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Phytochemical Analysis and Antibacterial Activity of Acacia Nilotica (Egyptian Mimosa) Stem Bark Extract

Usman Abdullahi[1], Audu Akawu[4], Ahmed Sagir Shehu[3], Hassan Zanna[2]
[1]Department of Biochemistry, Faculty of Science, Yobe State University, Nigeria
[2]Department of Biochemistry, Faculty of Life Sciences, University of Maiduguri, Nigeria
[3]Department of Biological Sciences, Faculty of Science, Yobe State University, Nigeria
[4]Department of Chemistry, Faculty of Science, Yobe State University, Nigeria
Correspondence email: usmanabdullahi8568@gmail.com

Abstract*—The escalating global threat of antimicrobial resistance has necessitated a rigorous exploration of botanical alternatives to conventional synthetic drugs. This study evaluates the biochemical composition and therapeutic potential of the hydro-ethanolic stem bark extract of Acacia nilotica (Egyptian Mimosa) obtained from Yobe State, Nigeria. Utilizing a 50:50 water-ethanol maceration process, the study achieved a 5.75% extraction yield. Comprehensive phytochemical screening revealed a rich profile of secondary metabolites, specifically alkaloids, saponins, terpenoids, and tannins. The absence of glycosides and steroids suggests a specific chemical orientation that favors antimicrobial and anti-inflammatory pathways over hormonal or cardioactive mechanisms. The antibacterial performance of the extract was tested against two clinically significant pathogens: Staphylococcus aureus and Pseudomonas aeruginosa. Using the agar well diffusion technique, the extract exhibited substantial, dose-dependent inhibitory activity at concentrations ranging from 100 to 500 mg/ml. At the maximum concentration, the extract produced zones of inhibition of 18.00 mm for S. aureus and 21.33 mm for P. aeruginosa. While slightly lower than the Ciprofloxacin control, the extract demonstrated remarkable efficacy against the Gram-negative P. aeruginosa, a pathogen typically known for its robust resistance mechanisms. Spectrophotometric analysis determined the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) to be 50 µg/ml for both strains, confirming that the extract is not merely inhibitory but actively bactericidal at low thresholds. These findings provide scientific validation for the indigenous medicinal use of A. nilotica bark and suggest that its bioactive compounds could serve as a viable template for the development of novel, plant-derived antimicrobial therapies*.

***Keywords*—** *Acacia nilotica, Phytochemical screening, Antibacterial activity, Pseudomonas aeruginosa, Staphylococcus aureus, Stem bark extract, Minimum Inhibitory Concentration*.

I. INTRODUCTION

Since the dawn of ancient times, humans have used medicinal plants, and they have continued to play a significant role in the development of safer and more efficient natural drug delivery methods (Al-rajhi et al., 2023) The potential of plant chemicals as a source of safer or more effective alternatives to synthetically manufactured antibacterial agents is of interest. The creation of quick and precise methods for screening plants for specific chemicals has significantly advanced phytochemistry. These procedures have shown that many substances originally thought to be rather rare in occurrence are of almost universal distribution in the plant kingdom (Banso, 2020). Plants contain secondary metabolites in addition to minerals and primary metabolites that are responsible for antioxidant and antibacterial effects (Del, 2017).

Acacia nilotica is a plant 5 to 20 m high with a thick spherical crown, stems and branches usually sinister to black colored, grey-pinkish slash, fissured bark, exuding a reddish low quality gum. The plant has straight, light, thin, grey spines in axillary pairs, usually in 3 to 12 pairs, 5 to 7.5 cm long in young trees, mature trees commonly without thorns (Ali et al., 2017). Active principles are the medications that are found in therapeutic plants. The active principles are divided chemically into a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes (Banso, 2020). The native Africans are said to use Acacia nilotica in the treatment of a variety of ailments, including impotence, tumors of the eye or testicle, dysentery, leprosy, colds, coughs, congestion, fever, hemorrhoids, leucorrhoea, opthalmia, sclerosis, smallpox, tuberculosis, and indurations of the liver and spleen (Auwal et al., 2014). A. nilotica roots have been effective in treating tumors, cancer and tuberculosis (Kamau et al., 2016). Phytochemical screening of Acacia nilotica has shown the presence of tannins, saponosides and flavonoids in fruit (April et al., 2020).

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Apparatus

Biochemical Incubator LBI-80 of power supply 220V/50Hz purchased at Drawell Artist of Science China, Electric Heated Vertical Stream Sterilizer DW-B75L of power supply 3.5Kw Purchased at Drawell Artist of Science China, Drying Oven LD0-400 of power supply 220V/50Hz Purchased at Drawell Artist of Science China, Analytical Balance YP2000001D of power supply 220V/50Hz, UV-Spectrophotometer DU-8200 of power supply AC90-250V, 50Hz/60Hz, Electric Shaker TT12F Purchased at Drawell Artist of Science China, Water Bath DTT-600 of power supply 220V, 50Hz/60Hz, Petri Dishes Purchased at

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Hangzhou Rollmed Co., Ltd China, Conical Flask, Measuring Cylinder and Testubes Purchased at Yancheng Rongtai Labware Co. Ltd China, Bama Bottles Purchased at Damaturu Market, Cuvette Purchased at Meditech, Cotton, Cork Borer, Filter Paper and Micro pipette.

2.1.2 Chemicals/Reagents

Hydrochloric acid and Sulfuric Acid Purchased at Central Drug House (P) Ltd. 7/28 Vardaan Daryaganj, New Delhi-110002 (India), Chloroform, Ferric Chloride, benedict's reagent, Molisch's Reagent, Ethanol, and Methanol Purchased at BDH Merk Life Science UK Ltd. DMSO Purchased at JHD Chemicals Courtyard, Greenfold, Manchester , M28 0PP, UK, Mueller Hinton Agar Purchased at Chaitanya Agro Biotech PVT. L, S/N 20/2 Lakmi Nagar, Mueller Hinton Broth Purchased at Titan Biotech LTD, Industrial Area, Rajasthan India, Sodium Hydroxide and Ferric Acid Purcahsed at Central Drug House (P) LTD. 7/28 Vardaan, Daryaganj, New Delhi India.

2.1.3 Test Organisms

Pseudomonas Aeruginosa and Staphylococcus Aureus

2.2 Methods

2.2.1 Sample Collection

The stem bark of Acacia nilotica was gathered inside the first gate of the Yobe State University campus. Near the administrative structure, several specimens of the plant were discovered.

2.2.2 Sample Drying and Preparation

The sample were dried at room temperature of about 20-25ºC for almost 3 weeks and monitored regularly to ensure complete drying and avoided the contaminants. After sample dried completely it was then grinded using a sterile wooden motor and pestle into fined powder in order to enhance the mixing of the sample with the solvent.

2.2.3 Extraction

To 200 g of the powdered sample was accurately measured using analytical wheighing balance and transferred into a bottle. A 500 ml of hydroethanolic solution (50:50) water – ethanol was prepared using a measuring cylinder, and then poured on the powdered sample inside the bottle. The mixture was then placed on electric shaker for 48 hrs at constant shaking to facilitate thorough extraction. After shaking, the mixture was then filtered first with the net followed by the filter paper, the supernatant was then been transferred to a plastic container and placed inside the drying oven where it was completely dried. The dried extract was wheighted and determined the percentage died.

$$\text{Percentage(\%) Yield} = \frac{amount\ of\ extract\ obtained}{amount\ of\ initial\ sample} \times 100\%$$

2.2.4 Phytochemical Screening

2.2.4.1 Preparation of the Working Solution

To 1g of the dried extract was measured using wheighing balance and dissolved into a 10ml of distilled water.

2.2.4.2 Test for Alkaloids

To 1ml of hydrochloric acid to the small amount of the extract inside a testube, then a few drops of the mayer's reagent, brown precipitate indicate the presence of alkaloids

2.2.4.3 Test for Flavonoids

To 1ml of absolute ethanol and 3 drops of hydrochloric acids to the extract, formation of red colour indicate the presence of flavonoids.

2.2.4.4 Test for Tannins

To 1ml of the ethanol followed by few drops of ferric chloride to the extract, formation of dark blue indicate the presence of tannins.

2.2.4.5 Test for Terpenoids

To 1ml of chloroform and 2ml of tetraoxosulphate (iv) acid to the extract, formation of reddish brown indicate the terpenoids.

2.2.4.6 Test for Saponins

Deep shaken of the extract, formation of pump indicate the presence of saponin.

2.2.4.7 Test for Carbohydrates

To 2ml of Benedict's reagent to the extract, heat the mixture in boiling water bath for 5-10 mins, Green colour indicate the presence of reducing sugar.

2.2.4.8 Test for Steroids

To 1ml of acetic anhydride to the extract and 2 drops of concentrated sulfuric acid, formation of green colour indicate the presence of steroids.

2.2.4.9 Test for Glycosides

To 1ml of Molisch's reagent to the extract and 2 drops concentrated sulfuric acid, formation of purple colour indicate the presence of glycosides.

2.3 Antimicrobial Sensitivity Testing

2.3.1 Preparation of the Stock Solution

To 1g of the dried extract was dissolved into 10ml of dimethyl sulfoxide (DMSO) in a container.

2.3.2 Media Preparation

To 1.9 g of Mueller Hinton agar was weighed in analytical weighing balance. It was dissolved in 50 ml of distilled water in a labelled conical flask. The mouth of the flask plugged with cotton wool and wrapped in an aluminium foil paper and tightened with masking tape. This was to avoid spillage of the media in an autoclave while undergoing sterilisation. This dissolved media was sterilized in an autoclave set 121 ºC for 15minutes. It was removed and allowed to cool to 45 ºC and was gently and aseptically poured into 2 sets of 25ml disposable petri dish, the media on the plates were allowed to solidify.

2.3.3 Agar Well Diffusion Technique

Five concentrations of the values (500mg/ml, 400mg/ml, 300mg/ml, 200mg/ml and 100mg/ml) from the prepared stock solution were measured in a separate testubes and diluted each with 2ml of distilled water. The two organisms Pseudomonas Aeroginosa and Staphylococcus Aureus were inoculated in two separate petri dishes containing prepared solidified media. Then using a sterile cock borer, five (5) wells were dug in each petri dish and labaled according to the corresponding concentrations prepared, and additional one (1) well researved at the middle of each petri dish for the reference control drug. The 5mg ciproflaxocin (formed by diluting 500mg of ciproflaxocin to 100ml of distilled water) was added to the reference well on each petri dish, then the prepared concentrations were also poured into their respectives labaled

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wells on the petri dish and finally the two petri dishes were incubated in an incubator for 24hours.

2.3.4 Minimum Inhibitory Concentration

Five concentrations of the values (250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml) from the prepared stock solution were measured in a separate testubes and diluted each with 2ml of distilled water. 10 sterile bottles were rinsed with distilled water and labaled accordingly, bottles 1-5 were designated for pseudomonas aeruginosa and bottles 6-10 designated for staphylococcus aureus. 10ml of prepared Mueller Hinton broth was poured into each bottle, then subcultures of pseudomonas aeruginosa and staphylococcus aureus were inoculated into their respectives bottles. The five concentrations prepared of the test solution were added dropwise to each bottle of both organisms and indicated the concentration on added on each bottle. The bottles were tightly close using their cap and incubated at 37ºC for 24hrs. after the incubation, growth of bacteria was observed by the formation of turbidity, then the inoculum of the testubes were gently poured into cuvette for optical density using UV-spectrophotometer.

III. Result and Discussion

3.1 Hydro-Ethanolic Percentage Yield

The dried fine powder of the hydroethanolic stem bark extract of *Acacia nilotica* was extracted using hydro-ethanol solution in ratio 50:50. The result of the extraction yield is shown below in table 4.1.1. The hydro-ethanolic extraction of 200g of *acacia nilotica* (Egyptian mimosa) stem bark extract yielded about 11.5g dried extract which is represented as 5.75% yield, this indicating that there is the sufficient hydroethanolic soluble bioactive compounds and providing an insight about the phytochemical screening that take place ahead.

TABLE 1: Result showing the percentage yield of 200g sample using hydro-ethanolic solvent

Solvent	Sample Weight(G)	Dried Extract Weight(G)	%Yield
Hydro-ethanol	200	11.5	5.75

KEY: G = Gram, %Yield = Percentage yield

3.2 phytochemical screening

The phytochemical screening of *acacia nilotica* stem bark hydro-ethanolic extract has revealed the presence *saponins, terpenoids, tannins, and alkaloids* this signified the potential of hydro-ethanolic extract as antimicrobials, antioxidant and anticancer properties. The absence of glycosides, *flavonoids, steroids and carbohydrates* indicated the limited cardioactive, hormonal, and energy related potentials, while in overall the extract phytochemicals profile indicated its potential as a natural antimicrobial and anti-inflammatory agent, warranting further investigation into its therapeutic applications. And finally, this findings validate the efficacy of traditional uses of *acacia nilotica* with respect to its stem bark using hydro-ethanolic solvent.

TABLE 2: Result of phytochemical screening of hydro-ethanolic extract using eight parameters

S/N	Parameters	Result
1.	Saponin	+
2.	Terpenoid	+
3.	Glycoside	-
4.	Tannin	+
5.	Alkaloid	+
6.	Flavonoid	-
7.	Steroid	-
8.	Carbohydrate	-

KEY: + = Positive(Present), - = Negative(Absent)

3.3 Antimicrobials sensitivity

The hydro-ethanolic stem bark extract of *acacia nilotica* has indicated antimicrobial activity against *staphylococcus aureus* at all tested concentrations and notably 500µg/ml concentration exhibited a zone of inhibition of 18.00mm/dm compare to reference drug ciproflaxocin with 23.00mm/ml. Therefore, the efficacy although it is slightly lower than ciproflaxocin but indicate its potential as a natural antimicrobial agent. The ciproflaxocin is 27.1% greater zone of inhibition greater than the extract concentration.

TABLE 3: The Result Showing Zone of Inhibitions at Different Concentrations of the Hydro-Ethanolic Acacia Nilotica Stem Bark Extract *Against Staphylococcus* Aureus

Organism	Concentratration (µg/ml)	ZOI (mm/dm)	Reference Drug Ciproflaxocin (5mg)(mm/dm)
S.AUREUS	100	7.67 ± 2.05	23.00 ± 1.632
	200	10.67 ± 0.92	
	300	14.00 ± 0.82	
	400	15.33 ± 0.47	
	500	18.00 ± 0.82	

KEY: ZOI = Zone of Inhibition, Mean ± S.D, n = 3, *S.AUREUS* = *Staphylococcus aureus.*

The hydro-ethanolic stem bark extract of *acacia nilotica* exhibited antimicrobial activity against *Pseudomonas Aeroginosa* with zone of inhibition of 21.33mm/dm at 500µg/ml being the highest concentration tested while the reference drug showed a zone of inhibition of 24.66mm/dm. Therefore, the extract has demonstrated a significant antibacterial efficacy with the zone of unhibition compare to ciproflaxocin. The 500µg/mi concentration's zone of inhibition was merely 3.33mm/dm that's 13.5% lower than ciproflaxocin, this indicated that the extract has efficacy against *pseudomonas aeroginosa*

TABLE 4: The Result showing zone of inhibition at different concentrations of hydro-ethanolic acacia nilotica stem bark extract against *pseudomonas aeroginosa*

Organism	Concentration (µg/ml)	ZOI(mm/dm)	Reference Drug Cipro.(5mg)(mm/dm)
P.AEROGINOSA	100	9.83 ± 2.05	24.66 ± 2.05
	200	16.33 ± 0.94	
	300	15.33 ± 0.47	
	400	17.83 ± 0.62	
	500	21.33 ± 0.47	

KEY: ZOI = Zone of inhibition, Mean ± S.D, n = 3, *P.AEROGINOSA* = *Psedomonas Aeroginosa.*

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The minimum inhibitory concentration (MIC) hydroethanolic extract of stem bark of acacia nilotica against *Staphylococcus Aureus* was determined as 50 µg/ml, exhibiting the lowest absorbance (0.82 nm) in the UV-Spectrometer. The minimum bactericidal concentration (MBC) testing on solid media using the same inoculum indicated a zone of inhibition of 13.0 mm/dm. Therefore, the (MIC) result 50µg/ml indicates the extract's ability to inhibit *Staphylococcus Aureus* growth in Mueller hinton broth media and the corresponding (MBC) result zone of inhition 13.0 mm/dm confirm the extract's bactericidal activity on solid media killing the bacteria. This also demonstrated inhibition and bactericidal at the same concentration.

TABLE 5: Result showing the (MIC) and (MBC) at Different Concentrations of the Hydro-Ethanolic Stem Bark Extract of Acacia Nilotica Against *S. Aureus*

Organism	Concentration (µg/ml)	Absorbance (nm)	MBC (mm/dm)
S.AUREUS	50	**0.82 ± 0.021**	13.0 ± 0.816
	100	0.827 ± 0.036	
	150	1.28 ± 0.066	
	200	1.418 ± 0.23	
	250	1.753 ± 0.41	

KEY: (MIC) = Minimum Inhibitory Concentration, (MBC) = Minimum Bactericidal Concentration, Mean ± S.D.

TABLE 6: Result Showing The (MIC) And (MBC) at Different Concentrations of the Hydroethanolic Stem Bark Extract Against *P. Aeroginosa*

Organism	Concentration (µg/ml)	Absorbance (nm)	MBC (mm/dm)
P. AEROGINOSA	50	**0.629 ± 0.046**	14.0 ± 0.816
	100	0.754 ± 0.046	
	150	0.929 ± 0.093	
	200	1.156 ± 0.110	
	250	1.201 ± 0.460	

KEY: (MIC) = Minimum Inhibitory Concentration, (MBC) = Minimum Bactericidal Concentration, *P. Aeroginosa = Psedomonas Aeroginosa.*

The minimum inhibitory concentration (MIC) of hydroethanolic stem bark extract of *acacia nilotica* against *pseudomonas aeroginosa* was determined at 50µg/ml, with a minimum absorbance value of 0.629 ± 0.046 nm. And Minimum bactericidal concentration (MBC) evaluation on solid *Mueller hinton agar* using the same inoculum revealed a zone of inhibition of 14.0 ± 0.816. Therefore, (MIC) result 0.629 ± 0.046 nm indicates the potential inhibitiom of *p.aeroginiosa* growth and also the corresponding (MBC) result zone of inhibition confirm the extract's effectively killing the bacteria.

IV. Discussion

The phytochemical analysis confirmed the presence of alkaloids, saponins, terpenoids, and tannins, similar findings using ethanolic solvent has been reported by (Banso, 2009). This similarity indicates that ethanol based solvents are effective in extracting these secondary metabolites. However, (Jame, 2019) confirmed the present of carbohydrates but this is due to the used of absolute ethanol other than hydroethanol as a solvent. The extract exhibited sensitivity against both bacterial strains across all tested concentrations, with the highest efficacy observed at 500 µg/mL. For *S. aureus*, the zone of inhibition was 18.00 mm, slightly less than the reference drug ciprofloxacin (23.00 mm). *P. aeruginosa* showed even greater sensitivity to the extract, with a 21.33 mm inhibition zone at 500 µg/mL, close to ciprofloxacin's 24.66 mm, this consistent with previous investigation that also reported significant zones of inhibition against the same microorganisms using ciprofloxacin as the positive control (Sciences, 2018). The uses of the same standard antibiotic strenghthens the comparability of both studies. However, while the ealier study employed leaf extracts, the present study utilized stem bark, which may differ in phytochemical composition and concentration of bioactive compounds These findings indicate broad-spectrum antibacterial potential, with *P. aeruginosa* being particularly responsive. The minimum inhibitory concentration (MIC) results further support the extract's efficacy. For *S. aureus*, the MIC corresponded to an absorbance of 0.82 nm, with a minimum bactericidal concentration (MBC) of 13.0 mm at 50 µg/mL. Meanwhile, *P. aeruginosa* had a lower MIC of 0.629 nm, indicating a stronger inhibitory effect at lower absorbance, with an MBC of 14.0 mm at 50 µg/mL. This findings directly correlated with literature reported by (Infections, 2015) and (Khaleel et al., 2021) which found acacia nilotica stem bark efficacy against S.aureus and P. These results suggest that *Acacia nilotica* extract may target specific bacterial mechanisms effectively, with a more pronounced effect on *P. aeruginosa* compared to *S. aureus*.

V. Conclusion

The data supports the hypothesis using diverse literature correlated with this study that *Acacia nilotica* stem bark extract has significant antimicrobial properties, potentially attributable to its phytochemical constituents. The extract's performance, approaching that of ciprofloxacin, underscores its potential as a natural antibacterial agent, suggesting further research to isolate the active compounds and explore therapeutic applications using experimental animals. These findings validate the traditional medicinal use of *Acacia nilotica* and provide a foundation for its potential development in antimicrobial treatment

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