TITLE -

Antimicrobial Product Development from *Acacia nilotica* Plant: Preclinical Studies and Product Filing.

EXECUTIVE SUMMARY

The alarming rise of multi-drug resistant (MDR) bacterial infections has become a formidable challenge in modern medicine, particularly in the treatment of upper and lower respiratory tract infections. Conventional antibiotics are increasingly losing efficacy, prompting an urgent need for novel therapeutic agents with potent antimicrobial properties. Among promising natural sources, the *Acacia* plant stands out due to its rich phytochemical profile and long-standing use in traditional medicine. Various species of *Acacia* have demonstrated significant antibacterial activity, attributed to bioactive compounds such as tannins, flavonoids, and alkaloids. Harnessing these compounds for drug development offers a sustainable and potentially effective strategy to combat MDR pathogens. This proposal explores the potential of *Acacia*-derived natural compounds in formulating new antimicrobial drugs targeted at respiratory tract infections. By integrating ethnobotanical knowledge with modern pharmacological techniques, this proposal aim to develop and formulate the crude extract from the bark of *Acacia nilotida* plant for clinical application. Previous studies from our laboratory have reported on its potent activity against a wide spectrum of pathogens, including: Gram-positive and Gram-negative bacteria (e.g., *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*), Fungal strains (*Candida albicans*), *Mycobacterium tuberculosis* and *M. bovis* (BCG). The development process will include rigorous *in-vivo* preclinical tests to ensure safety, efficacy, and resistance mitigation, coupled with the physicochemical stability of the drug product. The emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) strains of bacteria and tuberculosis underscores the urgent need for novel therapeutics. Standardized extracts from the *Acacia nilotica* plant offers a natural, low-toxicity alternative with broad-spectrum antimicrobial activity, proven efficacy against both common pathogens and tuberculosis strains, and a potential for formulation into

standardized drug products, either as standalone agents or in combination therapies. Thus, this research project seeks to contribute to the global fight against antibiotic resistance by offering a plant-based alternative with broad-spectrum antimicrobial potential.

INTRODUCTION

The search for new antimicrobial agents has become an urgent global priority due to the growing problem of antimicrobial resistance. Plants have long been recognized as a rich source of bioactive compounds, with the genus *Acacia* being one of the most widely distributed and traditionally used medicinal plant groups across Africa, Asia, and the Middle East. Within this genus, *Acacia nilotica* is one of the significant species abundantly available in Nigeria and has received significant scientific attention for its antimicrobial, antifungal, and antitubercular properties (Oladosu *et al*., 2013; Hafez *et al*., 2024). Our research group has carried out extensive studies on *A. nilotica* (Oladosu *et al*., 2013 and Oladosu *et al*., 2019) and the notable results obtained have prompted us to desire the further development of the crude extract into a pharmaceutical dosage form thereby transitioning from the laboratory bench to pharmacy shelves nationwide. The plant has been traditionally employed in the treatment of infectious diseases, skin conditions, wounds, and respiratory disorders. Modern pharmacological investigations have validated many of these traditional uses and have begun to elucidate the specific chemical constituents responsible for its therapeutic potential.

The antimicrobial efficacy of *A. nilotica* is primarily attributed to the presence of phenolic acids, tannins, flavonoids, and other secondary metabolites that disrupt microbial cell structures and inhibit enzymatic processes (Negi & Dave, 2010; Mishra *et al*., 2016). In *A. nilotica*, compounds such as gallic acid, methyl gallate, and catechin have been isolated and shown to possess strong antibacterial and antitubercular activities (Oladosu *et al*., 2013). These compounds act by multiple mechanisms, including the disruption of microbial cell membranes, inhibition of nucleic acid synthesis, and interference with oxidative phosphorylation (Al-Huqail *et al*., 2019). Such results provide strong evidence that the *A. nilotica* contains bioactive molecules with multi-targeted antimicrobial potential.

The process of developing an antimicrobial product from *A. nilotica* involves several key stages beginning with the extraction and standardization of the crude extract. Once standardized, the active extract is made to undergo *in-vitro* testing against a panel of bacterial and fungal pathogens to determine their minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). For instance, methyl gallate and catechin from *A. nilotica* have shown MIC values ranging between 20 and 62.5 μg/mL against *Mycobacterium tuberculosis*, *E. coli*, and *S. aureus* (Oladosu *et al*., 2013). These results suggest that the compounds possess significant pharmacologically relevant antimicrobial potency. In addition, the use of *in-vivo* animal models provides insights into the pharmacokinetics, safety, and therapeutic efficacy of the extracts before human application (Alli *et al*., 2015).

Toxicological evaluation is an essential component of preclinical studies. Although most *Acacia* extracts have demonstrated low acute toxicity, some studies have reported mild hepatic and renal effects at high doses (Alli *et al*., 2015). Therefore, standardized preclinical toxicity protocols such as acute, subacute, and chronic studies are required to ensure safety prior to regulatory submission. Herbal extracts should also undergo quality control testing for contaminants, heavy metals, and microbial content following the World Health Organization's Good Manufacturing Practices (WHO, 2007). Furthermore, to ensure reproducibility, herbal formulations must be standardized for active marker compounds such as gallic acid and catechin (Sharma, 2015). This ensures consistency between production batches and compliance with international quality standards.

Formulation development represents the transition from laboratory discovery to potential clinical application. Two principal development routes exist: the pharmaceutical route and the herbal formulation route. In the pharmaceutical pathway, isolated compounds can be synthesized or purified and formulated as drug candidates. This process requires full regulatory submission under the Investigational New Drug (IND) guidelines as specified by the U.S. Food and Drug Administration (FDA, 2016). In contrast, herbal products are developed from standardized plant extracts, which may be registered under traditional

medicine frameworks or as botanical drugs. The WHO (2007) emphasizes that both approaches must adhere to strict quality assurance, safety, and efficacy documentation. This dual approach allows for flexibility in development, ensuring that both traditional medicine practitioners and modern pharmaceutical industries can benefit from the therapeutic potential of medicinal plants. This project is intended to pursue the herbal formulation route.

The global burden of upper and lower respiratory tract infections, especially tuberculosis and the increasing incidence of multidrug-resistant strains highlight the need for novel anti-TB therapies derived from natural sources (Hafez *et al*., 2024). By employing a rigorous, evidence-based approach that integrates ethnomedicinal knowledge, phytochemical analysis, and preclinical validation, this study proposes a sustainable framework for developing *Acacia*-based antimicrobial products. Improved treatment options for respiratory tract infections—common and often deadly in sub-Saharan Africa—could lead to a substantial reduction in mortality rates, particularly among vulnerable groups like children and the elderly. *Acacia nilotica* is widely available across the region, making it a sustainable and culturally familiar resource for drug development. The affordability of plant-based treatments could greatly enhance access to effective healthcare, especially for low-income populations. The cultivation and processing of *Acacia nilotica* for pharmaceutical use may also stimulate local economies by creating jobs and supporting agro-industrial growth. This initiative encourages scientific innovation within the region, potentially inspiring further research into other native plants of therapeutic value. It could also attract investment in public health infrastructure and pharmaceutical research, enhancing disease surveillance and diagnostic capabilities.

In conclusion, the development of antimicrobial products from *Acacia* species offers significant promise for both pharmaceutical innovation and traditional medicine advancement. Compounds such as gallic acid, methyl gallate, and catechin have demonstrated potent antimicrobial and antitubercular activity, supporting their potential for drug and herbal formulation development. Preclinical studies will establish their safety and efficacy profiles, while existing WHO and FDA guidelines provide clear pathways for

product standardization and filing. With further support through the proposed grant application, *Acacia nilotica*-derived antimicrobial agents could emerge as valuable tools in the fight against infectious diseases and antimicrobial resistance worldwide. Moreover, validating traditional medicinal knowledge through scientific research fosters community trust and strengthens the integration of indigenous practices into modern medicine. This approach aligns with the principles of sustainable drug discovery, leveraging indigenous biodiversity while meeting modern regulatory and scientific standards. This project is also in agreement with Mr. President's Renewed Hope Agenda through self-reliance in Nigeria's health care delivery.

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AIM

To develop an alternative antimicrobial product to resistant bacteria from *Acacia nilotica* plant by 2027.

OBJECTIVES

- To obtain the crude extract of *Acacia nilotica* from the plant's dried leaves.
- To confirm the plant's strong antimicrobial activity against antibiotic resistant strains of bacteria in this crude extract.
- To carry out preclinical studies of crude extract such as acute and chronic toxicity.
- To formulate capsules from the crude extract as a drug product.
- To file the drug product at NAFDAC for pre-registration purposes.

Materials and Reagents

The following materials and reagents will be used for the extraction, isolation, antimicrobial testing, and formulation of products derived from *Acacia nilotica*.

Plant Material: Mature pods, bark, and leaves of *Acacia nilotica* will be collected from authenticated botanical sources. The plant will be identified and verified by a botanist, and a voucher specimen will be deposited in a recognized herbarium.

Chemicals and Solvents: Analytical and HPLC-grade solvents such as methanol, ethanol, n-hexane, ethyl acetate, chloroform, and distilled water will be procured from Sigma-Aldrich (Germany). Standards including gallic acid, methyl gallate, and catechin will be obtained for calibration and quantification. Other reagents include dimethyl sulfoxide (DMSO), nutrient agar, Sabouraud dextrose agar, and Lowenstein–Jensen medium for microbial culture preparation.

Microbial Strains: The antimicrobial screening will be carried out against clinical and

standard strains, including *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10231), and *Mycobacterium tuberculosis* H37Rv strain for antitubercular assays.

Equipment and Instruments: Rotary evaporator, Soxhlet apparatus, analytical balance, UV–Vis spectrophotometer, high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), nuclear magnetic resonance (NMR) spectrometer, incubators, biosafety cabinets, and laminar flow hoods will be used. Capsule filling machine, tablet hardness tester, and dissolution apparatus will be employed for formulation studies.

Pharmaceutical Capsule Formulation: Isolated active compounds (gallic acid, methyl gallate, catechin) will be combined with excipients such as microcrystalline cellulose, lactose monohydrate, magnesium stearate, and starch as fillers and flow agents. The mixture will be homogenized and encapsulated into hard gelatin capsules of 500 mg per unit using a semi-automatic capsule filler. Dissolution and disintegration tests will be performed according to pharmacopeial standards.

Herbal Capsule Formulation: A standardized ethanolic extract of *Acacia nilotica* fruits will be concentrated, dried, and granulated. The extract will be standardized using gallic acid as a marker compound. Excipients including maltodextrin, pregelatinized starch, and magnesium stearate will be added for capsule formulation. Each capsule will contain 400 mg of the standardized extract, packed in airtight containers, and stored under controlled humidity conditions.

Storage Conditions: All extracts and formulated capsules will be stored at 4°C in amber glass bottles until further analysis to prevent degradation. Microbial assays will be performed under aseptic conditions to ensure result validity.

Table 1: Plant Materials

S/N	Product	Quantity	Unit Price	Amount
1	Mature pods of *Acacia nilotica*	**50 kg**	**2,000**	**100,000**
2	bark of *Acacia nilotica*	50 kg	2,000	100,000
3	leaves of *Acacia nilotica*	50 kg	2,000	100,000

Table 2: Chemicals and Solvents

S/N	Product	Quantity	Unit Price	Amount
1	Methanol	**250 L**	**2,500**	**625000**
2	ethanol	**250 L**	2,500	**625000**
3	n-hexane	**250 L**	2,500	**625000**
4	ethyl acetate	**250 L**	2,500	**625000**
5	chloroform	**250 L**	2,500	**625000**
6	distilled water			
7	gallic acid	100 mg	150,000	150,000
8	methyl gallate	100 mg	150,000	150,000
9	catechin	100 mg	150,000	150,000
10	dimethyl sulfoxide (DMSO),	10 L	10,000	100,000
11	nutrient agar	500g x 2	150,000	300,000
12	Sabouraud dextrose agar	500g x 2	150,000	300,000
13	Lowenstein–Jensen medium	500g x 2	200,000	400,000
14	Bovine Serum fraction V	500g	300,000	300,000
15	Glycerol	10L	25,000	250,000

16	Rifampicin	100 mg	150,000	150,000
17	Isoniazide	100 mg	150,000	150,000
18	96 Microwell plates	100	300,000	300,000

Table 3: Microbial Strains

S/N	Product	Quantity	Unit Price	Amount
1	*Escherichia coli* (ATCC 25922)	**10 vials**	**50,000**	**500,000**
2	*Staphylococcus aureus* (ATCC 25923),	**10 vials**	**50,000**	**500,000**
3	*Bacillus subtilis* (ATCC 6633)	**10 vials**	**50,000**	**500,000**
4	*Pseudomonas aeruginosa* (ATCC 27853)	**10 vials**	**50,000**	**500,000**
5	*Candida albicans* (ATCC 10231)	**10 vials**	**50,000**	**500,000**
6	distilled water			
7	*Mycobacterium tuberculosis* H37Rv strain	**10 vials**	**50,000**	**500,000**

Table 4: Equipment and Instruments

S/N	Product	Quantity	Unit Price	Amount
1	Rotary evaporator	**1**	**400,000**	**400,000**
2	Soxhlet apparatus	2	200,000	200,000
3	analytical balance	1	250,000	250,000
4	UV–Vis spectrophotometer	1	500,000	500,000
5	high-performance liquid chromatography (HPLC)	1		
6	liquid chromatography–mass spectrometry (LC-MS)	1		
7	nuclear magnetic resonance (NMR) spectrometer			
8	incubators	1	400,000	
9	biosafety cabinets	1	1,000,000	
10	laminar flow hoods	1	200,000	

11	Capsule filling machine			
12	dissolution apparatus			

8. PROJECT TEAM MEMBERS

S/N	Name	Institution / Department	Expertise Coordination	Qualification	Contribution to Project
1.	Prof. Peter Oladosu	Microbiology and Biotechnology department, NIPRD	Microbiology and Biotechnology	Ph.D	Principal Investigator
2.	Dr. Tiwalade Adelakun	Medicinal Chemistry and Quality Control department, NIPRD	Medicinal Plant Chemistry	Ph.D	Investigator
3.	Dr. Henry Eghareva	Medicinal Plant Research & Traditional Medicine department, NIPRD	Plant Chemistry	Ph.D	Investigator
4.	Dr. Bulus Adzu	Pharmacology and Toxicology department, NIPRD	Natural Products Pharmacology and Toxicology	Ph.D	Investigator
5.	Dr. K.B Mustapha	Medicinal Chemistry and Quality Control department, NIPRD	Pharmaceutical Chemistry and Biopharmaceutics	Ph.D	Investigator

6.	Dr. Mercy Aboh.	Microbiology and Biotechnology Department, NIPRD	Microbiology and Biotechnology	B.Sc	Investigator
7.	Mr. Abdulakeem Olayanju	Medicinal Plant Research & Traditional Medicine department, NIPRD	Taxonomist and Species authentication	B.Sc	Plant species identification, authentication and plantation contact.