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Phytochemical, HPTLC finger printing and antibacterial activity of *Acacia nilotica* (L.) Delile

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Abstract

The leaves of *Acacia nilotica* (L.) Delile was extracted with methanol and aqueous medium and studied for *in vitro* antimicrobial property. The methanol extract was found to be most active against all the bacterial species tested except *S.aureus.* Active methanol extract was further studied for HPTLC fingerprint and by phytochemical analysis. HPTLC analysis confirmed segregation of four individual compounds with individual R_f values and peak area percentage. The results of phytochemical screening of extract revealed the presence of Tannins, Carbohydrates and Glycosides. This analysis revealed the high antibacterial activity in the methanol extract of *Acacia nilotica* (L.) Delile

Key words: HPTLC finger printing, methanol, MIC, microorganisms

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 of 58% (Arokiyaraj *et al*., 2009). More than hundreds of plants worldwide are used in traditional medicine as treatments for bacterial infection (Doss *et al*., 2009). Although many have been treated by conventional pharmaceutical approaches, there is a growing interest in the use of natural products by the general public (Ghosh and Playford, 2003). In addition to the pharmaceutical industry continues to investigate their potential as sources of novel growth factor, immunomodulatory and antimicrobial activity (Sasikumar *et al*., 2006).

Acacia nilotica (L.) Delile commonly known as babul, kikar or Indian gum Arabic tree, has been recognized worldwide as a multipurpose tree. It is widely distributed throughout arid and semi-arid zones of the world. Presently about 20% of the total geographical area of India is wasteland. Growing demand for fuel, fodder, wood and food has extensively depleted or eliminated protective plant cover and exposed soils to processes of degradation resulting in partial to complete loss of soil productivity (Venkataswamy, 2010). *A. nilotica* species have been reported to possess anti-hyperglycemic (Zourata Lompo-Ouedraogo *et al*., 2004), antimicrobial (Venkataswamy *et al*., 2010), antiplasmodial (El-Tahir *et al*., 1991), anti-inflammatory, analgesic and antipyretic properties (Dafallah and Al-Mustapha, 1996).

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Literature study showed antibacterial activity of this plant, but activity against clinical isolates has not so far been reported. Considering the medicinal value of this plant, we evaluated the antibacterial potential, HPTLC fingerprint and phytochemical analysis of *Acacia nilotica* (L.) Delile (Leaves).

2. Materials and methods

2.1**Plant Collection**

Leaves of *A.nilotica* were collected from Ayyasamy Hills, Coimbatore District, Tamilnadu. A voucher specimen was deposited at the department herbarium, Government Arts College, Coimbatore. Collected materials were washed thoroughly, shade dried in open air and grounded into powder.

2.2. Preparation of organic and aqueous extracts

Dried powder of each plant (250g) was placed in 500 ml of water for 24 hours for maceration. After 24 hours the mixture was boiled for 1 hour and then filtered. The filtrate thus obtained was cooled and concentrated to dryness. Soxhelet extractor was used for alcoholic extraction of each drug. 250 gm of dry powder of each drug was packed in a filter paper and inserted into the control tube of the extractor where the solvent got circulated continuously at constant temperature. After 24 hours of extraction the mixture was filtered and the filtrate thus obtained was concentrated to a residue in a distilling unit. The obtained residues were weighed and kept in bottles and were used for further phytochemical and biological screening studies.

2.3. HPTLC Finger Printing

Chromatography was performed on 3 × 10 cm HPTLC plates coated with 0.25 mm layer of silica gel 60 F_{254} (Merck, Germany). The plate was prewashed with methanol and activated at 110° C for 5 min. The methanol extracted sample was applied as 10 mm bandwidth using a Camag (Muttenz, Switzerland) Linomat IV sample applicator equipped with 100 µl Syringe. A constant application rate of 20 µl/sec was used. Mobile phase was Toluene: Ethyl acetate: Acetic acid (12.5: 7.5: 0.5) and chromatogram were scanned at 365 nm.

2.4. Phytochemical Analysis[10]

Phytochemical screening of methanol extract was carried out to detect the presence of secondary metabolites, like Alkaloids, Glycosides, Carbohydrates, Saponins, Steroids, Tannins and Proteins.

2.5. Microorganism

Staphylococcus aureus NCIM 5021, *Bacillus subtilis* NCIM 2010, *Escherichia coli* NCIM 2118, *Pseudomonas aeruginosa* NCIM 5029, *Klebsiella pneumoniae* NCIM 2707, and *Proteus vulgaris* NCIM 2027 were collected from The National Chemical Laboratory, Pune and stored in the Department of Microbiology, RVS College of Arts and Science, Sulur, Coimbatore, TamilNadu.

2.6. Antibacterial Screening

The antibacterial test was performed by following agar disc diffusion method (Bauer *et al*., 1996; Nair and Chanda, 2005) using Mueller Hinton Agar No. 2 medium for the assay. The zone of inhibition diameters (m.m) were determined for the respective drug groups and tabulated in the table 1 .

2.7. Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) was determined by adopting the standard reference method NCCLS (2002). The extracts were dissolved in 2% dimethyl sulfoxide (DMSO). A stock solution of each extract was serially diluted in 96-well microtiter plate with Mueller Hinton broth to obtain a concentration ranging from 8 mg/ml to 0.125 mg/ml. A standardized inoculum for each bacterial strain was prepared so as to give an inoculum size of 10^5 CFU/ml in each well. Ciprofloxacin was used as a standard antibiotic for comparative analysis with the effectiveness of various extracts against tested clinical isolate and drug resistant bacteria. Microtiter plate was kept at 37°C and incubated for 24 h. Following incubation, the MIC was calculated as lowest concentration of the extracts inhibiting the visual growth of the test cultures on the agar plate. Three replications were maintained.

3. Results and Discussion

In the HPTLC fingerprinting of methanol extract gave four spots with the following Rf values: 0.08 (24.74%), 0.21 (22.18%), 0.40 (9.81%), 0.50 (9.33%). Purity of the sample extract was confirmed by comparing the absorption spectra at start, middle and end position of the band. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. The HPTLC chromatogram of the methanol extract is presented in Figure 1.

The phytochemical analysis of methanol extract showed the presence of glycosides, carbohydrates and tannins (Table 3). Secondary metabolites of plant origin appear to be one of the alternatives for the control of antibiotic resistant human pathogens. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. This antibacterial activity may be due to the presence of secondary metabolites (Arokiyaraj *et al*., 2009).

The antibacterial activities of extracts of *A.nilotica* against clinically important microbial pathogens are summarized in Table 1. The MICs of the extracts ranged between 0.125 and 2 mg/ml. Among the two extracts, the methanol was found to be most active against all the tested bacterial species except *Staphylococcus aureus* [*Klebsiella pneumonia* (0.125mg/ml), *Bacillus subtilis* (0.500 mg/ml), *E.coli* (2.0 mg/ml), *Proteus vulgaris* (2.0 mg/ml), *Pseudomonas aeruginosa* (1.0 mg/ml)] (Table 2). Aqueous extract was less active than methanol extract. Compare to standard ciprofloxacin, the extracts showed good activity against all the tested bacteria strains. Several plants which are rich in tannins have been shown to possess antimicrobial activity against a number of microorganisms. The ability of tannin compounds to cause the bacterial colonies to disintegrate probably results from their interference with the bacterial cell wall synthesis, thereby inhibiting the microbial growth (Doss *et al*., 2009).

Table 1: Antibacterial activity of the leaf extracts of *Acacia nilotica* (L.) Delile

Extracts	**Conc.**	**Zone of Inhibition (mm)**					
	(mg/ml)	*B.subtilis*	*S.aureus*	*E.coli*	*P.vulgaris*	*K.pneumoniae*	*P.aeruginosa*
Methanol	250	10	-	12	10	15	13
	500	11	-	15	12	18	16
Aqueous	250	-	-	11	11	12	12
	500	-	-	13	14	16	14
Standard (Ciprofloxacin)		28	29	30	22	20	27

Table 2: Minimum Inhibitory Concentration of the leaf extracts of *Acacia nilotica* (L.) Delile

Extracts	Minimum inhibitory concentration (mg/ml)					
	B.subtilis	***S.aureus***	***E.coli***	***P.vulgaris***	***K.pneumoniae***	***P.aeruginosa***
Methanol	0.500	-	2.0	0.500	0.125	0.125
Aqueous	2.0	-	2.0	1.0	0.250	0.500

Table 3: Preliminary phytochemical screening of the leaf extracts of *Acacia nilotica* (L.) Delile

S.No.	Phytochemicals	Tests	Results
1	Alkaloids	Dragendroff's test	-
		Hager's test	-
		Wagner's test	-
		Mayer's test	-
2	Glycosides	Baljet test	+
		Legal test	+
3	Carbohydrates	Molisch's	+
		Fehling's	+
		Benedict's	+
		Barford's	+
4	Saponin	Foam test	-
		Blood Haemolysis test	-
5	Steroids	Libermann Burcherd test	-
6	Tannins	Ferric chloride test	+
7	Proteins	Biurette test	-

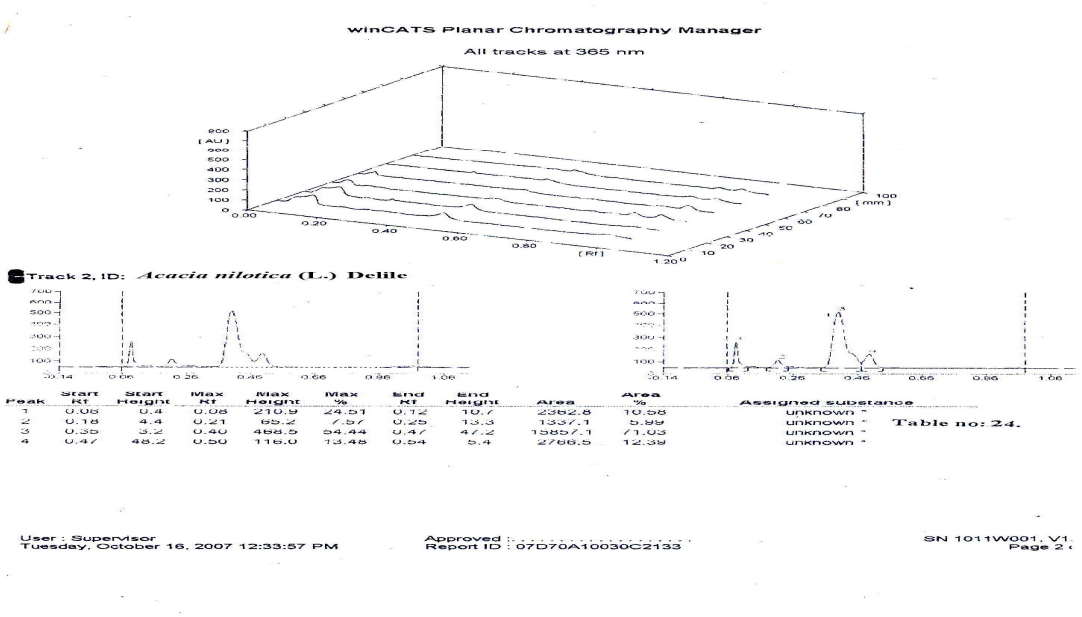

Figure 1: HPTLC fingerprint profile of ethyl acetate extract of *Acacia nilotica* (L.) Delile

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