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Antimicrobial Activity of *Acacia nilotica*

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The antimicrobial activity of *Acacia nilotica* was tested by disc diffusion method against a panel of human pathogenic microorganisms. The chloroform extract of *A. nilotica* stem bark, at a concentration of 400 µg/disc showed the zones of inhibition ranging from 13 to 24 mm against 1×10^7 microbial cells with largest zone against *S. aureus* indicating that this herbal extract was strongly inhibitory towards this microorganism and least to *A. niger*. The minimal inhibitory concentrations (MICs) were determined by broth dilution technique which ranged between 200–1600 µg/mL, *S. aureus* with lowest MIC value and *P. aeruginosa* and *A. niger* with highest MICs indicating that *S. aureus* was strongly inhibited and *P. aeruginosa*, *A. niger*, the least. The biochemical alterations induced by this plant extract were assessed by performing several biochemical tests. There was inhibition of protease and amylase activities of these microorganisms following treatment with the extract. These results indicate that *A. nilotica* possesses potent broad spectrum antimicrobial activity against all microorganisms tested.

Key Words: Antimicrobial activity, *Acacia nilotica*, Hemolytic activity.

INTRODUCTION

Acacia nilotica (Fam. Mimosaceae) is a fairly large evergreen tree with a geographical distribution of tropical and subtropical regions of South East Asia. Its colloquial name is "Nalla tumma" in Telugu regional language and trade name is "Babul". It is a tall tree reaching a height of 10 to 20 metres and crowned with a dome-shaped shoot system. The stem exudates a gum resin sold in Indian markets as gum arabic. The extracts of leaf, stem, bark and gum exhibit various pharmacological activities. The bark is useful in the treatment of helminthiasis[1], diarrhoea, dysentery, leprosy, oral ulcers and several other ailments like cancer[2]. Polyphenolic phlobaphenes consisting predominantly of catechol, pyrogallol and four derivatives of (+) catechin-5-gallate (I, II, III, IV) with gallic acid, its methyl ester and naringenin have been isolated from bark of *A. nilotica*[3]. Several medicinal plants have been found to have antimicrobial activity[4–8]. But the antimicrobial activity of *A. nilotica* was not studied on these aspects to date.

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Therefore, an attempt was made to study the antimicrobial activity of *A. nilotica* and the biochemical alterations induced by this plant extract.

EXPERIMENTAL

Collection of plant material and preparation of chloroform extracts: In the present investigation the stem-bark of *A. nilotica* was collected from an aged tree at Gollaprolu, East Godavari District, Andhra Pradesh, India. For preparation of chloroform extract. the shade dried bark weighing 6 kg was extracted with chloroform in a 10 L aspirator bottle by percolation every two days. The process was repeated 11 times for extractable organic compounds. The resultant solid residue (6.0 kg) was obtained. The stock solution was prepared by dissolving 10 mg residue in 1 mL absolute ethanol which was placed in appendorf tube and stored in refrigerator before use.

Microorganisms: The following microbial cultures were obtained from MTCC, Chandigarh, India: *Staphylococcus aureus* (ATCC 23564), *Salmonella typhi* (ATCC 10749), *Salmonella typhimurium* (ATCC ATCC 23564), *Yersinia enterocolitica* (ATCC 9610), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 25619), *Candida albicans* (ATCC 2091). *Streptococcus pneumoniae* is an isolate of urinary tract infection and *Bacillus subtilis* and *Aspergillus flavus* were isolated from skin infection of human leg. *Serratia marcescence* and *Aspergillus niger* were free gifts from Prof. K.K. Rao, Head of the Department of Biotechnology, GITAM.

Microbial culture conditions: The bacterial cultures were maintained on Nutrient agar slants or plates (peptone 0.5%, beef extract 0.3%, NaCl 0.5%, agar 2.0%) and fungal cultures on Sabouraud's agar slants or plates (mycological peptone 1%, dextrose 2–4%, agar 2%). Overnight cultures were used in all experiments by inoculating a single colony of each type of culture in respective 5 mL broth and incubating at 37°C for 18–24 h (bacteria) or at room temperature for 48 h (fungi).

Disc diffusion method: Nutrient agar plates or Sabouraud's agar plates were inoculated with 0.1 mL of each fresh culture containing 10^7 cells by spread plate method. Later two sterile filter paper discs (5 mm) and reference antibiotic discs such as ampicillin (10 μg) and nystatin (100 units/disc) procured from Himedia, Mumbai, were placed in corresponding plates. On one filter paper disc, 400 μg of chloroform extract, each in 20 μL volume and on the other disc, 20 μL of absolute ethanol (control) was put. Later the plates were kept at room temperature for 1 h for the plant extracts to be diffused into the medium. Then the plates were incubated at 37°C for 18–24 h (bacteria) and at room temperature (29°C) for 48 h (fungi). The zone of inhibition was measured and expressed in mm.

Determination of minimal inhibitory concentrations (MICs): The minimal inhibitory concentrations were determined by broth dilution technique. Duplicates of serial dilutions of respective broth and various concentrations of plant extracts were made in sterile test tubes for 1 mL. The controls did not receive any extract. 50 μL of culture containing 10^3 cells was added to each test tube. The suspension was mixed well by rotating between the palms and poured on to the respective agar plates which were later incubated in an incubator at 37°C for

18–24 h or at room temperature for 48 h in case of fungi. Later the lowest concentration of the plant extract that inhibited the growth of microorganisms was noted as minimal inhibitory concentration by counting the colonies.

Biochemical tests: These were performed according to the methods of Cappuccino and Sherman[9]. The tests include Gram staining, motility, mannitol fermentation, lactose fermentation, IMVic, catalase, oxidase, urease, amylase, protease, hemolysis and quantitation of protease, amylase production. The tests were conducted for these microorganisms by growing in the presence and absence of plant extract. The respective MIC concentrations were used in the experiments.

RESULTS AND DISCUSSION

The results are summarized in Tables 1–3. The largest zone of inhibition was formed against *S. aureus* (Table-1) *i.e.*, 24 mm and minimal inhibitory concentration was less (200 µg/mL) for this organism indicating that this was strongly inhibited by the plant extract. Later, second largest zone was found for *P. aeruginosa* isolated from throat infection (23 mm) and 200 µg/mL of MIC value indicating that *A. nilotica* was strongly effective to this pathogen. The MIC values ranged from 200–1600 µg/mL with few species of highest value less sensitive to this extract. To some organisms the MICs were moderate, *i.e.*, 400–800 µg/mL indicating that *A. nilotica* extract was moderately effective towards these pathogens. *Y. enterocolitica* was positive to amylase and urease tests. *Pseudomonas* was positive to amylase, protease, α-hemolysin and lipase. *E. coli* was positive to indole and methyl red tests. The biochemical tests when performed qualitatively on respective agar plates (Table-2) did not show clear distinction between control and experimentals, where to one half of the line inoculum a loopful of extract was added. This may be due to insufficient dosage. But when amylase and protease activities were quantitated in broth, the actual difference between plant extract treated and untreated control was clear. The above activities were inhibited by this palnt extract (Table-3). There was 660 µg/mL of amylase production by control *Y. enterocolitica* when cultured in amylase producing medium at 37°C which was absolutely reduced to zero indicating 100% inhibition of amaylase production following *A. nilotica* extract treatment. Amylase activity was inhibited in all organisms tested by treatment with this plant extract. The protease activity showed that in controls as the protease breaks the protein and when the amount of protein retained was less when compared to the experimental samples treated with plant extracts, the protease activity was inhibited and the remaining protein content measured was high, indicating inhibition of protease activity. The protease production was highest in *Serratia* species, *i.e.*, 362.5 µg tyrosin/mL/h. There was 60% inhibition of protease activity by treatment with plant extract in *Serratia*. There was around 80% inhibition in protease production of *P. aeruginosa*. These results indicate that *A. nilotica* chloroform extract was inhibitory to amylase and protease enzyme production. The results of the present study differ from our earlier results[5] where *Caesalpinia dygina* was very effective against *S. aureus* forming larger zone. It can be attributed that *A. nilotica* was a potential broad spectrum antimicrobial agent.

TABLE-1
ANTIMICROBIAL ACTIVITY OF *A. NILOTICA*

Organism	Zone of inhibition (mm)	Ampicillin	Ethanol	MIC (μg/mL)
S. aureus	24.0	25.0	10.0	200
S. pneumoniae	18.0	22.0	11.0	400
B. subtilis	15.0	18.0	12.0	800
S. typhi	15.0	14.0	10.0	800
S. typhimurium	15.0	14.0	10.0	800
E. coli (UTI)	16.0	0.0	15.0	800
E. coli (drinking water)	13.5	12.0	14.0	1600
Y. enterocolitica	20.0	18.0	10.0	400
P. aeruginosa	23.0	0.0	13.0	200
Pseudomonas species	14.0	0.0	16.0	1600
S. marcesceens	16.0	24.0	6.0	800
C. albicans	16.0	20.0*	11.0	800
A. niger	13.0	17.0*	10.0	1600
A. flavus	18.0	15.0*	12.0	400

*nystatin.

TABLE-2
MORPHOLOGICAL AND BIOCHEMICAL ALTERATIONS INDUCED BY *A. NILOTICA*

Test	SA		STM		ECU		ECE		YE	
	C	E	C	E	C	E	C	E	C	E
Gram staining	+	+	–	–	–	–	–	–	–	–
Motility	–	–	+	±	+	±	+	±	+	±
Indole	–	–	–	–	+	+	+	+	–	–
Methyl red	+	+	+	+	+	+	+	+	–	–
MacConkey agar	–	–	–	–	+	+	+	+	–	–
Coagulase	+	–	–	–	–	–	–	–	–	–
Mannitol salt agar ferment	+	–	–	–	–	–	–	–	–	–
Catalase	+	+	+	+	+	+	+	+	+	+
Oxidase	–	–	–	–	–	–	–	–	+	+
Urease	–	–	–	–	+	+	–	–	+	+
Amylase	–	–	–	–	–	–	–	–	+	±
Protease	–	–	–	–	–	–	+	–	+	–
Lipase	+	+	–	–	–	–	–	–	–	–
Fermentation of sugars	A,G	A,G	A,G	A,G	A, G	A,G	A, G	A, G	A, G	A, G
Sucrose	NT		NT		+ +	++	++	++	– –	– –
Glucose	NT		NT		++	++	++	++	++	++
Mannitol	+ –	+ –	NT		++	++	++	++	– –	– –
Dextrose	+ –	+ –	NT		++	++	++	++	– –	– –

SA = *S. aureus*; STM = *S. typhimurium*; ECU = *E. coli* (uropathogen); ECE = E. *coli* (enteropathogen); YE = *Y. enterocolitica*; + = positive; – = negative; q = faintly positive; NT = not tested; A = acid formation; G = gas formation.

TABLE-3
EFFECT OF *A. NILOTICA* ON PROTEASE AND AMYLASE PRODUCTION

Organism	Protease (μg/mL/h)	% inhibition	Amylase (μg/mL/h)	% Inhibition
P. aeruginosa	125	90	20	100
Y. enterocolitica	ND	—	660	100
A. niger	ND	—	120	100
B. subtilis	200	27	275	100
S. marscescence	362	62	ND	100
Psedomonas sp	337.5	11	80	100

ND = not determined

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