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STUDIES ON ANTIBACTERIAL ACTIVITY OF *ACACIA NILOTICA*

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Abstract: This study was conducted as the first step to explore the possibility of isolating active ingredient(s) of *Acacia nilotica* as antibacterial agents. Two types of extracts from air-dried *Acacia nilotica* leaves were prepared through solvent extraction method using methanol and hexane. The disc diffusion method was used to screen 10 different pathogenic bacteria for their sensitivity to the leaf extracts. *Acacia nilotica* was found to be effective against *Salmonella typhi*, *Shigella boydii*, *Shigella flexneri*, *Shigella sonnei*, *Vibrio cholerae 0139* and *Hafnia*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these extracts were determined. In case of methanol extract, the MIC values were 700μg ml^{-1} against Salmonella *typhi* and 800 μg ml^{-1} for each of *Shigella boydii*, and *Shigella flexneri* , whereas the corresponding MIC values for hexane extract were found 800, 800 and 900 μg ml^{-1} respectively. The MBC values of methanol extract were found to be 800 μg ml^{-1} for *Salmonella typhi*, 900 μg ml^{-1} against each of Shigella *boydii* and *Shigella flexneri* and in case of hexane extract the MBC values were found 900, 1000 and 1200 μg ml^{-1} respectively.

Key words: *Acacia nilotica*, enteropathogen, antibacterial activity

Introduction

Diarrheal disease like cholera, dysentery and enteric fever (typhoid) are very common bacterial infection Bangladesh. A majority of bacteria in nature are non-pathogenic and sensitive to antibiotics. Due to evolutionary and man made antibiotic pressure sensitive bacteria acquire resistance gene and evolves to a resistant one (Kapil, 2005) .Introduction of newer antibiotic is usually followed sooner or later by emergence of bacterial resistance to antimicrobial agents (Shanahan *et al.,*1994). Innate genetic properties, mutation, dissemination of R-factor among the genera of the pathogenic bacteria are the causes of drug resistance (Stainier *et al*., 1993).The introduction of third generation cephalosporin in the 1980s was quickly followed by the emergence of resistance in gram negative bacilli (Burwen *et al*., 1994). In Bangladesh, it has been reported that 65.5% of commensal enterobacteriaceae and organism isolated from cases of urine infection are resistant to ampicillin, tetracycline, co-trimoxazole and other commonly used antibiotics (Chowdhury *et al*., 1994.).From prehistoric era people of this subcontinent have been using plants and plant materials to combat disease like typhoid, dysentery, and other diarrhoeal

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infections. To keep up with the changing antibiogram of bacterial pathogen there is desperate need for searching plants for antibacterial activity.

In Bangladesh, *Acacia nilotica* (=*Acacia arabica*) is known as Babla, Babul, Babur or Kikar. It is a xerophytic evergreen tree, grows as wild species and as well as cultivated in dry places. It belongs to the family of Mimosaceae in the order Liguminaceae. It is widely distributed throughout Bangladesh, greater part of India, Beluchistan, Arabia, Egypt, and tropical Africa (Shukla and Misra, 1997). Current investigation on *Acacia nilotica* was carried out beause of the following usages of the plant in different treatise.

In Ayurveda, Younani, Blatter, Masker and Caius the plant (leaves, bark, flower, gum) is used as astringent, alexipharmic, antidysenteric, antipyretic, liver and brain tonic, demulcent and is used in the treatment of a diverse number of cases like bronchitis, vata, piles, fractures, leucoderma, diarrhoea, cough, biliousness, burning sensation, reproductive tract infection, leprosy and ophthalmia, eye sores, dyspnoea, insanity, lung troubles, burns, leucorrhoea, ulcers, diabetes mellitus, throat and chest troubles, snake bite etc (Kirtkar and Basu,1987).

So far our knowledge goes, no *In vitro* studies have been carried out to find out antibacterial activity of *Acacia nilotica* against common enteric pathogen. So, our present study was aimed at: (i) to screen antibacterial activity of *Acacia nilotica* against a group of enteropathogenic bacteria; and (ii) to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the solvent extract(s).

Materials and Methods

In this investigation, screening of different solvent extracts of *Acacia nilotica* for their antibacterial activity were carried out by disc diffusion method (Bauer *et al*., 1966). After initial screening minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined.

Collection of plant materials and preparation of solvent extracts: The fresh leaves of *Acacia nilotica* were collected from the campus of Khulna University. Powdered material was extracted successively with two different solvents using the concept of the nature of solubility and distribution of the active ingredients. For this, methanol and hexane were used as polar and non-polar solvent respectively. Powder was mixed with methanol and preserved for several days in an airtight flask. The liquid portion of the mixture was taken into a separation funnel and the debris was discarded. In separation funnel, appropriate amount of hexane was given and occasionally shaken several times. The hexane portion was then separated and collected in beaker and this procedure was repeated three times. Liquid portion in the beaker was air-dried. In the same way methanol extract was obtained using methanol portion of the separation flux.

Screening of Acacia nilotica leaf extracts for antibacterial activity against pathogenic bacteria: The antibacterial activity of *Acacia nilotica* leaf extracts was done by the disc diffusion method (Bauer *et al.,* 1966). Hospital isolates of ten pathogenic bacteria were collected from International Center for Diarrhoeal Disease Research' Bangladesh (ICDDR'B) for the test. They are listed in Table 1.

Table 1. List of organisms used in the study.

Shigella boydii	*Vibrio cholerae* O1
Shigella flexneri	*Vibrio cholerae* 0139
Shigella sonnei	*Plesiomonas shigelloides*
Shigella dysenteriaetype-1	*Hafnia* spp
Salmonella typhi	*Proteus* spp

Preparation of the discs: Paper discs were placed in a sterile blank petridish. The discs were impregnated separately with the solution of plant extracts so that each disc contain 1000 µg of the test material and left for a period of time in an aseptic condition for the complete evaporation of the solvents. Control discs were also prepared in order to determine whether remaining solvent (if any) in disc shows any antimicrobial activity. For the preparation of control disc equal volume of solvent without test material was used to soak the paper disc. Kanamycin was used as standard antibiotic disc (Oxoid Ltd).

Procedure of disc diffusion method for screening antibacterial activity: From overnight culture plate, small portion of a fresh colony was transferred to test tube containing Muller-Hinton broth and incubated at 37 °C until the growth reached the log phase (~5×10^6 cfu ml^{-1}). After optimum growth, plates were seeded properly by pouring the culture broth with a pasteur pipette to make a bacterial lawn. The excess culture-broth were discarded from the plates with a pasteur pipette and allowed to dry for 5 minutes. Discs impregnated with different solvent extracts were placed at a proportionate distance from each other using a sterile needle. The plates were incubated overnight at 37 °C and zone of inhibition (if any) was measured in mm.

In vitro determination of minimum inhibitory concentration (MIC) of the Acacia nilotica leaf extracts: The minimum inhibitory concentration (MIC) of methanol extract was determined by the broth dilution test tube method (Blair *et al.*, 1997). In brief, an increasing concentration of solvent extracts (200, 300, 400, 500, 600, 700, 800, 900, 1000 and 1200 µg ml^{-1}) were added to different test tube, each containing 3 ml of tryptone soy broth. Then 10 µl inoculum from young liquid culture (~5×10^6 cfu ml^{-1}) was added. Additional test tubes were also included as control to check the sterility of the media, inhibitory effect of the solvent itself and viability of inoculum. All the tubes were incubated overnight at 37 °C and checked for growth. Tubes with lowest concentrations of extracts without any turbidity were considered MIC of the respective solvent extracts. However, in case of colored extract, subculture was made from the tubes to determine the MIC.

In vitro determination of Minimum Bactericidal Concentration (MBC) of methanol and hexane extracts: The minimum bactericidal concentration (MBC) of solvent extracts were determined by measuring the viability loss of bacterial culture with increasing concentrations of extract added. Test tubes containing higher concentrations of leaf extracts than MIC were plated out on to TSA plate to check the viability. Suspension from the tube containing lowest concentration of extracts producing no colonies after overnight incubation indicated MBC value.

Results

Yield of solvent extraction: From solvent extraction method 28.19 gm Methanol extract and 3.48 gm hexane extract were obtained from 250 gm of air-dried powdered leaf of *Acacia nilotica*. Yield was 11.27% and 1.39% for methanol and hexane extraction respectively (Table 2).

Table 2. Extraction yield of the leaves of *Acacia nilotica*.

Weight of plant material (g)	Weight of methanol extract (g)	Weight of hexane extract (g)	Extraction Yield	
			Methanol %	Hexane %
250	28.19	3.48	11.27	1.39

Antibacterial activity: In the preliminary experiment, methanol and hexane extracts showed positive result. Discs of methanol extract (1000 µg) produced zones of 12 mm against *Salmonella typhi* and 10 mm against each of *Shigella boydii*, *Shigella flexneri*, and *Shigella sonnei* (Table 3). Antimicrobial activity of methanol extract against O139 *Vibrio cholerae and Hafnia* were also noteable (8 mm zone).The bacterial isolates of *Vibrio cholerae* O1, *Shigella dysenteriae type1, Plesiomonas shigelloides* and *Proteus sp* were fully resistant to methanol extract (Table 3). Hexane extract also showed noteable activity against *Shigella boydii* (11 mm zone) and 10 mm zone for each of

Table 3. Sensitivity of enteric pathogen against methanol and hexane extract.

Name of bacteria	Zone of inhibition (in mm)	
	Methanol extract (1000 µg $disc^{-1}$)	Hexane extract (1000 µg $disc^{-1}$)
Shigella boydii	10	11
Shigella flexneri	10	10
Shigella sonnei	10	10
Shigella dysenteriae type-1	0	0
Salmonella typhi	12	10
Vibrio cholerae O1	0	0
Vibrio cholerae 0139	8	0
Plesiomonas shigelloides	0	0
Hafnia spp.	8	10
Proteus spp.	0	0

Salmonella typhi, Hafnia, Shigella flexneri and *Shigella sonnei* (Table 3). But no activity was found against *Shigella dysenteriae type-1*, *Plesiomonas shigelloides, Proteus* sp *and Vibrio cholerae* of *0139 and* O1serogroup (Table 3). Though the extracts were crude but the zones of inhibition were very clear indicating the presence of active compound against bacteria.

The MIC of leaf extracts: Some selected bacteria from those showed sensitivity against methanol and hexane extracts in preliminary screening were used for MIC test. The MIC of methanol extracts were found 700µg ml^{-1} for *Salmonella typhi*, 800µg ml^{-1} for each of *Shigella boydii* and *Shigella flexneri* respectively (Table 4). Higher MIC values were obtained in case of hexane extract and 800 µg ml^{-1} against each of *Salmonella typhi* and *Shigella boydii* and 900 µg ml^{-1} for *Shigella flexneri* (Table 4). In the MIC assay, a gradual decrease of bacterial growth were observed from the initial concentration.

Table 4. Determination of MIC value by bacterial growth inhibition with increasing concentrations of methanol and hexane extracts.

Tube no.	Conc. (µg ml^{-1})	Inoculum (10^6 cells ml^{-1}) added in µl	Observation					
			Methanol extract			Hexane extract		
			S. typhi	*S. boydii*	*S. flexneri*	*S. typhi*	*S. boydii*	*S. flexneri*
1	200	10	G	G	G	G	G	G
2	300	10	G	G	G	G	G	G
3	400	10	G	G	G	G	G	G
4	500	10	G	G	G	G	G	G
5	600	10	G	G	G	G	G	G
6	700	10	NG	G	G	G	G	G
7	800	10	NG	NG	NG	NG	NG	G
8	900	10	NG	NG	NG	NG	NG	NG
9	1000	10	NG	NG	NG	NG	NG	NG
10	1200	10	NG	NG	NG	NG	NG	NG
11(C_s)	00	10	G	G	G	G	G	G
12(C_i)	00	10	G	G	G	G	G	G
13(C_m)	00	00	NG	NG	NG	NG	NG	NG

G = Growth, NG = No Growth, C_s = Solvent (8% Methanol) + Medium + inoculum, C_i = Inoculum + Medium, C_m = Only medium.

The MBC of leaf extracts: By measuring the viability loss of inoculum from the tubes of MIC experiment, the minimum bactericidal concentrations (MBC) were determined against *Salmonella typhi*, *Shigella boydii*, and *Shigella flexneri*. Gradual loss of viability of bacteria was observed with increasing concentration of extract and from a certain concentration no viable bacteria was found.

The concentrations were then plotted against the log of respective bacterial counts to study the nature of the depletion of bacterial growth with the increasing concentration (Fig. 1 and 2). The curves indicate that the rates of initial decrease of bacterial growth for all the organisms with increasing concentration are lower for both methanol and hexane extracts. The rate of bacterial growth then rapidly falls with the increasing concentration and finally become zero. For the methanol extract (Fig.1), the viable count becomes zero for *Salmonella typhi,* at a concentration of 800 µg ml^{-1} and that for *Shigella boydii* and *Shigella flexneri* at a concentration of 900 µg ml^{-1} indicating their MBC values. Similarly, for hexane extract (Fig. 2), the viable count of *Salmonella typhi* becomes zero at a concentration of 900 µg ml^{-1} and that for *Shigella boydii* and *Shigella flexneri* at a concentration of 1000 µg ml^{-1} and 1200 µg ml^{-1} respectively. Hence, the MBCs of hexane extracts for *Salmonella typhi*, *Shigella boydii* and *Shigella flexneri* were found to be 900, 1000 and 1200 µg ml^{-1} respectively.

Table 5. Determination of MBC by viability loss of *Salmonella typhi, Shigella boydii* and *Shigella flexneri* with increasing concentration of methanol and hexane extracts.

Tube no.	Conc. µg ml^{-1}	Observation (Count) cells ml^{-1}					
		Methanol extract			Hexane extract		
		Salmonella typhi	*Shigella boydii*	*Shigella flexneri*	*Salmonella typhi*	*Shigella boydii*	*Shigella flexneri*
1	200	1.2×10^{9}	2×10^{9}	1.8×10^{9}	1×10^{9}	1.5×10^{9}	2.6×10^{9}
2	300	1.7×10^{8}	3.1×10^{8}	7×10^{8}	4×10^{8}	5×10^{8}	1.7×10^{9}
3	400	3.9×10^{7}	1.8×10^{8}	2.1×10^{8}	2.3×10^{7}	2.9×10^{8}	3.5×10^{8}
4	500	1.2×10^{7}	1.4×10^{8}	3.5×10^{7}	1.1×10^{7}	3.1×10^{8}	1.2×10^{8}
5	600	2.5×10^{6}	4.5×10^{7}	3.3×10^{7}	2.6×10^{6}	4×10^{7}	4.6×10^{7}
6	700	1.1×10^{5}	2.9×10^{6}	1.9×10^{6}	1.3×10^{6}	3.5×10^{6}	2.8×10^{7}
7	800	00	3×10^{5}	9×10^{5}	3×10^{5}	1.1×10^{5}	1.4×10^{6}
8	900	00	00	2×10^{4}	00	2.3×10^{4}	1.9×10^{5}
9	1000	00	00	00	00	00	1.1×10^{4}
10	1200	00	00	00	00	00	00
11(Cs)	00	2×10^{9}	2.5×10^{9}	2×10^{9}	1.9×10^{9}	2×10^{9}	2.8×10^{9}
12(C_i)	00	2×10^{9}	2.5×10^{9}	2.1×10^{9}	2×10^{9}	2×10^{9}	3×10^{9}
13(C_m)	00	00	00	00	00	00	00

C_s = Solvent (8% Methanol) + Medium + inoculum, C_i = Inoculum + Medium, C_m = Only medium

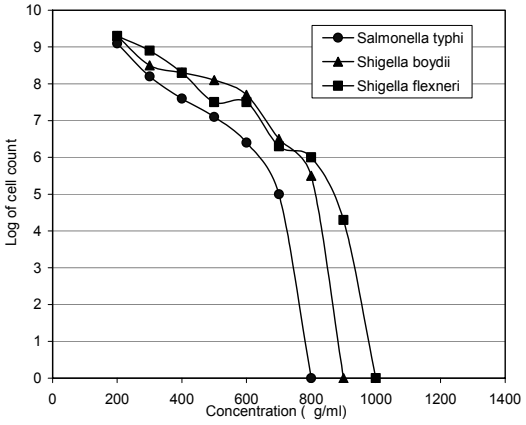

Fig. 1. Curves for viability loss in MBC test using methanol extract (concentration of extract vs. log of viable count).

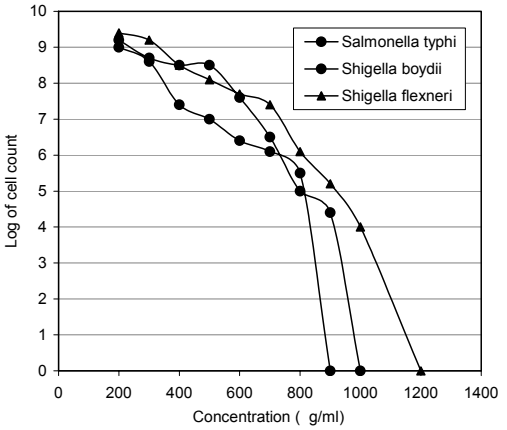

Fig. 2. Curves for viability loss in MBC test using hexane extract (concentration of extract vs. log of viable count).

Discussion

Plant material was extracted with a very polar solvent to get all the components present in the sample. Extraction yield of methanol is higher than that of the hexane. Out of ten isolates of enteric bacteria tested seven showed various level of sensitivity to methanol and /or hexane extract (Fig. 2). The O1 and O139 isolate of *Vibrio cholerae* varied in their sensitivity to methanol extract. Screening of a good number of *Vibrio cholerae* isolates from both the serotypes is required to conclude whether this difference is serotype specific. The over all MBCs of methanol extract are lower than the hexane extract indicates that the methanol extract possesses stronger antibacterial activity than the hexane extract. It could be argued from this result that the antibacterial compound is very much accumulated in the methanol extract.

Conclusion

Extracts of *Accacia nilotica* seems to have antibacterial activity to diverse species of enteric bacteria. Inclusion of other species of the enterobacteriaceae family for screening, together with isolation of active ingredients from methanol and hexane extracts will further clarify the assumption.

References

Bauer, A.W.; Kirby, W.M.H.; Scherris, J.C. and Turck, L.L. 1966. Antibiotic susceptibility testing by a standardized single method. *American Journal of Clinical Pathology,* 45:493-496.

Blair, J.E.; Lennte, E.D.M. and Truant, J.P. 1997. *Manual of Clinical Microbiology*. American Society for Microbiology, Bethesda, 306 pp.

Burwen, D.R.; Banerjee, S.N. and Gaynes, R.P. 1994. The national nosocomial infections surveillance system. Cerftazidime resistance among selected nosocomial Gram negative bacilli in the United States. *Journal of Infectious Diseases,* 170: 1622-1625.

Chowdhury, M.A.Q.; Rahman, K.M.; Amin, M.R. and Haq, J.A. 1994. Transferable drug resistance (R-factor) among the enterobacteriaceae in urinary tract infection: a study at an urban hospital in Bangladesh. *Journal of Tropical Medicine and Hygiene*, 97: 161-166.

Kapil, A. 2005. The challenge of antibiotic resistance: Need to contemplate. *Indian Journal of Medical Research,* 121: 83-91.

Kirtikar, K.R. and Basu, B.D. 1987. *Indian Medicinal Plants*. Vol-II, 2nd edn., International Book Distributors, New Delhi, pp. 919-935.

Shanahan, P.M.A.; Thompson, C.J. and Amyes, S.G.B. 1994. The global impact of antibiotic resistant bacteria: their sources and reservoirs. *Reviews in Medical Microbiology*, 5: 174-182.

Shukla, P. and Misra, S.P. 1997. *An Introduction to Taxonomy of Angiosperms*. Vikas Publishing House Pvt. Ltd., Calcutta, pp. 255-259.

Stanier, R.Y.; Ingraham, J.L.; Wheelis, M.L. and Painter, P.R. 1993. *General Microbiology*. 5th edn., The Macmillan Press Ltd., London, pp. 274-278.