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Original article

Phytochemical Analysis, Antimicrobial, Antioxidant, Anti-Inflammatory, and Cytotoxic Properties of *Acacia nilotica* Leaf Extract

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Abstract
Acacia nilotica is a medicinal plant widely used in traditional medicine. This study evaluated the phytochemical composition and antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of the ethanolic leaf extract. Leaves were collected from Khartoum, Sudan, authenticated, dried, and extracted with 75% ethanol using maceration. Phytochemical screening was performed using standard test methods. The total phenolic content (TPC) and total tannin content (TTC) were determined spectrophotometrically. Antibacterial activity was tested against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* using the agar well diffusion method. Antioxidant activity was assessed via DPPH radical scavenging, anti-inflammatory activity via egg albumin denaturation inhibition, and cytotoxicity via the brine shrimp lethality assay. The extract contained alkaloids, flavonoids, tannins, terpenoids, coumarins, and sterols. TPC was 169.62 ± 0.06 mg GAE/g, and TTC was 63.85 ± 0.05 mg TAE/100 g. The extract exhibited moderate antibacterial activity, with inhibition zones ranging from 13.0 to 17.0 mm. Antioxidant IC_{50} was 13.7 µg/mL, and anti-inflammatory IC_{50} was 280.7 µg/mL. The cytotoxicity against *Artemia salina* showed an LD_{50} of 183.3 µg/mL, indicating high toxicity. These findings suggest that *A. nilotica* leaf extract possesses promising bioactive properties, supporting its traditional medicinal use.
Keywords. *Acacia nilotica*, Phytochemicals, Antimicrobial, Antioxidant, Anti-inflammatory, Cytotoxicity.

Introduction

Medicinal plants have been a cornerstone of human healthcare for thousands of years, serving as the primary sources of therapeutic agents across diverse cultures [1]. The World Health Organization estimates that approximately 80% of the global population, particularly in developing countries, relies on traditional medicine for primary healthcare needs [2]. In recent decades, there has been a resurgence of interest in medicinal plants owing to growing concerns regarding the side effects of synthetic drugs, the emergence of multidrug-resistant pathogens, and the need for more affordable therapeutic alternatives [3]. Sudan, with its unique geographical location and diverse climatic zones, harbors a rich flora of medicinal plants, many of which remain scientifically underexplored [4].

Among these, *Acacia nilotica* (L.) Delile (family Fabaceae), commonly known as "garad" or "qarad" in Sudan and "babul" in India, is a multipurpose tree widely distributed across the tropical and subtropical regions of Africa, Asia, and the Arabian Peninsula [5]. The plant thrives in arid and semi-arid environments and is well adapted to the climatic conditions of central and eastern Sudan [6]. Traditionally, various parts of *A. nilotica* ,including leaves, pods, bark, and gum ,have been employed in folk medicine to treat a wide spectrum of ailments. These include respiratory infections, gastrointestinal disorders (diarrhea, dysentery), wound infections, sore throat, diabetes, and inflammatory conditions such as rheumatism and arthritis [7,8]. Additionally, the plant has been used in dental care as a toothbrush stick (miswak) and in livestock management as an anthelmintic agent [9].

Phytochemical investigations have revealed that *A. nilotica* is a rich source of secondary metabolites, particularly phenolic compounds, flavonoids, tannins, alkaloids, saponins, sterols, and terpenoids [10,11]. Among these, tannins and flavonoids are considered the major bioactive constituents responsible for the astringent, antimicrobial, antioxidant, and anti-inflammatory properties of plants [12,13]. In particular, the high tannin content has been linked to the traditional use of this plant in treating diarrhea and as a wound-healing agent [14].

Numerous pharmacological studies have been conducted on A. nilotica extracts from different geographical regions. The antimicrobial activity of various bacterial and fungal pathogens has been documented [9,15]. The antioxidant potential of the plant has been demonstrated using several in vitro

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models, including DPPH, ABTS, and FRAP assays [7,13]. Anti-inflammatory and analgesic effects have been reported in animal models [8]. Furthermore, cytotoxic and antiproliferative activities against several cancer cell lines have been observed, suggesting potential anticancer properties of these compounds [11]. However, the chemical composition and biological activities of medicinal plants can vary significantly depending on several factors, including geographical origin, climatic conditions, soil type, time of harvest, and extraction methods [5]. Therefore, findings from studies conducted on *A. nilotica* in other regions (e.g., India, Pakistan, Nigeria, and Egypt) cannot be directly extrapolated to the Sudanese variety without verification [6].

To the best of our knowledge, a comprehensive phytochemical and multi-bioactivity profiling of *A. nilotica* leaves collected from Khartoum, Sudan, has not been adequately documented. Most published studies on Sudanese Acacia species have focused on gum arabic (*Acacia senegal*) rather than A. nilotica leaves [4]. This represents a significant research gap, as locally sourced medicinal plants require scientific validation to support their traditional use and identify potential lead compounds for drug discovery.

Given this context, the present study was designed to achieve the following objectives: (1) to perform qualitative phytochemical screening of the ethanolic leaf extract of *A. nilotica* collected from Khartoum, Sudan; (2) to quantitatively determine its total phenolic and total tannin contents; (3) to evaluate its antibacterial activity against clinically relevant bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*; (4) to assess its antioxidant potential using the DPPH radical scavenging assay; (5) to determine its anti-inflammatory activity using the egg albumin denaturation inhibition method; and (6) to evaluate its cytotoxic profile using the brine shrimp (*Artemia salina*) lethality bioassay.

Materials and methods

Plant Collection and Authentication

Fresh leaves of *Acacia nilotica* (Figure 1) were collected from Khartoum, Sudan, between February and April 2022. The plant material was identified and authenticated by Dr. Mubarak Siddig, a taxonomist at the Herbarium of the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), National Centre for Research, Khartoum, Sudan. A voucher specimen (No. MAPTMRI-2022-045) was deposited in a herbarium for future reference.

Figure 1. Photograph of an Acacia nilotica plant.

Preparation of Ethanolic Extract

The collected leaves were shade-dried for one week and then ground into a fine powder. Extraction was performed using the overnight maceration method described by Harborne [16]. Briefly, 100 g of dried powder was soaked in 1000 mL of 75% ethanol for 72 h at room temperature, with intermittent stirring every 24 h. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 55°C. The resulting extract was dried in a fume hood. The extraction yield was calculated as follows:

$$\text{Yield (\%)} = (\text{Weight of extract} / \text{Weight of dry plant material}) \times 100$$

Phytochemical Analysis

Qualitative Screening

The ethanolic extract was subjected to standard qualitative tests for the identification of secondary metabolites, according to established protocols [16,17]. Alkaloids were detected using Mayer's test, flavonoids using the alkaline reagent test, tannins using ferric chloride solution, saponins using the froth

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test, sterols using the Liebermann-Burchard test, terpenoids using the Salkowski test, anthraquinones using Borntrager's test, and coumarins using the KOH test.

Quantitative Analysis

Total Phenolic Content (TPC)

The Folin-Ciocalteu method described by Singleton et al. [19] was used. A 0.5 mL aliquot of the extract (1 mg/mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10) and 2 mL of 7.5% sodium carbonate. After 90 min of incubation in the dark, the absorbance was measured at 765 nm using a Jasco V-630 UV-Vis spectrophotometer. The results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

Total Tannin Content (TTC)

Tannin content was determined using the Folin-Ciocalteu method after polyvinylpolypyrrolidone (PVPP) adsorption, as described by Julkunen-Tiitto [19]. The tannin concentration was calculated by subtracting non-tannin phenolics from total phenolics and expressed as milligrams of tannic acid equivalent per 100 g of dry extract (mg TAE/100 g).

Antimicrobial Activity

Test Microorganisms

The antibacterial activity was evaluated against one Gram-positive bacterium (*Staphylococcus aureus* ATCC 25923) and two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). All strains were obtained from the culture collection of the Department of Microbiology, MAPTMRI, and were subcultured on Mueller-Hinton agar (MHA) at 37°C for 18–24 h prior to testing.

Agar Well Diffusion Assay

Antimicrobial susceptibility was assessed using the agar well diffusion method, as outlined by Kavanagh [20], with minor modifications. Bacterial suspensions were adjusted to the 0.5 McFarland standard (~10^8 CFU/mL). One milliliter of each suspension was mixed with molten MHA (100 mL of molten MHA, and 20 mL portions were poured into sterile Petri dishes. After solidification, wells with a diameter of 6 mm were bored using a sterile cork borer. Each well received 100 µL of ethanolic extract solution (100 mg/mL in 10% DMSO). The negative controls received 10% DMSO, while the positive controls received azithromycin (standard antibiotic). The plates were left at room temperature for 30 min for pre-diffusion and then incubated at 37°C for 18–24 h. All tests were performed in triplicates. Inhibition zone diameters were measured using a digital caliper, and the results were recorded as mean ± standard deviation (SD).

Antioxidant Activity

The free radical scavenging activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method described by Brand-Williams et al. [21] with modifications. Different concentrations of the extract (10–100 µg/mL) were mixed with 0.1 mM DPPH in methanol. The mixture was incubated in the dark at 37°C for 30 min, and the absorbance was measured at 517 nm using a microplate reader. Gallic acid was used as the reference standard. The percentage of scavenging activity was calculated as follows:

$$\% \text{ Scavenging} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

The half-maximal inhibitory concentration (IC_{50}) was determined using nonlinear regression analysis.

Anti-inflammatory Activity

The anti-inflammatory effect was assessed using the albumin denaturation inhibition method described by Mizushima and Kobayashi [22]. The reaction mixture contained 200 µL of 1% w/v egg albumin, 1400 µL of phosphate-buffered saline (pH 6.4), and 400 µL of the extract at concentrations ranging from 62.5 to 1000 µg/mL. The mixture was incubated at 37°C for 15 min and then heated at 70°C for 5 min. After cooling, the turbidity was measured at 660 nm. Ibuprofen was used as a reference drug. The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Cytotoxicity Assessment

Cytotoxicity was evaluated using the brine shrimp (*Artemia salina*) lethality bioassay, as described by Meyer et al. [23]. *Artemia salina* cysts were hatched in artificial seawater under continuous aeration and light for 48 h. Ten mature nauplii were transferred into vials containing 5 mL of seawater at extract concentrations of 10, 100, and 1000 µg/mL. Etoposide was used as a positive control. After 24 h, the number of dead nauplii was recorded. The percentage mortality was calculated, and the median lethal concentration (LD_{50}) was determined using probit analysis. Toxicity classification followed McLaughlin et al. [24]: LD_{50} < 249 µg/mL = highly toxic; 250–499 µg/mL = moderately toxic; 500–1000 µg/mL = slightly toxic; >1000 µg/mL = non-toxic.

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Statistical Analysis

All experiments were performed in triplicate (n = 3), and the results are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 9.0.

Results

Extraction Yield and Phytochemical Composition

Maceration of 100 g of dried *A. nilotica* leaf powder in 75% ethanol yielded 25.4 g of crude extract, corresponding to an extraction yield of 25.4% (w/w). Qualitative phytochemical screening (Table 1) revealed the presence of alkaloids, flavonoids, tannins, terpenoids, coumarins, and sterols, while saponins and anthraquinones were absent in the extracts.

Table 1. Qualitative phytochemical analysis of A. nilotica leaf extracts.

Secondary Metabolite	Test used	Result
Alkaloids	Mayer's test	+ve
Anthraquinones	Borntrager's test	-ve
Coumarins	KOH test	+ve
Flavonoids	Alkaline reagent test	+ve
Saponins	Froth test	-ve
Sterols	Liebermann-Burchard test	+ve
Tannins	Ferric chloride test	+ve
Terpenoids	Salkowski test	+ve

Key: +ve = present; -ve = absent.

Quantitative analysis (Table 2) revealed a total phenolic content of 169.62 ± 0.06 mg GAE/g dry extract and a total tannin content of 63.85 ± 0.05 mg TAE/100 g.

Table 2. Quantitative phytochemical content of A. nilotica leaf extract.

Scientific Name	Phenolic (mg GAE/g)	Tannin (mg/100 g)
A. nilotica	169.62 ± 0.06	63.85 ± 0.05

Antibacterial Activity

The ethanolic extract of *A. nilotica* (100 mg/mL) exhibited moderate antibacterial activity against the pathogens tested. The largest inhibition zone was observed against *S. aureus* (17.0 ± 0.05 mm), followed by *E. coli* (16.0 ± 0.08 mm) and *P. aeruginosa* (13.0 ± 0.01 mm). The standard antibiotic, azithromycin, produced larger inhibition zones, ranging from 22.0 to 25.0 mm (Table 3).

Table 3. Antibacterial activity of A. nilotica ethanolic extract (100 mg/mL).

Bacterial Strain	*A. nilotica*	Azithromycin
	Zone of Inhibition (mm, mean ± SD)	
S. aureus	17.0 ± 0.05	25.0 ± 0.00
E. coli	16.0 ± 0.08	22.0 ± 0.00
Ps. aeruginosa	13.0 ± 0.01	24.0 ± 0.00

Antioxidant Activity

The DPPH radical scavenging assay showed that the *A. nilotica* extract exhibited strong antioxidant activity. At a concentration of 100 µg/mL, the percentage of inhibition was 78.9 ± 0.02%. The calculated IC_{50} value was 13.7 µg/mL, which was slightly lower (indicating higher potency) than that of gallic acid (15.2 µg/mL) (Table 4).

Table 4. Antioxidant activity and IC_{50} of A. nilotica extract.

Scientific name	% Inhibition (Mean ± SD)	IC_{50} (µg/ml)
A. nilotica	78.9±0.02	13.7
Gallic acid (Reference)	74.0±0.00	15.2

Anti-inflammatory Activity

The extract inhibited heat-induced albumin denaturation in a concentration-dependent manner. At 500 µg/mL, the inhibition was 57.87 ± 0.01%, comparable to that of ibuprofen at the same concentration (56.03 ± 0.02%). The IC_{50} values were 280.7 µg/mL for the extract and 335.8 µg/mL for ibuprofen (Table 5).

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Table 5. Anti-inflammatory activity of A. nilotica extract.

Concentration (µg/mL)	*% Inhibition (Mean ± SD)*	*IC_{50} (µg/ml)*
125	38.37±0.01	280.7
250	48.86±0.05	
500	57.87±0.01	
Ibuprofen (500)	56.03±0.02	335.8

Cytotoxicity Effect

The extract induced concentration-dependent mortality in *Artemia salina* nauplii, with mortality rates of 20%, 40%, and 70% at 10, 100, and 1000 µg/mL, respectively. The calculated LD_{50} was 183.3 µg/mL, which is below 249 µg/mL, indicating high toxicity according to McLaughlin's classification. The positive control etoposide showed an LD_{50} of 7.463 µg/mL (highly toxic) (Table 6).

Table 6. Cytotoxicity of A. nilotica extract on Artemia salina.

Concentrations (µg/mL)	**Mortality Rate (%)**	**LD_{50} (µg/mL)**	**Toxicity Level**
10	20	183.3	Highly Toxic
100	40		
1000	70		
Etoposide (control)		7.463	Highly Toxic

Discussion

The present study investigated the phytochemical composition and multiple biological activities of an ethanolic leaf extract of *Acacia nilotica* collected from Sudan. The extraction yield (25.4%) was relatively high compared to previous reports on the same species, which may be attributed to differences in geographical origin, season of collection, and solvent polarity [25].

Qualitative phytochemical screening revealed the presence of several bioactive secondary metabolites, including alkaloids, flavonoids, tannins, saponins, sterols, terpenoids, and coumarins in the extracts. These findings are largely consistent with those of earlier studies on *A. nilotica* [9,10]. However, the presence of saponins in our extract differed from that in some reports where saponins were absent [26]. This variation could be due to differences in plant parts, extraction methods, or environmental factors. The absence of anthraquinones aligns with previous findings that this class is not characteristic of *A. nilotica* leaves [27].

The total phenolic content (169.62 mg GAE/g) was notably higher than that reported for *A. nilotica* from other regions, such as India (approximately 98–120 mg GAE/g) [28]. This suggests that Sudanese *A. nilotica* may be particularly rich in phenolic compounds, possibly owing to adaptive stress responses in arid climates [5]. Phenolics are well-known for their antioxidant and antimicrobial properties, which likely contribute to the observed effects [29].

The antibacterial assay demonstrated moderate activity against both Gram-positive and Gram-negative bacteria. The extract was most effective against *S. aureus* (17.0 mm), consistent with previous studies reporting the higher susceptibility of Gram-positive bacteria to plant extracts owing to the absence of an outer membrane barrier [30]. The relatively lower activity against *P. aeruginosa* (13.0 mm) is not surprising, as this organism is inherently resistant to many antimicrobial agents. [31]. Although the inhibition zones were smaller than those of azithromycin, the extract exhibited clinically relevant activity, supporting its traditional use in treating wound infections [7].

The DPPH radical scavenging assay revealed potent antioxidant activity (IC_{50} = 13.7 µg/mL), which was slightly better than that of gallic acid (15.2 µg/mL). This high antioxidant capacity is likely attributable to the elevated levels of phenolics and tannins in the extract [32]. Antioxidants protect cells from oxidative stress, which is implicated in aging, inflammation, and chronic diseases, such as cancer and cardiovascular disorders [33].

The anti-inflammatory activity, assessed by the inhibition of albumin denaturation, showed a dose-dependent response. At 500 µg/mL, the extract (57.87% inhibition) was comparable to that of ibuprofen (56.03%). Denaturation of tissue proteins is a well-established marker of inflammatory processes, and agents that inhibit this denaturation are considered potential anti-inflammatory drugs [34]. The IC_{50} of the extract (280.7 µg/mL) was lower than that of ibuprofen (335.8 µg/mL), indicating its superior potency in this in vitro model. This finding supports the traditional use of A. nilotica in managing inflammatory conditions such as rheumatism and arthritis [8].

The brine shrimp lethality assay yielded an LD_{50} of 183.3 µg/mL, categorizing the extract as highly toxic according to McLaughlin's criteria [24]. This level of toxicity is not necessarily detrimental; rather, it is often correlated with anticancer or potent bioactivity [23]. Many clinically used anticancer agents, such as etoposide (LD_{50} = 7.46 µg/mL in this assay), exhibited high toxicity in this model. Therefore, the observed cytotoxicity warrants further investigation into the possible antiproliferative effects against cancer cell lines, while also signaling caution regarding unmonitored high-dose consumption.

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Despite these promising findings, this study had several limitations. First, the antibacterial activity was assessed using only the agar well diffusion method, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were not determined. Second, the anti-inflammatory activity was evaluated solely using an *in vitro* protein denaturation assay, which does not fully replicate the complex *in vivo* inflammatory response. Third, cytotoxicity was tested only on brine shrimp (*Artemia salina*), which, although a useful preliminary screening tool, cannot replace mammalian cell line studies. Fourth, the active compounds responsible for the observed bioactivities have not been isolated or characterized. Finally, the study did not include *in vivo* experiments to confirm the efficacy and safety profiles of the extract in animal models.

Conclusion

The ethanolic leaf extract of *Acacia nilotica* from Sudan is rich in phenolic compounds, flavonoids, tannins, and other bioactive metabolites. It demonstrated moderate antibacterial activity, strong antioxidant capacity, promising anti-inflammatory effects, and high cytotoxicity in a brine shrimp model. These findings validate the traditional medicinal uses of this plant, particularly for infections, oxidative stress-related conditions, and inflammation. The high toxicity suggests potential anticancer properties but also indicates the need for careful dosing in therapeutic applications. Future studies should isolate and characterize the active principles responsible for these activities and evaluate their safety and efficacy *in vivo*.

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Conflicts of Interest

The authors declare no conflict of interest.

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