p)	50	0	0	0	0	0	16.77±0.19
	100	0	0	0	0	0	
	200	10.33±0.33^{d}	10.66±0.33^{d}	10.77±0.50^{c}	0	0	
	400	11.11±0.19^{c}	11.66±0.18^{c}	11.22±0.45^{c}	10.33±0.33^{c}	0	
	500	12.55±0.22^{b}	14.11±0.19^{b}	13.11±0.19^{b}	11.77±0.39^{b}	11.22±0.19^{b}	
S.typhae	50	0	0	0	0	0	16.55±0.39
	100	0	0	0	0	0	
	200	0	0	0	0	0	

	400	10.55±0.50^{c}	0	10.22±0.39^{c}	0	0	
	500	12.00±0.33^{b}	11.88±0.50^{b}	10.66±0.33^{b}	14.44±0.50^{b}	11.11±0.19^{b}	
K.pneumonia	50	0	0	0	0	0	
	100	0	0	0	0	0	
	200	10.33±0.33^{d}	0	0	0	0	13.55±0.69
	400	11.11±0.19^{c}	11.66±0.57^{c}	12.00±0.33^{c}	10.22±0.39^{c}	0	
	500	12.77±0.38^{b}	13.44±0.50b	13.56±0.19^{b}	11.66±0.33^{b}	0	

Table 2. Antibacterial activity of *A. corniculatum* leaves extracts based on agar well diffusion method.

Name of bacteria	Conc. µg/ml	WB	MB	EB	CB	PB	Std.
E.coli(u)	50	0	0	0	0	0	
	100	10.66±0.33^{d}	0	0	0	0	
	200	10.77±0.19^{d}	10.66±0.57^{d}	10.33±0.33^{c}	10.33±0.33^{d}	0	15.33±0.33
	400	11.44±0.19^{c}	11.66±0.33^{c}	10.66+0.33^{c}	11.33±0.66^{c}	10.88±0.50^{c}	
	500	12.22±0.22^{b}	13.44±0.38^{b}	11.77±0.19^{b}	12.55±0.50^{b}	11.44±0.51^{b}	
E.coli(p)	50	0	0	0	0	0	
	100	0	0	0	0	0	
	200	0	0	10.66±0.33^{d}	0	0	14.66±0.33
	400	0	10.44±0.50^{c}	11.77±0.19^{c}	10.66±0.33^{c}	10.33±0.33^{c}	
	500	12±0.33^{b}	13.11±0.51^{b}	12.77±0.21^{b}	12.00±0.34^{b}	11.88±0.50^{b}	
B.subtilis	50	0	0	0	0	0	
	100	0	10.11±0.19^{d}	0	0	0	
	200	0	10.44±0.2^{d}	0	10.33±0.33^{d}	0	16.33±0.33
	400	11.22±0.50^{c}	11.66±0.33^{b}	0	11.88±0.38^{c}	10.33±0.33^{c}	
	500	11.55±0.38^{b}	13.00±0.33^{c}	12.88±0.19^{b}	14.11±0.39^{b}	11.66±0.38^{b}	
P.auroginosa (u)	50	0	0	0	0	0	
	100	0	0	10.44±0.50^{d}	0	0	
	200	0	0	11.77±0.51^{c}	0	0	16.88±0.39
	400	11.44±0.19^{c}	12.22±0.19^{c}	12.22±0.19^{c}	11.55±0.50^{b}	10.22±0.19^{c}	
	500	13.00±0.33^{b}	13.89±0.2^{b}	13.55±0.22^{b}	12.00±0.33^{b}	11.22±0.69^{b}	
P.auroginosa (p)	50	0	0	0	0	0	
	100	0	0	0	0	0	
	200	0	0	10.33±0.33^{d}	0	0	16.77±0.19
	400	10.33±0.33^{c}	10.33±0.33^{c}	10.77±0.38^{c}	11.33±0.33^{c}	0	
	500	12.66±0.33^{b}	14.55±0.38^{b}	11.88±0.19^{b}	12.67±0.48^{b}	11.88±0.38^{b}	
S.typhae	50	0	0	0	0	0	
	100	0	0	0	0	0	
	200	0	0	0	0	0	16.55±0.39
	400	10.22±0.19^{c}	10.33±0.33^{c}	0	11.00±0.00^{c}	0	
	500	12.00±0.88^{b}	12.11±0.19^{b}	11.89±0.50^{b}	12.55±0.50^{b}	11.77±0.19^{b}	
K.pneumonia	50	0	0	0	0	0	
	100	0	0	0	0	0	
	200	0	0	11.66±0.33^{d}	0	0	13.55±0.69
	400	10.11±0.19^{c}	10.00±0.00^{c}	14.11±0.19^{c}	11.00±0.33^{c}	0	

	500	**13.22±0.22^b**	**12.11±0.19^b**	**14.66±0.33^b**	**12.89±0.19^b**	**10.11±0.19^b**	

Table 3. MIC and MBC of bark extract:

Name of bacteria	MIC (µg /ml)						MBC(µg /ml)					
	WB	MB	EB	CB	PB	kan	WB	MB	EB	CB	PB	kan
E.coli(u)	250	250	250	125	250	125	500	500	500	250	500	250
E.coli(p)	250	125	125	250	250	62.50	500	250	125	500	500	125
B.subtilis	250	250	250	125	500	31.25	125	500	250	125	500	62.50
P.auroginosa(u)	250	125	250	250	250	31.25	250	125	500	250	500	62.50
P.auroginosa(p)	125	125	250	250	250	31.25	250	250	250	500	500	31.25
S.typhae	250	500	500	125	500	62.50	500	500	1000	250	1000	125
K.pnumonia	125	125	62.50	250	250	125	250	250	125	500	500	250

Table 4. MIC and MBC of leaves extract:

Name of bacteria	MIC (µg /ml)						MBC(µg /ml)					
	WL	ML	EL	CL	PL	kan	WL	ML	EL	CL	PL	kan
E.coli(u)	250	250	250	125	250	250	500	500	500	250	500	500
E.coli(p)	250	125	250	250	250	500	500	500	500	500	500	500
B.subtilis	500	125	250	62.5	500	31.25	1000	250	250	62.5	500	62.50
P.auroginosa(u)	125	125	500	500	500	31.25	250	250	500	500	1000	62.50
P.auroginosa(p)	500	250	125	500	500	31.25	500	500	250	500	500	31.25
S.typhae	250	250	500	125	500	62.50	500	500	1000	250	1000	125
K.pnumonia	250	125	125	500	500	125	500	250	250	500	500	125

CONCLUSION –

Aegiceras corniculatum showed that the various degree of inhibitory activity against the bacteria tested. The obtained results indicated that the *Aegiceras corniculatum* is having a good antibacterial. Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

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