

# Fingerprinting of the *Acacia nilotica* (L.) Bark Extract Having Antibacterial Property

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**Abstract:** *Acacia nilotica* (L.) Delile, commonly known as “Babul”, is reported for its medicinal property since ages. Plant is recognized for its medicinal use in traditional practices. For the study, phytochemicals were extracted as well as identified from four different solvents and their antibacterial property was determined. The fingerprinting was accomplished using HPTLC and the MIC (Minimum Inhibitory Concentration) was elucidated using standard protocols. Two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria were selected for the antibacterial screening. The major phytochemicals identified in the extract of the bark of *A. nilotica* were catechin/epicatechin, ellagic acid and m-digallic acid. Excellent antimicrobial activity was found in chloroform (40 µg/mL) and methanol (60 µg/mL) extracts against *Pseudomonas aeruginosa*. The chloroform extracts consist of higher number of phytochemicals/bands as compared with other extracts and demonstrated significant antibacterial activity against all the test bacterial species.

**Key words:** *Acacia nilotica*, antimicrobial screening, fingerprinting, bacteria, HPTLC.

## 1. Introduction

The plant, *Acacia nilotica*, belongs to the Mimosaceae family. It is commonly known as “Babul” and is a native of India but is also found in western Asia and Africa. It was introduced to Australia-Northern Territory, South Australia, and Queensland in the late 1800s. Different parts of the *A. nilotica*, such as the bark, gum, leaves, and pods are used in different parts of world for their medicinal properties. *A. nilotica* bark extract is used in curing various ailments such as cancer, cold, congestion, cough, diarrhoea, dysentery, fever, hemorrhage, hemorrhoids, leucorrhoea, ophthalmia, sclerosis, smallpox, asthma, bronchitis, diabetes, dysentery, diarrhea, skin diseases and tuberculosis [1, 2]. A series of experiments has been

conducted earlier to evaluate the medicinal properties of plants in India [4], northeast México [5], South Africa [6-8], Cameroon [9]. Zampini and coworkers [14] have studied aqueous extracts of some plants against multi drug resistance bacteria and found good activity of many plants.

SRISTI (Society for Research and Initiative for Sustainable Technologies and Institutions, www.sristi.org) has scouted various practices since 1993 and maintains more than 20,000 examples of grassroots innovations and traditional knowledge practices in its data base. To validate the claims made by the traditional healers (as mentioned in Table 1) about the use of *A. nilotica* L., an initiative was undertaken to determine the growth inhibition effect of various extracts from the bark of *A. nilotica* against two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram negative

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**Table 1** List of traditional knowledge holders as per SRISTI data base (www.sristi.org) using bark of *Acacia nilotica* for the cure of their cattle.

Sr. no	Name of knowledge provider	Bark used for
1	Baldevbhai Okabhai Desai, Mehsana; Prakashbhai Kevaldas Makwana, Dahod; Gokalbhai Amarabhai Rabari, Surendranagar; Veljibhai Surjibhai Asari, Sabarkantha; Babubhai Bhayalbhai Patel, Valsad; Maansing Gavjibhai Garasiya, Dahod; Chithrabhai Ukabhai Dihora, Bhavnagar; Khodabhai Vajabhai Bharvad, Surendranagar; Thiru Perisaminy, Thullukkankulam.	Diarrhoea in animal
2	Lubhabhai Dulabhai Hathiya, Bhavnagar; Raysing Arjanbhai Padhiyar, Junagadh; Gokulbhai Rambhai Rabari, Surendranagar.	Wound in Animal
3	Lubhabhai Dulabhai Hathiya, Mahuva, Bhavnagar	Abscess wound
4	Chamnbhai Kesaribhai Chauhan, Banaskantha; Hirabhai Narayanbhai Parmar, Ahmedabad; Hirabhai Meghabhai Rajput, Bhavnagar.	Maggot wound

(*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria.

In the study, phytochemicals catechin/epicatechin, ellagic acid, and m-digallic acid in the bark extract (organic and aqueous) of *A. nilotica* were identified using HPTLC. The authors' studies strengthen the claims of grass roots traditional knowledge.

## 2. Materials and Methods

### 2.1 Collection and Extraction of Plant Material

The bark of *A. nilotica* was collected from its natural habitat (semi arid regions of India), dried under shade and finally powdered using domestic grinders. The plant was identified and verified by the taxonomist at the Botanical Survey of India, Arid Zone Circle, Jodhpur (India). Aqueous extract and organic extracts were prepared using standard protocols [10] and extracts were further diluted with DMSO (dimethylsulphoxide). Amikacin, tetracycline and cefepime at concentrations 30 µg/mL were used as positive control; whereas DMSO was used as a negative control in these experiments [11].

### 2.2 Phytochemical Analysis

Quantitative and qualitative analysis were performed using HPTLC system (Camag, Muttenz, Switzerland) consisting of (1) TLC scanner connected to a PC running WinCATS software under MS Windows NT; (2) Linomat V Sample applicator; and (3) Photo documentation system Camag, Reprostar III as described by Patel and Kumar (2008) [12]. The chromatographic estimation was performed by spotting

the extracts (6 mm band) on a precoated silica gel 60 F254 aluminum TLC plate (10 cm × 20 cm). Extracts (volume-2 µL) were applied to the TLC plate. Solvent system consisted of Toluene:Ethyl acetate:Diethylamine:Methanol:Chloroform in the ratio of 10:6:2:2:1 v/v. After spotting the plate; it was subjected to linear ascending development up to a distance of about 95 mm in the solvent system in Camag, Solvent system consisted was different for all plants. Densitometric scanning was carried out using Camag TLC Scanner III in the absorbance mode of wavelength 200-450 nm, with a slit dimension of 6.00 × 0.30 mm, micro and scanning speed of 20 mm/s, data resolution 100 µm/step. After the scanning; images of the plate were taken at three different wavelengths of light (254 nm by UV lamp, 366 nm by mercuric lamp, 400-800 nm by white lamp) with the help of Photo documentation system Camag, Reprostar III [13].

### 2.3 Antimicrobial Screening

The standard strains used were *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741) and *Bacillus subtilis* (MTCC 441). A standard inoculum 0.1 mL volume ( $10^6$ - $10^7$  CFU) of the test bacterial strain was spread on MHA (Mueller Hinton Agar) with a sterile glass spreader and allowed to dry. Then, 6 mm-diameter wells were bored in the MHA. Plant extracts were introduced into each well and allowed to stand for 1 h at room temperature to diffuse before incubation at 37 °C for 24 h. The IZD (Zone of Inhibition) was measured by the antibiotic zone reader

to the nearest mm [11]. The MIC (Minimum Inhibitory Concentration) was determined using the agar diffusion by plotting the graph of  $IZD^2$  ( $mm^2$ ) against the log of concentration and point of intersection on the x-axis was recorded [14]

### 3. Results and Discussion

#### 3.1 Phytochemical Analysis

Bark of *A. nilotica* was extracted in water and three organic solvents. Data (Table 2) shows that the minimum extract yield was found in petroleum ether (0.002%) whereas maximum yield was found in water (33.28%). The literature survey showed that major phytoconstituents found in the plant were: l-arabinose, catechol, galactan, galactarabin, galactose, N-acetyldjenkolic acid, sulphoxides pentosan, saponin, tannin and sugar, whereas the bark specifically contains epigallocatechin, catechol, pyragallo, m-Digallic acid, gallic acid, and ellagic acid [15, 16]. The TLC scanning of all four extracts (methanol, chloroform, petroleum ether, and water) of the bark of *A. nilotica* is shown in Fig. 1. The authors' phytochemical analysis of the *A. nilotica* bark extract displayed presence of three known compounds; catechin/epicatechin, ellagic acid, and m-digallic acid based on their  $R_f$  values. The  $R_f$  value of catechin is 0.10, ellagic acid is 0.45, m-digallic acid is 0.55 and there was no overlap with any other component in the analyzed sample in the range between 200-450 nm. Chromatogram analysis showed that chloroform

extract contains more numbers of phytochemicals; whereas petroleum ether extract contains only two compounds; m-digallic acid and catechin. In methanol extract m-digallic acid, ellagic acid and catechin were found. It is necessary to purify the compounds present in all the extracts for further studying their anti-bacterial properties.

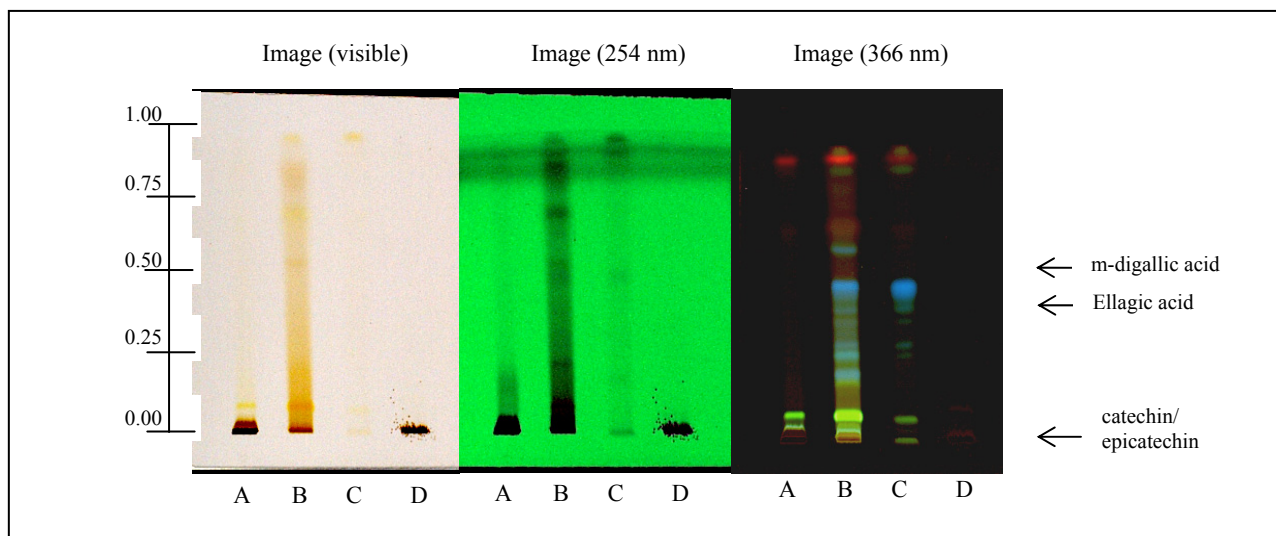
#### 3.2 Antimicrobial Activity

The results (Table 2) indicate that maximum inhibition was observed in chloroform extract against *P. aeruginosa* (MIC: 40  $\mu g/mL$ ), followed by methanol extract against the same bacterial species (MIC: 60  $\mu g/mL$ ). Methanol extract against *E. coli* and petroleum ether extract against *P. aeruginosa* displayed very good inhibition (MIC: 70 $\mu g/mL$ ). Other extracts also displayed significant antibacterial activity against screened pathogens except for methanol extract against *B. subtilis* and water extract against *P. aeruginosa*. These results are similar to the findings reported by Bagchi and colleagues [17]. Their group had reported that extracts of *A. nilotica* effectively inhibited growth of *E. coli* and *P. aeruginosa* but did not affect the growth of *B. subtilis*. Srinivasan and his colleagues [18] had earlier reported that aqueous extract of *A. nilotica* was effective against only *B. subtilis* but ineffective against other three bacterial species (*Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*). The authors' results are contrary to their results in which water extracts was found effective against all the

**Table 2** Antimicrobial activity of bark of *Acacia nilotica* extracts.

Extracts name and control (% yield of extract)	Zone of inhibition (mm) at concentration 1 mg/ml excluding disc (well) region			
	<i>E. coli</i> *	<i>S. aureus</i> *	<i>P. aeruginosa</i> *	<i>B. subtilis</i> *
Petroleum ether extract (0.002)	4.07 ± 0.04 (140)	0.53 ± 0.06 (1080)	6.1 ± 0.06 (70)	2.42 ± 0.2 (100)
Chloroform extract (1.01)	3.06 ± 0.24 (200)	3.7 ± 0.06 (120)	7.44 ± 0.16 (40)	3.22 ± 0.02 (150)
Methanol extract (4.55)	4.85 ± 0.76 (70)	3.7 ± 0.05 (490)	6.79 ± 0.4 (60)	--
Water extract (33.28)	2.85 ± 0.85 (120)	4.52 ± 0.82 (110)	--	1.45 ± 0.95 (390)
Tetracycline (30 $\mu g/mL$ )	16.9 ± 0.04	4.02 ± 0.02	17.9 ± 0.06	23.06 ± 0.02
Amikacin (30 $\mu g/mL$ )	18.1 ± 0.02	8.1 ± 0.01	10.0 ± 0.02	16.1 ± 0.02
Cefepime (30 $\mu g/mL$ )	6 ± 0.04	0.45 ± 0.22	19.6 ± 0.05	14.03 ± 0.04
Dimethylsulfoxide	--	--	--	--

--: No growth inhibition. \*: Minimum inhibitory concentration (MIC; in  $\mu g/ml$ ) is given in parenthesis.



**Fig. 1** Chromatograms obtained from different extracts of *Acacia nilotica*.

Four extracts (aqueous and organic solvent) were spotted. Spot abbreviations are described below with their application volume. A = Methanol extract 2  $\mu$ l, B = Chloroform extract 2  $\mu$ l, C = Petroleum ether extract 2  $\mu$ l and D = Water extracts 2 $\mu$ l. Catechin/Epicatechin is visible at  $R_f$  value of-0.10; Ellagic acid at-0.45 and m-digallic acid at-0.55.

above mentioned bacterial species except *Ps. Aeruginosa*. Deeni and Sadiq reported that methanol extract was found inhibitory to *B. subtilis*, *Ps. aeruginosa* and *S. aureus* but not effective towards *E. coli*; whereas in studies methanol extract can inhibit the growth of *E. coli*, *S. aureus*, and *Ps. aeruginosa*, whereas *B. subtilis* is resistant to the methanol extract [19].

The present study has highlighted the bark extract of *A. nilotica* to control pathogenic bacteria grown based on the basic information of grass roots traditional knowledge as per Table 1. Results clearly indicate the antibacterial activity of the aqueous and organic extract of *A. nilotica*. The study will help to develop potential herbal medicine for infections such as extra intestinal infections, skin diseases, acute food poisons and post surgery infections commonly caused by *E. coli*, *S. aureus*, *B. subtilis*, and *Ps. aeruginosa*. The food and medicines of the indigenous people mostly constituted of native plants, which may have helped them to combat many microbial diseases. The current study supports this hypothesis.

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