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Phytochemical and Antimicrobial Analysis of *Acacia nilotica* Bark Extract

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Abstract: *Acacia nilotica*, a traditionally valued medicinal plant, was subjected to phytochemical screening and antimicrobial evaluation to explore its bioactive potential. Various solvent extracts, including ethyl acetate, methanol, ethanol, and aqueous, were analyzed for the presence of secondary metabolites. The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, glycosides, saponins, phenols, and steroids, with methanol and aqueous extracts showing the most diverse profile. Antibacterial activity of the crude bark extract was assessed against clinically significant pathogens such as *Staphylococcus aureus*, *S. mutans, and C albicans*. Activity decreased with lower concentrations, indicating a dose-dependent effect. The results suggest that *Acacia nilotica* possesses potent phytochemicals with significant antimicrobial properties, supporting its traditional use and potential for development into therapeutic agents.

Keywords: Antimicrobial, phytochemicals, *Acacia nilotica*, C. *albicans, S. aureus* and *S. mutans.*

INTRODUCTION

Dental caries is one of the oldest known human illnesses, with archaeological evidence indicating its presence dating back to between 12,000 and 3,000 BC (Ferreira Zandoná, *et al*., 2012). Records from back to 5000 BC mention a "tooth worm" as the thought cause of dental caries in ancient civilizations, including India, Egypt, Japan, and China (Santosh and Ogle 2017). In ancient China, individuals developed several traditional methods for dental caries prevention. For example, arsenic trioxide was used to relieve tooth pain, a practice that persisted well into modern society (Siderov and Duggan 2010). Dental caries, also referred to as “tooth decay,” is the most widespread chronic infectious disease in the oral cavity (Peres, *et al*., 2019). Dental caries is the predominant cause of tooth loss or tooth decay in children and young adults, and the key factor contributing to root decay in the old people. According to data from the World Health Organization (WHO), its prevalence ranges from 60–80% in children and nearly 100% in adults (Petersen, *et al*., 2005). The oral cavity delivers a different ecological environment for microorganisms, also known as oral biofilm, several of which colonize dental surfaces to form dental plaque. Carcinogenic bacteria, which ferment carbohydrates to produce acids, play an important role in the growth of dental caries by causing demineralization of tooth surfaces (Beighton, 2009; Lippert, 2012). *Streptococcus mutans, Candida albicans*, Thomas, *et al*., (2016), and *Staphylococcus aureus*, Smith, *et al*., (2003), and some other anaerobic bacteria are examined to be the main cariogenic agents involved in the development of dental caries (Thomas, *et al*., 2016).

Acacia nilotica is a genus of shrubs and trees that belongs to the subfamily Mimosoideae of the family Fabaceae or Leguminosae. It is also known as ‘Babul’ or ‘Kikar’, this moderately sized tree with a dispersal crown is broadly spread across tropical and subtropical regions. It is native to the Indian subcontinent, as well as tropical Africa, Sri Lanka, Burma, Saudi Arabia, Egypt, and both West and East Sudan. In India, natural babul forests are usually found in states such as Maharashtra, Gujarat, Rajasthan, Andhra Pradesh, Karnataka, and Haryana (Singh, *et al*., 2013).

Hence, the main aim of the present research is to identify the phytochemical analysis or antimicrobial activity of *Acacia nilotica* bark against *Streptococcus mutans and Staphylococcus aureus.*

MATERIALS AND METHODS

Collection of plant material and identification

The bark of *Acacia nilotica* has an accession number 1671, was identified from BSI, Dehradun. The bark of the plant was collected from Bahadrabad (29.8685°N, 78.0281°E), Haridwar. The experiments were carried out in Department of Botany Meerut college Meerut.

Extraction of the bark material

The fresh bark of *Acacia nilotica* was accurately collected, washed with water, air-dried, and then ground into a coarse powder. Around 150 grams of bark powder were placed in a Soxhlet apparatus for extraction utilizing 1500 ml of ethyl acetate, ethanol, methanol, and water separately. The solvents were subjected to heating, resulting in vaporization and following condensation, which smoothed the collection of the extract back into the

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flask of the Soxhlet apparatus. The extract was then concentrated by evaporation at 70°C and dried (Chaudhary, *et al*., 2023).

The percentage plant yield of the bark extract was calculated using the formula:

$$Percentage\ yeild = \frac{Weight\ of\ the\ extract}{Weight\ of\ the\ sample} \times 100$$

Test microorganism

The tested culture of *Staphylococcus aureus* MTCC-744 and *Candida albicans*-227 was obtained from the stock samples preserved at the Department of Botany & Microbiology, Gurukula Kangri (Deemed to be University); Haridwar, and *Streptococcus mutans* MTCC-497 was obtained from Chandigarh.

Qualitative phytochemical analysis of *Acacia nilotica* bark

Tannins – The 2 ml of the bark extract was occupied in a test tube, and a few drops of 1% ferric chloride solution were added to it. Formation of the Blue-green precipitate confirmed the presence of tannins (Ujah, *et al*., 2021).

Alkaloids

Dragendroff's reagent test: 0.5 g of the bark extract was dissolved in 1% hydrochloric acid and filtered through filter paper; 2 ml of the filtrate was treated with Dragendorff's reagent. The formation of the red precipitate confirmed the presence of the alkaloids (Ujah, *et al*., 2021).

Phenol

Lead acetate test: The 10 mg bark extract was tested with a few drops of lead acetate solution. The yellow coloured precipitate confirmed the presence of phenol (Santhi and Sengottuvel, 2016).

Test for saponin- 0.5 mg of bark extract was shaken with 5 ml of distilled water. The frothing formation (appearance of creamy small bubbles) indicated the presence of saponins (Santhi and Sengottuvel, 2016).

Test for glycosides- 5 ml of bark extract, 0.3 ml of Fehling's A and B solutions were added to the solution until it turns red colour, indicating the presence of glycoside (Ujah, *et al*., 2021).

Amino acid- 1 ml of bark extract was placed in a test tube, and some drops of Ninhydrin were added to it. The purple colour formation indicates the presence of amino acids (Al-Hashemi, *et al*., 2016).

Steroid- 2 ml of the bark extract was taken in the test tube and dissolved in 2 ml of chloroform, and 2 ml of concentrated sulfuric acid was added to it. A red colour was formed, indicating the presence of steroids (Raphael, 2012).

Flavonoids-

Pew's test: 5 ml of bark extract of *Acacia nilotica* was dissolved with 0.1 g of metallic zinc and 8 ml of concentrated sulphuric acid. A red colour formation indicates the presence of flavonoids (Santhi and Sengottuvel, 2016).

Coumarin- 3 ml of 10% NaOH was added to a bark extract, and a yellow colour was observed. This indicates the presence of coumarins (Le Thi, *et al*., 2021).

Antibacterial activity of plant extract

The antimicrobial activity of the plant extract was determined by agar well diffusion method of different solvents of bark extracts, as displayed by (Daoud, *et al*., 2015). Nutrient broth was used to culture bacteria, as well as a fresh overnight culture of inoculum. The test cultures were spread on Mueller–Hinton agar (MHA) plates and permitted to dry for 10 min. Wells of 6 mm diameter were punctured and filled with 50µl (100 mg/ml, 125 mg/ml, and 250 mg/ml) of herbal extracts. DMSO was used as a negative control, and standardised Streptomycin was used as a positive control. The culture plates were incubated for 24 hours in an incubator at 37ºC. The diameters of the zone of inhibition were measured in millimetres (mm).

RESULTS

Percentage yield of the bark extract in different solvents

Table 1 presents the extract percentage yield for *Acacia nilotica* using various solvents. Ethanol provided the highest yield at 16.60%, producing 30 grams of extract. Ethyl acetate followed with a yield of 10.00%, yielding 20 grams of extract. Methanol resulted in a 15.40% yield, extracting 23.70 grams of plant material, while water produced the lowest yield at 3.60%, with 7 grams of extract. According to Singh (2016), the percentage yield of extracts obtained from different solvents. Methanol obtained 20.93%, yielded the highest yield of ethanol at 19.41%, and butanol at 17.02%. In contrast, hot extraction significantly increased the yield, with methanol again producing the highest yield at 48.49%, followed by ethanol at 35.65% and butanol at 26.78%. The percentage yield of the *Acacia nilotica* extracts obtained using different solvents

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from 200 grams of plant material. Among the solvents used, ethanol produced the highest yield at 8.16%, followed closely by water at 8.05%. Chloroform yielded 6.52%, benzene gave 6.42%, while petroleum ether showed the lowest yield at 5.16%. These results indicate that ethanol and water are more effective solvents for extracting a higher quantity of phytoconstituents compared to the others (Shakya, *et al*., 2012).

Table 1: Extract percentage yield of *Acacia nilotica*

S. No.	Solvent	Weight of plant extract in grams	% yield
1.	Ethyl acetate	20.00	10.00
2.	Ethanol	30.00	16.60
3.	Methanol	23.70	15.40
4.	Water	07.00	03.60

Phytochemical Analysis of Bark Extract

Table 2 presents the results of the qualitative phytochemical analysis of *Acacia nilotica* using different solvents. Tannins and phenols were detected in only ethanol and methanol solvents. Alkaloids were found in ethanol and water, while glycosides were only present in methanol. Saponins were identified in ethanol, methanol, and water. Steroids were detected in ethyl acetate, ethanol, methanol, and water, while amino acids were found only in ethanol. Coumarin and flavonoids were absent in most solvents, with flavonoids detected in both ethyl acetate and ethanol. Phytochemical screening showed alkaloids in methanol and aqueous extracts, flavonoids in all extracts with the highest in methanol, glycosides consistently present across all, tannins abundant in ethanol and aqueous, terpenoids slightly present in all, saponins absent in methanol but strong in ethanol and aqueous, and steroids moderately in methanol and weakly in others (Byakod 2023). The phytochemical screening of different solvents shows diverse profiles of bioactive compounds. Methanol and aqueous extracts showed the broadest range of phytochemicals, with both containing tannins, steroids, saponins, glycosides, phenols, and flavonoids, while methanol alone showed the presence of alkaloids and aqueous extract uniquely contained terpenoids. Chloroform extract revealed the presence of tannins, steroids, and glycosides, but lacked other major phytochemicals. The n-hexane extract contained only steroids, while the petroleum ether extract showed no detectable presence of any tested phytochemicals. These results highlight methanol and aqueous solutions as the most effective solvents for extracting a wide range of phytoconstituents (Attahiru , *et al*., 2024).

Table 2: Phytochemical analysis of the plant extract

S. No.	Phytochemicals	Solvents			
		Ethyl acetate	**Ethanol**	**Methanol**	**Dist. Water**
1.	Tannins	-	+	+	-
2.	Alkaloids	-	+	-	+
3.	Phenol	-	-	-	-
4.	Glycosides	-	-	+	-
5.	Sponins	-	+	+	+
6.	Steroid	+	+	+	+
7.	Amino acid	-	+	-	-
8.	Coumarin	-	-	-	-
9.	Flavanoids	+	+	-	-

Antimicrobial activity of *Acacia nilotica* bark against *S. mutans*, *S. aureus*, and *C. albicans*

Table 3 outlines the results of zone of inhibition tests for extracts of *Acacia nilotica* against *Staphylococcus aureus* and *Streptococcus mutans*, using ethyl acetate, ethanol, methanol, and distilled water as solvents at concentrations of 250 mg/ml, 150 mg/ml, and 100 mg/ml. For *S. aureus*, the ethyl acetate extract demonstrated the highest zone of inhibition, ranging from 16.46 ± 0.32 to 20.00 ± 0.00 mm, while ethanol and methanol extracts demonstrated lower activity. Distilled water extract did not shows significant inhibition. Similarly, for *S. mutans*, the ethyl acetate extract showed the largest inhibition zones, ranging from 16.26 ± 0.77 to 21.73 ± 0.30mm, while the ethanol and methanol extracts showed slightly lower activity. Distilled water extracts again showed no significant inhibition. Whereas the *C. albicans* ethyl acetate extract demonstrated the highest zone of inhibition, ranging from 14.26 ± 0.77 to 18.73 ± 0.00. Distilled water extracts also demonstrated the

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antifungal activity against C. albicans, ranging from 6.33 ± 0.22 to 10.22 ± 0.30 mm. The extracts of *Acacia nilotica* demonstrated antimicrobial activity with zones of inhibition ranging from 6 mm to 22 mm, showing significant effectiveness against all clinically relevant bacterial and fungal species. The highest inhibition zone (22 mm) was observed with the methanol and chloroform (75:25) extract against *Bacillus subtilis*, while the lowest (4 mm) was recorded with the methanol and chloroform (50:50) extract against *Drechslera avenacea* (Kaur, *et al*., 2016). The crude extract of *Acacia nilotica* demonstrated significant antibacterial activity against various bacterial strains, with zones of inhibition increasing with extract concentration. The highest activity was observed against *Staphylococcus aureus* at 1000 mg/ml, showing a zone of 32.00 ± 0.00 mm, followed by *Pseudomonas aeruginosa* (27.00 ± 0.00 mm), *Bacillus subtilis* (26.00 ± 0.00 mm), and *Escherichia coli* (25.67 ± 0.58 mm). As the concentration decreased, the zones of inhibition gradually reduced across all strains. At the lowest concentration (50 mg/ml), *S. aureus* still showed notable sensitivity (22.33 ± 0.58 mm), while other strains exhibited reduced zones. The positive control (TCF) recorded the highest zone of inhibition (40.00 mm for *P. aeruginosa*), while DMSO (negative control) showed no activity, confirming the antimicrobial potential of the plant extract (Jodi, *et al*., 2020).

Table 3: Zone of inhibition *Acacia nilotica*

Microorganisms	Extract conc. (mg/ml)	Diameter of the Zone of Inhibition (mm)			
		Ethyl acetate	Ethanol	Methanol	Dist. water
S. aureus	**250**	20.00 ± 0.00	15.56 ± 0.49	NA	NA
	150	18.63 ± 0.32	13.76 ± 0.75	NA	NA
	100	16.46 ± 0.32	11.43 ± 0.37	NA	NA
S. mutans	**250**	21.73 ± 0.30	16.66 ± 0.11	15.96 ± 0.20	NA
	150	19.66 ± 0.57	15.13 ± 0.73	13.43 ± 0.15	NA
	100	16.26 ± 0.77	12.36 ± 0.35	10.33 ± 0.15	NA
C. albicans	**250**	18.73 ± 0.00	12.23 ± 0.63	NA	10.22 ± 0.30
	150	16.63 ± 0.57	10.46 ± 0.33	NA	8.43 ± 0.35
	100	14.26 ± 0.77	8.13 ± 0.57	NA	6.33 ± 0.22

CONCLUSION

These findings align with the traditional knowledge of local communities and provide preliminary scientific validation for the antimicrobial use of *Acacia nilotica* bark, highlighting the significance of their conservation and sustainable use. The study advises that the extract of neem leaves contains several active compounds capable of controlling the pathogens.

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