JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2011. Vol. **5**(2), p. 953-957

Antibacterial Activity of *Cleome viscosa, Hygrophila auriculata*, *Aristida stricta* on Isolated Characterised *Lactobacillus* and *Streptococcus* from Milk

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(Received: 18 February 2011; accepted: 21 April 2011)

Lactobacillus, Streptococcus **are gram positive bacteria. These two bacteria isolated from milk.** ***Lactobacillus, Streptococcus*** **could be identified from milk through various biochemical test. In the present study an attempt has been made to carry out screening for the preliminary antibacterial activity of plants used in Indian folk medicine. The aim of the study was to select an active plant extract may be useful in developing new lead compounds to control diseases. Three plants were selected for preliminary screening of their antibacterial potentiality , viz,** ***Cleome viscosa, Hygrophila auriculata, Aristida stricta.*****The antibacterial activity was done by Disc diffusion method against** ***Lactobacillus and streptococcus.***

Key words: *Lactobacillus, Streptococcus, Cleome viscosa, Hygrophila auriculata, Aristida stricta.*

Bacteria are prokaryotic unicellular microorganisms which divide by binary fission and do not possess chlorophyll and true branching, except in the so called "higher Bacteria" (Actinomycetes). Their size varies from 0.5 to 15 ìm in length and 0.2 to 2 ìm in breadth[1]. *Streptococci* are non motile and non spring, most strains belonging to group A and C possess capsule composed of hyaluronic acid, while members of group B and D show polysaccharide capsules[2]. They are aerobes, facultative anaerobes and grow best at a temperature of 37^0C, group D *Streptococci* grow well at and between 10° C. Pathogenic *streptococci* are most exacting in nutritive requirements. Growth is poor on solid media containing blood, sugars and 10 % carbon di oxide in the environment promotes growth and haemolysis.

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Streptococcus groups A, C and viridians *Streptococci* can occur as commensals in the upper respiratory tract. *Enterococci* form part of the normal flora of intestinal tract. Pathogenecity of *streptococcus* 1) Respiratory tract infection as a) Sore throat (acute tonsillitis / and or pharyngitis) is the commonest of *Streptococcal* diseases. The bacteria adhere to the pharyngeal epithelium by means of lipiteichoic acid covering surface pili. b)Scarlet fever occurs as a complication of *Streptococcal* infection. When the infecting strain produces erythrogenic toxin and the patient has got no antitoxic immunity (Usually a child) 2)Skin infection as a) Impetigo: Impetigo is a superficial discrete crushed spot, specially in children, usually less than one inch in dimeter. Streptococcal erythema is a more severe type of local skin lesion with an ulcerated area of similar size having a raised and edematous edge. b)Erysipelas : Erysipelas is an acute spreading lesion. Infected area of skin shows massive browny oedema with erythema .3)Other suppurative infection a) Puerperal sepsis b) Sepsis : Infection of wounds, burns and chronic skin lesions. c) Lymphadenitis: Septicaemia, acute

endocarditis, abscess in internal organs. d) Necrotising fascitis is characterized by extensive fascial and subcutaneous necrosis. e) Otitis media, sinusitis, mastoiditis, bacteraemia and pneumonia are less commonly seen. f) Streptococcal toxic shock syndrome is a result of systematic toxicity resembling staphylococcal toxic shock syndrome Group A, β- *Haemolytic streptococci* are very sensitive to penicillin, Erythromycin[3].

Lactobacilli are widely distributed as saprophytes in fermenting animal and vegetable material (Eg : milk, Cheese);others form common flora of man and animals in the mouth gut and vagina. *Lactobacilli* grow best under microaerophilic conditions in presence of 5% carbon dioxide and at pH 6.0. Better growth is obtained in media enriched with glucose or blood colonies are small on nutrient agar after 4 hrs incubation. *Lactobacillus* are prominent as indigenous flora in vagina and colon and present in smaller numbers in the mouth. They are only occasionally involved on human infections , usually pleuropulmonary infections are dental caries, and almost always as part of a mixed bacterial flora, they have been isolated although rarely from urinary tract infection bacteremia, endocartidis, local suppurative infection and chorioamnionitis[4]. Some *Lactobacillus* species are widely used for the production of yougurt, cheese, sauerkraut, pickles, beer, wine, cider, kimchi, chocolate and other fermented food as well as animal feeds such as silage. Some *Lactobacillus* and other lactic acid bacteria may possess potential therapeutic properties including anti inflammatory and anti cancer activities[5]. Most of the anaerobes are sensitive to penicillin *(except B. fragilis)*, chloramphenicol, fucidin, trimethoprim and tetracycline.

Medicinal plants represents a rich source of antimicrobial agent. Plants also used medicinally in different countries and are a source of many potent and powerful drug . *Cleome viscosa* is a *Cleomaceae* family. This medicinal herb is an erect herb of waste places to 60 cm high, with viscous foliage, occurring widespread over the whole region from Senegal to Nigeria, and commonly across Africa and throughout the tropics. The plant has an acrid taste. The leaves are used as a counter-irritant for headache or local pain, the leaves being rubbed on the parts affected, or applied as a poultice, or rubbed in the hands and inhaled like smelling-salts. Decoction of the plant is used for colic and even advanced dysentery. The seeds are anthelimitic, carminative, constipating, febrifuge and cardiac stimulant and are useful in fever, diarrhoea, worm infection, cardiac disorders and dyspepsia[6].

Hygrophila auriculata is a *Acanthaceae* family. This medicinal herb is an erect, hispid, semiwoody, annual with numerous faciculate sub quadrangular stems; leaves in verticels of six ata node. Diuretic properties of seeds are due to large amount of mucilage and potassium salts. Seeds contains 23% of a yellow semi drying oil, they also contain diastase, lipase, protease. Xylose and uronic acids from oil of seeds; lupenol, stigma sterol and straight chain hydrocarbon from lipid extract of plant palmitic (7.2), steric (0.8), oleic (11.9) and linoleic (0.82 – 1 %) acids from seed oil are separated.The roots are sweet, sour, bitter, refrigerant, diuretic, anti inflammatory, aphrodisiac and toxic. They are useful in dropsy of chronic Bright's disease, inflammation, ascites, hyperdipsia, vesical calculi, jauntice, flatulence, dysentery and vitiated conditions of veta[7]. The leaves are sweet, sour, bitter, anti inflammatory, aphrodisiac, opthalemic and toxic. They are useful in jaundice, dropsy, rheumatisum, lumbago, arthralgia, anasarca, disease of the urogenital tract, arthritis, cough, vitiated conditions of pitta, gastropathy, anemia and opthalmopathy.

Aristida stricta is a *poaceae* family. This medicinal herb is a tufted annual with thin culms 15-30 cm high. Leaf-blades flat or folded 2.5-10 cm long, up to 2 mm wide. Panicle linear up to 10 cm long, composed of a few long spikelets. Lower glume 20-30 mm long and 3-6 mm longer than the upper glume but both long attenuate; body of the lemma up to 4 mm long, awn column articulated below, 16-45 mm long. It is distributed in Kenya; also from Senegal to Somalia then through Saudi Arabia to India. Mature pineland threeawn is low quality forage. Compared with other native forage grasses, it is poorly digested and nutritionally inferior .Nutritional quality is greatly increased by prescribed burning.

MATERIAL AND METHODS

Collection of milk , plants sample

Milk sample and the three plants samples are *Hygrophila auriculata, Cleome Viscosa* and *Aristida stricta* were collected from Peraiyur Village, Mannargudi Taluk, and Thiruvarur District. Plants are collected in early morning of December and shade dried for 10 days, then powdered.

Preparation of plant extracts

The shade dried plant material was powdered using kitchen blender and that powder was subjected to Soxhlet extraction with aqueous, methanol and ethanol (60°C) for 24 hrs. Each solvent extract was distilled and condensed at 40°C. The condensed extract was stored at room temperature in air tight bottles and used for further studies.

Isolation

Several different methods are used for the isolation of pure culture of microorganisms. These methods involve separating microorganisms on a solid medium into individual cells that are then allowed to reproduce to form a colony. A colony is a visible clone of microorganisms.Cultivation is the process of propagating organisms by providing the proper environmental conditions. Bacteria were grown on Nutrient agar medium.

Isolation of bacteria - serial dilution technique

The nutrient agar medium were prepared and sterilized. The medium was poured in sterile petriplates and allowed to solidify. 10ml of the milk sample was added to 90ml of the distilled water in a flask. It was shaked vigorously and 1ml was transferred in test tube containing 9ml of distilled water. The content was mixed well and 1ml was transferred from 10^{-1} dilution to the next dilutions up to 10^{-9} dilution. After solidifying, the nutrient agar plates with dilution 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} were taken. 0.1ml sample was poured in petriplates using spread plate technique. The plates were incubated for bacteria at 37°C for 24 hrs. After incubation, the plates were observed.

The following tests were performed to characterize the isolates

Gram staining method,Motility test-hanging drop method, Indole test, Methyl red test, Voges –proskauer test, Citrate utilization test, Urease test, Triple sugar ion test, Catalase test, Oxidase test, Nitrate reduction test.

Confirmation test

The test organisms are inoculated in the selective medium and the results were observed. The isolated cultures of bacteria are maintained at 4 ^{0}C in nutrient agar. The antimicrobial activity of plant extract against different pathogens was determined by Agar disc diffusion. The plant extracts were further subjected to antibacterial study. Using sterile inoculation loop pure colonies of the test organism are transferred to 5ml of sterile nutrient broth and incubated at 37 ^{0}C overnight for 18hrs. Then this bacterial culture were suspended in saline solution (0.85%Nacl) and adjusted to a turbidity of 0.5 Mac Farland standards (10^8cfu/ml). This suspension was used for preliminary screening of anti bacterial activity.

Preliminary screening for anti bacterial activity

Dip the sterile swab into bacterial suspension compared to standard then swab onto the surface of Mueller Hinton Agar using Overlapping technique . Allow the organism to be absorbed by the medium. Place Ethanol, Methanol, Aquous extracts of *Cleome viscosa ,Hygrophila auriculata,Aristida stricta*. Incubate for 24 hrs at 37^{0}C.Measure the diameter of the Zone of Inhibition (area wherein there is no growth around the discs) using the millimeter of a ruler.

RESULTS AND DISCUSSION

The *Lactobacillus* and *Streptococcus* were isolated from milk sample. The milk sample was serially diluted, after serial dilution were spread on plates of selective media and incubated at the particular conditions. All the inoculated plates were incubated anaerobically at 30° C for 48hrs. MRS was used as a selective medium for *Lactobacillus* [8]. TAS was used as a selective medium for *Streptococcus* [9]. After incubation at room temperature, the plates showed off-white cream colour colonies. The quantity of colonies is high in 10^{-4} and 10^{-5} dilution. Colonies were selected and the result showed in table 1.The isolates are subjected to biochemical test and the result showed in table 2.

Steptococcus is Gram positive, Non motile, Cocci in shape, TSI test is Ammonia gas produced, Methyl red test is positive. Voges proskauer test, citrate test, Indole test, Urease test ,Catalase test, Oxidase test , Nitrate reduction test is negative.

Table 1. Colony Morphology of Bacterial isolates on Specific media

S. No.	Organism	Media Used	Colony Morphology
1.	*Lactobacillus*	MRS Media	lactose Circles
2.	*Streptococcus*	TSA Agar	β-haemolysis

Table 2. Characterization of Bacteria

Tests	*Streptococcus*	*Lactobacillus*
Gram staining	GP	GP
Motility	-	+
Shape	Cocci in Chain	Bacilli
Iodole	-	+
Methyl Red	+	+
Voges Proskauer	-	-
Citrate	-	-
Urease	-	-
TSI	A gas	A gas
Catalase	-	+
Oxidase	-	-
Nitrate Reduction	-	-

Table 3. Anti bacterial activity of *Cleome viscosa.*,*Hygrophila auriculata.*, *Aristida stricta.*, on isolated,characterised *Lactobacillus* and *streptococcus* from milk

Species	Group	Zone of inhibition (mm)	
		Lactobacillus	*Streptococcus*
Cleome viscosa	Control	0	0
	Aqueous	11	13
	Methanol	14	12
	Ethanol	12	15
	Tetracycline	25	25
Hygrophila auriculata	Control	0	0
	Aqueous	9	11
	Methanol	15	9
	Ethanol	16	10
	Tetracycline	23	23
Aristida sticta	Control	0	0
	Aqueous	20	11
	Methanol	21	15
	Ethanol	22	11
	Tetracycline	24	24

Lactobacillus is Gram positive, motile, Bacilli in shape, TSI test is Ammonia gas produced, indole test , Catalase test ,Methyl red test is positive. Voges proskauer test, citrate test, Urease test, Oxidase test, Nitrate reduction test is negative.

The different extracts of (Aqueous,Methanol,Ethanol) the three plant samples namely *Cleome viscosa*,*Hygrophila auriculata* and *Aristida stricta* were assessed for the antibacterial activity on *Lactobacillus* and *Streptococcus* in Table 3.

According to Williams, 2003[10], *Cleome viscosa* has anti-bacterial, anti-fungal, contact insecticidal and nematicidal activity against *Bacillus subtilis* (Gram-positive) and *Pseudomonas fluorescens* (Gram-negative), respectively. In this study the plant *C. viscosa* also having the antibacterial effect against the selected bacteria.Methanol extract of *C. Viscosa* produce better inhibitory zone against *Lactobacillus* than other extracts. Ethanol extract of *C. viscosa* produce better inhibitory zone against *Streptococcus* than other extract. In the study, conclude *C. viscosa* acts against the harmful effect of *Streptococcus* than the harmful effect of *Lactobacillus.*

Hygrophila auriculata was extensively used in traditional system of medicine for various ailments like rheumatism, inflammation, jaundice, hepatic obstruction, pain, etc. The extracts exhibited anti-nociceptive activity by central and peripheral mechanism. extract was exhibited significant hepatoprotective and antioxidant activities [11]and also possess the anti bacterial property against intestinal bacteria. Ethanol extract of *H. auriculata* possess potential activity against *Lactobacillus* than other extracts. Aqueous extract of *H. auriculata* possess potential activity against *Streptococcus* than other extract[10]. In the study, conclude *H. auriculata* act potential antibacterial activity against the harmful effect of *Lactobacillus* than the harmful effect of *Streptococcus.*

Methanol extract of *Aristida stricta* exhibit highest activity against *streptococcus* than other extracts. Ethanol extract of *A. stricta* exhibit on highest activity against *Lactobacillus* than other extracts. In the study, conclude *A.stricta* acts against the harmful effects of *Lactobacillus* than the harmful effect of *Streptococcus*. When compared these three plants the *A. stricta* has high antibacterial activity against *Lactobacillus.* From this study, conclude that three plants possess the antibacterial activity and prevent the intestinal infections in child due to the intake of milk.

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