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Evaluation of the Antibacterial Potential of various solvent extracts of *Acacia nilotica linn.* Leaves

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Abstract

Plan: *Bioactive compounds of leaves of Acacia nilotica Linn. (Malvaceae) have been studied for their antimicrobial activities using disc diffusion assay.*
Methodology: *Four Gram negative bacteria (Escherichia coli MTCC 2961, Pseudomonas aeruginosa MTCC 4676, Klebsiella pneumoniae MTCC 432 and Salmonella typhi MTCC 733), two Gram positive bacteria (Staphylococcus aureus, Bacillus subtilis) were selected for the study. Minimum inhibitory concentration (MIC) of the active compounds was evaluated by micro broth dilution method.*
Outcome: *All the active compounds exhibited broad spectrum antimicrobial activity. Alkaloids and Flavonoides were found to be more potent. S. aureus was found to be most susceptible organism followed by E. coli and S.typhi. Our findings confirm that the traditional therapeutic claims for this plant, in near future surely be able to replace the conventional antimicrobial agents to which there is increased incidence of drug interactions and the study suggests that this plant is promising for development of phytomedicines with antibacterial properties.*
Key words: *Acacia nilotica leaves, Alkaloids, Flavonoides, Saponins, Antibacterial property, Disc diffusion method.*

1. Introduction

Infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Death from infectious diseases ranked 5th in 1981, has become the 3rd leading cause of death in 1992, with an increase 58% (Venkataswamy *et al*., 2010). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Doss *et al*., 2009). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increases in the incidence of new and re-emerging infectious diseases (Anand *et al*., 2011).

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind (Mahesh and Satish, 2008). Medicinal plant based antimicrobials represent a vast untapped source of pharmaceuticals and further exploration of plant antimicrobials need to occur for treatment of infectious diseases both in plants and humans while simultaneously for mitigating many of the side effects that are often associated with synthetic antimicrobials.

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Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agents. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals (Banso, 2009).

Acacia nilotica (L.) Willd. ex Del. is also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 2000 m and withstand at extreme temperature (>50^{o} C) and air dryness but sensitive to frost when it is young. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India. The plant is a tree with yellow mimosa-like flowers and long grey pods constricted between seeds. The bark and branches are dark with fissures. The branches bear spikes about 2 cm long. The leaves are five and densely hairy with 3 - 6 pairs of pinnae consisting of 10 - 20 pairs of leaflets that are narrow with parallel margins that are rounded at the apex and with a central midrib closely crowded. The inflorescence consists of bright yellow flowers in auxillary head on stalks that are half way up. The flowering period of the plant is between November and March (Mann *et al*., 2003).

Acacia species contains secondary metabolites including amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, nonprotein amino acids, terpenes (including essential oils, diterpenes, phytosterol and triterpene genins and saponins), hydrolyzable tannins, flavonoids and condensed tannins (Seigler, 2003). Babul plant is therapeutic used as Anti-cancer, anti tumours, Antiscorbutic, Astringent, anti-oxidant, Natriuretic, Antispasmodial, Diuretic, Intestinal pains and diarrhea, Nerve stimulant, Cold, Congestion, Coughs, Dysenter Fever, Hemorrhages, Leucorrhea, Ophthalmia and Sclerosis (Sapna Malviya *et al*., 2011). The present study was carried out to determine the antibacterial activity of different bioactive compounds of the leaves on gram positive and gram negative micro organisms against Chloramphenicol.

2. Materials and methods

2.1. Plant Material

The botanical identity of *Acacia nilotica* Linn. was confirmed by Dr.V.Sampath Kumar, Scientist – C, Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu. A voucher specimen has been deposited at the Department of Microbiology, RVS College of Arts and Science, Sulur, Coimbatore, Tamilnadu, India.

2.2. Extraction of alkaloids

Alkaloids were extracted from leaves of the selected plant by well established methods (Geeta Singh and Padma Kumar, 2011) after preliminary detection of alkaloids. Leaves of *A.indicum* were taken for the extraction. Finely powered sample (100g) was extracted with 10% acetic acid in ethanol for 4 h. Extracts were concentrated and were making alkaline by Ammonium hydroxide. Precipitate thus obtained was collected by centrifugation, washed with 1% Ammonium hydroxide, dried in vaccuo and weighed. Extracts thus obtained were stored at 4°C in air tight glass vials for further use.

2.3. Extraction of saponins

The shade dried and powdered plant material (500 g) was extracted with water by soxhlation for 8 h. The aqueous extract was concentrated under vacuum. The dark colored residue (80 g) was refluxed with n-butanol for 2 h and n-butanol soluble constituents were separated by filtration. The n-butanol layer was sequentially washed with distilled water, alkali (2% KOH) and distilled water again. The n-butanol layer was evaporated and dried under vacuum to obtain a dark green color powder. Charcoal treatment was given to the powder and the filtrate was further dried under vacuum to give rich saponins. The extract was tested for Libermann- Burchard test (Rama Manohar Reddy *et al*., 2011).

2.4. Preparation of the Polysaccharide, Proanthocyanidin and Flavonoid fractions

The hot water extract was prepared by boiling 200 g of fresh leaves with 1 ltr. of distilled water for 3h. The final volume was reduced to 200 ml. The water extract was centrifuged and the supernatant was obtained. Excess of ethanol was added to the supernatant to precipitate the high molecular weight polysaccharides fraction, which was filtered and concentrated in vacuum and extracted with ethyl acetate. The ethyl acetate soluble fraction (flavonoids) and the aqueous fraction (Proanthocyanidin) were obtained (Doss *et al*., 2011a).

2.5.Bacterial Strains

Microorganisms were obtained from the Microbial Type Culture Collection Centre (MTCC), Chandigarh, India. Amongst six microorganisms investigated, two Gram-positive bacteria were *Staphylococcus aureus*, *Bacillus subtilis* while four Gram-negative bacteria were *Escherichia coli* MTCC 2961, *Pseuodomonas aeruginosa* MTCC 4676, *Klebsiella pneumoniae* MTCC 432 and *Salmonella typhi* MTCC 733. All the microorganisms were maintained at 4^0C on nutrient agar slants.

2.6. Antimicrobial activity

Antimicrobial activity was carried out by the disc diffusion method. Sterile paper discs (6 mm in diameter) prepared from Whatman No 1 filter paper was impregnated with drug containing solution and placed on the inoculated agar.

Negative and Positive controls used were Dimethylsulfoxide (DMSO) and Chloroamphenical (Doss *et al*., 2011b). The inoculated plates were incubated at 37^0C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone for the test microorganisms.

2.7. Minimum Inhibitory Concentration

For determination of MIC, 1 ml of broth medium was taken into 10 test tubes for each bacteria. Different concentrations of plant extracts ranging from 0.125-8 mg ml^{-1} concentration were incorporated into the broth and the tubes were then inoculated with 0.1 ml of inoculum of respective bacteria (10^5 CFU ml^{-1}) and kept at 37°C for 24 h.

The test tube containing the lowest concentration of extract which showed reduction in turbidity when compared with control was regarded as MIC of that extract (Mubarack *et al*., 2011).

3. Results and Discussion

Herbal medicine is still the mainstay of about 70-80% of world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with human body with lesser side effects. The World Health Organization (WHO) has listed more than 21,000 plants, which are used for many medicinal purposes around the world. They observed that about 74% of 119 plant-derived pharmaceutical medicines are used in modern medicine. It also estimates that 4 billion people (80 percent of the world population) presently use herbal medicine for health care (Sapna Malviya *et al*., 2011).

Antimicrobial activity (assessed in terms of inhibition zone and activity index) of the bioactive compounds, tested against selected microorganisms were recorded (Table 1). In the present study total 5 active compounds of leaves of selected plant were tested for their bioactivity, among which alkaloids showed significant antimicrobial potential against test microbes. However, two active compounds (anthacyanins and polysaccharides) showed no significant activity against any selected microorganism. Most susceptible organism in the investigation was *S.aureus against* which, most of the active compounds showed inhibition zone and best activity was observed by bound alkaloids, where IZ of 15.5 mm, and MIC value of 0.125 mg/ml was recorded. The range of MIC of extracts recorded was 0.125 – 1.0mg/ml (Table 2). In the present investigation lowest MIC value 0.125 mg/ml was recorded against *S. aureus* whereas, MIC against *E. coli* and *S.typhi* was 0.250 mg/ml indicating significant antimicrobial potential of test extracts. MIC values were found same for four extracts of plant indicating high potential at low concentration.

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds of plant that serve as defence mechanisms against predation by many microorganisms, insects and herbivores. The antibacterial activity of flavonoids is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Doss *et al*., 2011).

Antimicrobial activity of phenolic compounds present in plants change according its structure; flavone, quercetin and naringenin were effective in inhibiting the growth of *Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* and *Staphylococcus epidermidis* while gallic acid inhibited only *P. aeruginosa*; rutin as well as catechin did not show any effect on the tested microorganisms.

Several reports are available in support of antimicrobial activity of saponins against bacterial and fungal pathogens (Gopish khanna and Kannabiran, 2008). The alkaloids are known to have antimicrobial and antiparasitic properties. Verpoorte (1998) have reported about 300 alkaloids showing such activity.

Similar results on antibacterial activity were reported on related species of the genus *Mahonia* by Duraiswamy *et al*. (2006), Livia *et al*. (2004) and Li et al. (2007).

In the present study IZ, AI, MIC and TA have been evaluated for each compound. For most of the compounds MIC values recorded were very low, indicating strong bioefficacy of the plant. In an overview of the bioactivity data obtained from the current investigation, it can be highlighted that the tested compounds have great potential to inhibit bacteria. Crude alkaloids of *A.nilotica* leaves had higher inhibitory potential against tested bacterial pathogens. The inhibition exerted by *A.nilotica* alkaloids against the oppturnistic pathogen, *S. aureus* is of considerable importance because it is considered to be one of the major causative agents for numerous hospital and community acquired infections.

Table No. 1. Antibacterial effects of bioactive compounds of *Acacia nilotica* Linn. Leaves

Microorganisms	*Zone of Inhibition (mm)*					
	Alkaloids	*Flavonoids*	*Saponins*	*Polysaccharides*	*Proanthocyanidins*	*Pos.control**
S.aureus	15.5	12.5	10.5	9.5	-	22
B.subtilis	10.5	10	10	9	-	17
E.coli	12.5	10	9.5	-	-	21
S.typhi	10	9.5	-	-	-	16
P.aeruginosa	-	-	9.5	-	-	18
K.pneumoniae	-	-	-	-	-	20

*Positive control (Chloramphenicol)

Table No. 2. Minimum Inhibitory Concentration of certain bioactive compounds of *Acacia nilotica* Linn. Leaves

Microorganisms	*Minimum Inhibitory Concentration (mg/ml)*				
	Alkaloids	*Flavonoides*	*Saponins*	*Polysaccharides*	*Proanthocyanidins*
S.aureus	0.125	0.500	0.500	1.0	-
B.subtilis	0.500	1.0	1.0	1.0	-
E.coli	0.250	0.500	-	-	-
S.typhi	0.250	-	-	-	-
P.aeruginosa	-	-	-	-	-
K.pneumoniae	-	-	-	-	-

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