A PROPOSAL SUBMITTED TO NASENI GRANTS PROGRAMS

TITLE: SCALING UP PRODUCTION AND ADVANCING CLINICAL TRIALS OF A PLANT-BASED ANTIFUNGAL OINTMENT: A Health Innovation for Accessible and Affordable Fungal Infection Treatment

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SCALING UP PRODUCTION AND ADVANCING CLINICAL TRIALS OF A PLANT-BASED ANTIFUNGAL OINTMENT: A Health Innovation for Accessible and Affordable Fungal Infection Treatment

Executive Summary

Fungal infections represent a pressing but often underappreciated global health challenge, with both superficial and systemic manifestations contributing significantly to morbidity and, in some cases, mortality. Globally, more than one billion people are affected by superficial fungal infections annually, and approximately 150 million people suffer from serious or life-threatening fungal diseases[1]. In Nigeria and other tropical countries, the warm, humid climate creates conditions that favor the persistence and spread of dermatophytes and opportunistic fungal pathogens, amplifying the burden of disease in already resource-limited health systems[2]. Despite the availability of conventional antifungal drugs, their clinical effectiveness is undermined by multiple challenges including emergence of antifungal resistance, toxicity concerns, high cost of therapy, and limited availability of certain drugs in rural and peri-urban communities[3]. Herbal medicines on the other hand, are relatively cheap, easily affordable and accessible in addition to being readily accepted due to its purported claim of relative safety, these can bridge the gap between rising demands and limited therapeutic options. By utilizing the healing properties of medicinal herbs, we have created a new herbal antifungal ointment (NIPRIFAN^{TM}) that has shown great potential as a potent topical antifungal agent in initial laboratory studies. However, clinical research on the efficacy of this formulation is lacking.

NIPRIFAN^{TM} is a formulation of the extract of the aerial parts of the plant *Mitracarpus villosus*, locally known as “Irawo-ile” by the Yoruba tribe of Nigeria, “Obuobwa” in Igbo, “Gududal” by the Hausas and Fulani is traditionally, the plant is used in treatment of fungal skin infections. Based on the hypothesis that NIPRIFAN^{TM} is efficacious and cheap for the treatment of topical fungal infection, the goal of this project is to scale up, validate, and clinically evaluate a novel herbal antifungal ointment, ensuring its stability, safety, and efficacy for widespread and affordable use in the management of fungal infections.

The objectives of the project are:

1. To scale up the production of NIPRIFAN^{TM} to pilot-scale manufacturing ensuring reproducibility, cost-effectiveness and Good Manufacturing Practices (GMP).
2. To conduct accelerated and real-time stability testing under varying storage conditions to establish physicochemical integrity, quality, shelf-life and storage requirements of the ointment

3. To assess the safety, tolerability and therapeutic effectiveness of the herbal antifungal ointment in patients with superficial fungal skin infections through Phase I clinical trials
4. To generate and compile necessary data for regulatory approval laying the foundation for introduction to the drug market.

This concept is unique because it seeks to develop a completely indigenous product and scale-up same to make it assessable and available to the Nigerian populace. The proposed pilot scale-up production and clinical trials will be initiated and completed within 24 months. The project is estimated to cost two hundred and thirty-five million, six hundred and thirty-five thousand naira (₦235,635,000.00) only. Upon completion of this project, it is expected that a safe, effective, affordable and accessible antifungal topical product with potential to strengthen local manufacturing and reduce dependence on imported products would have been developed.

BACKGROUND

Fungal infections represent a diverse spectrum of diseases caused by yeasts, dermatophytes, and molds. They range from superficial infections, such as tinea corporis and onychomycosis, to invasive systemic infections caused by opportunistic fungi like *Candida spp*. and *Aspergillus spp*.[4]. These infections contribute significantly to the global disease burden with increasing incidence and prevalence, particularly in low- and middle-income regions such as Asia and Sub-Saharan Africa. The World Health Organization (WHO) estimates that approximately 1.7 billion individuals suffer from superficial fungal infections each year, and over 150 million people experience severe fungal diseases with high mortality rates[1].

In Sub-Saharan Africa, including Nigeria, the burden is magnified due to environmental and socio-economic factors. Warm and humid climates favour fungal growth, while limited access to healthcare, overcrowding, and poverty contribute to the persistence and spread of infection. A recent survey in Nigeria found dermatophytic infections to be one of the most common skin diseases among children and adults, with prevalence rates ranging from 10–35% depending on the region[2]. In immunocompromised individuals particularly those living with HIV/AIDS, cancer, or diabetes, fungal infections pose a significant threat, often leading to systemic complications.

Over the past several decades, antifungal therapy has relied heavily on polyenes (e.g., amphotericin B), azoles (e.g., fluconazole, itraconazole), echinocandins (e.g., caspofungin), and allylamines (e.g., terbinafine). These drugs have revolutionized clinical practice, but they are not without limitations such as high cost, limited accessibility, emergence of drug-resistant strains, adverse reactions like nephrotoxicity and poor patient adherence[3]. These health trend analysis highlights the critical need for effective, accessible, and cost-effective antifungal therapies, including options derived from herbal and natural sources to address this public health challenges.

Phytomedicines, derived from plant-based bioactive compounds, offer a promising avenue for addressing the limitations of conventional antifungals. Ethnomedicinal practices across cultures have long utilized plants like *Punica granatum* (pomegranate), *Eucalyptus globulus*, *Azadirachta indica* (neem), *Syzygium aromaticum*, essential oils from cinnamon (*Cinnamomum zeylanicum*) and *Peganum harmala* for treating fungal infections, with modern studies validating their efficacy.

The plant *Mitracarpus villosus*, family, Rubiaceae which was formerly referred to as *Mitracarpus scaber* Zac is a small herbaceous plant that is widely distributed in the tropical countries including Nigeria. It is locally known as “Irawo-ile” by the Yoruba tribe of Nigeria,

"Obuobwa" in Igbo, "Gududal" by the Hausas and Fulani and traditionally used against ringworm, eczema, scabies, and other dermatophytic infections[5]. Preliminary phytochemical and pharmacological investigations confirm that the aerial parts of the plant contain bioactive compounds such as tannins, flavonoids, and saponins, which have demonstrated antimicrobial and antifungal properties[6]. Scientific studies have also demonstrated that incorporation of the extracts of *Mitracarpus villosus* into various cream bases have inhibited *Trichophyton, Microsporum, Epidermophyton species, Candida albicans* and *Aspergillus flavus* [7,8]. However, despite its traditional popularity and scientific claims, the antifungal property of the plant remains underexplored in terms of standardized pharmaceutical formulation and clinical evaluation.

In response to this identified gap, we have developed and trademarked a novel herbal ointment with demonstrated antifungal activity. The product (NIPRIFAN$^{TM)}$ has been trademarked by the National Agency for Food and Drug Administration and Control (NAFDAC) and is now ready to move forward into large manufacturing, stability validation and clinical testing. The National Institute for Pharmaceutical Research and Development (NIPRD) where this product originates from has extensive background in herbal formulation and pharmaceutical development as such, it is uniquely positioned to lead and advance this project. Promotion of our innovation (NIPRIFAN^{TM}) would improve access to affordable topical antifungal treatment, reduce dependency on costly imported antifungal products, boost local manufacturing and contribute to sustainability of public health impact.

PROBLEM STATEMENT

Topical fungal infections are a major public health issue, despite their global prevalence and significant socioeconomic burden. In Sub-Saharan Africa, it affects mostly school-aged children and young adults while also contributing to morbidity and mortality in immunocompromised populations[2]. In Nigeria, challenges with treatment of fungal infections includes high cost of conventional agents, poor affordability, reduced accessibility, particularly in rural and underserved areas. Rise of antifungal resistance especially among *Candida species* compromises conventional therapeutic effectiveness, making some of these agents unreliable[3] while nephrotoxicity, and drug–drug interactions, restrict their clinical utility. Although herbal medicines offer promising antifungal activity, most remain confined to traditional use without validation for stability, safety, and efficacy. This lack of standardized, clinically tested herbal antifungal products creates a gap that limits their integration into mainstream healthcare. This project addresses this critical gap by advancing the scale up of a novel, already trademarked

herbal antifungal ointment (NIPRIFAN^{TM}), from laboratory development to clinical validation. Thus, reducing dependence on costly imported antifungal products, boosting local pharmaceutical manufacturing and improving health outcome of the Nigerian populace.

SIGNIFICANCE OF THE STUDY

Superficial fungal infections are a persistent challenge in the tropical and subtropical regions including Nigeria. These infections not only cause physical discomfort but also contribute to absenteeism, reduced productivity, and social stigma as a result of their visible manifestations. Conventional antifungal agents like the azoles although widely effective are limited by challenges of high costs, limited accessibility, emerging resistance and undesirable adverse effects. This underscore the urgent need for alternative therapeutic options that are affordable, safe, culturally acceptable, and effective. Our herbal topical antifungal preparation; NIPRIFAN^{TM}, has been developed to meet this need. Advancing NIPRIFAN^{TM}, to clinical effectiveness offers a practical intervention capable of to reducing morbidity, improving quality of life, and mitigating the psycho-social burden associated with these skin conditions. This innovation also serves to reduce dependence on imported synthetic drugs and provides an alternative with lower treatment cost for patients. In addition, this advancement represents an important step toward strengthening local pharmaceutical innovation with a potential to contribute to the diversification and expansion of the National Formulary with a credible plant-based topical alternative for treatment of fungal infections. Significantly, this endeavor aligns with the World Health Organization's advocacy for the integration of scientifically validated herbal medicines into formal healthcare systems[1]. Summarily, advancing the development of NIPRIFAN^{TM} holds considerable public health, pharmaceutical, socioeconomic, and scientific significance.

PROJECT GOALS AND OBJECTIVES

The overall **goal** of this project is to prepare our herbal antifungal ointment, NIPRIFAN^{TM}, for commercialization by scaling up production, validating stability, and confirming its safety and tolerability through a Phase I clinical trial for the management of fungal skin infections.res compliance with Good Manufacturing Practices (GMP), reproducibility and production of high-quality batches of the ointment

1. To carry out quality control and standardization of the ointment by establishing analytical parameters that ensures batch-to-batch consistency.

2. To conduct stability studies of the herbal antifungal ointment under various storage conditions following the ICH guideline to determine its shelf-life and optimal storage conditions.
3. To evaluate the safety, tolerability and preliminary clinical efficacy of the herbal antifungal ointment in patients with superficial fungal infections through Phase I clinical trials.
4. To generate scientific data for regulatory purposes that can facilitate wider approval, market expansion, and possible export of the product.
5. To build local capacity for the scientific development, testing, and commercialization of indigenous herbal medicines, especially those of topical formulations, thereby contributing to national self-reliance and healthcare improvement.

METHODOLOGY

a. Collection of raw materials and preparation of extract

The aerial parts of *Mitracarpus villosus* will be collected and validated as per earlier methods. The ethyl-acetate extract will be prepared by earlier standardized methods and stored appropriately.

b. Validation and optimization of the benchtop production of NIPRIFAN^{TM}

NIPRIFAN^{TM} is a topical ointment containing the ethyl-acetate extract of aerial parts of *Mitracarpus villosus*. The ointment, weighing 100 g per batch, will be prepared following already established standardized methods according to the composition in Table 1. Appropriate quantities of emulsifying wax, BP, would be melted over a water bath at 70- 80 ^{o}C, appropriate quantities of liquid paraffin will be heated to 70- 80 ^{o}C and the white soft paraffin will be melted in the paraffin at 70- 80 ^{o}C. The melted liquid paraffin mixture would be incorporated into the melted wax at the same temperature (70- 80 ^{o}C). The melted bases would be integrated in the appropriate quantity of extract and homogenized to give a consistent mixture. The prepared ointment (NIPRIFAN^{TM}) will be packaged into air-tight containers and stored until further analysis.

Table 1: Composition of ingredients for the preparation of NIPRIFAN™

Ingredients	Quantity
Extract of *M. villous* (g)	1.5
Emulsifying wax BP (g)	30
White soft paraffin (g)	48.50
Liquid paraffin (g)	20
Total (g)	**100**

c. **Pilot scale production of NIPRIFAN™**

The above method will be directly scaled up by a factor of 1000. In-process quality control will be carried out during production and packaging cycles. Final quality control and shelf-life determination will be carried out on the finished products randomly selected from the batches. The product of this pilot scale up production will be used for the clinical trial.

d. Quality control

Various parameters like color, consistency, texture, temperature monitoring and mixing speed would be evaluated during production. The final product would be evaluated for appearance, consistency, pH, viscosity, spreadability test, assay of active marker in extract (HPLC/UV) and microbial limit test.

e. Stability testing

Accelerated stability studies would be conducted on the prepared ointment according to ICH guidelines[9] for 6 months while real-time stability testing would be conducted at room temperature for 12 months. Samples would collected at 0, 3, 6 and 0, 3, 6, 9 and 12 months respectively for accelerated and real-time stability testing. Collected samples would be evaluated for appearance, consistency, pH, viscosity, assay of extract active marker and microbial limit test. Data obtained would be used to determine storage conditions and shelf-life of the product.

CLINICAL TRIALS; PHASE 1 FOR NIPRIFAN™

The goal of this Phase 1 trial is a randomized, double-blind, placebo-controlled ascending doses study to evaluate the safety, tolerability and preliminary clinical efficacy of NIPRIFAN™ in healthy subjects and subjects with atopic dermatitis.

Introduction

NIPRIFAN™ is a phytomedicine from the decoction of its aerial parts of *Mitracarpus villosus*. It has been studied and found to be indicated for the treatment atopic dermatitis and superficial fungal infections caused by *Tinea cruris* (ringworm, groin), *Tinea corporis* (ringworm, body) *Tinea capitis* (ringworm, head) and *Tinea uguium* (nail).

This is a Phase I clinical study of NIPRIFAN™ topical ointment an Investigational Medicinal Product (IMP). The current study is designed to evaluate the safety and tolerability, pharmacokinetics and preliminary efficacy of ascending doses of the NIPRIFAN™ in healthy volunteers and participants with atopic dermatitis. This is an ascending dose escalation study to test the safety, tolerability and preliminary efficacy of NIPRIFAN™ in first-in-human subjects. The safety, tolerability and pharmacokinetic data and results obtained from this study will determine the potentially efficacious doses of the NIPRIFAN™ topical ointment in the subsequent efficacy studies. Physical well-being, including laboratory investigations, will be closely monitored and evaluated.

Objectives

Specific Objective 1: To determine the safety of NIPRIFAN™ in healthy volunteers attending NIPRD Clinic, Abuja, Nigeria.

Specific objective 2: To determine the safety of NIPRIFAN™ in subjects with atopic dermatitis attending NIPRD Clinic, Abuja, Nigeria.

Research Question: Is NIPRIFAN™ safe to use for topical application in healthy adults and adults with atopic dermatitis?

Hypothesis: NIPRIFAN™ is safe for topical use when applied on an intact skin in healthy participants and those with atopic dermatitis.

Study site

The clinical trial will be carried out in NIPRD Research Clinic and other accredited laboratory. The use of appropriate health diary by the patients will be introduced in the study.

Study design

This is a two-part, randomized, blinded, vehicle-controlled study to determine a safe and tolerable dose of NIPRIFAN™. Part A will assess a single ascending dose of NIPRIFAN™ in cohorts of healthy volunteers, while Part B will assess multiple ascending doses of placebo/ NIPRIFAN™, to be determined (TBD) based on Part A safety and tolerability, in patients with mild-to-moderate dermatitis. Results from Part A and B will characterize safety, tolerability and pharmacokinetics. Part B patients will be assessed for changes in pruritus based on a numerical rating scale (NRS) of pruritus at baseline and on Day 15. Study duration is for 4 weeks with 2 weeks follow up. The participant and investigator will be blinded with the exception of unblinded dispensing pharmacy in part A. This same protocol will be same for part B as done for part A. The placebo will be a product with identical package, smell, and appearance as the investigational medicinal product (NIPRIFAN™).

Table 2: Arms and Interventions

Participant Group/Arm	**Intervention/Treatment**
PART A	
Experimental: dose to be determined NIPRIFAN™ or Placebo (6 subjects NIPRIFAN™, 2 subjects placebo)	
NIPRIFAN™ single application in 7 days or Placebo	NIPRIFAN™ or Placebo
NIPRIFAN™ (1st high dose) single application in 7 days or Placebo	NIPRIFAN™ or Placebo
NIPRIFAN™ (2nd high dose) single application in 7 days or Placebo	NIPRIFAN™ or Placebo
NIPRIFAN™ (3rd high dose) single application in 7 days or Placebo	NIPRIFAN™ or Placebo
NIPRIFAN™ (4th high dose) single application in 7 days or Placebo	NIPRIFAN™ or Placebo
PART B	
Experimental: dose to be determined NIPRIFAN™ Cohort 1 or Placebo (6 subjects NIPRIFAN™, 2 subjects placebo)	
NIPRIFAN™ Cohort 1 daily application for 15 days or Placebo	NIPRIFAN™ or Placebo
NIPRIFAN™ (1st high dose) Cohort 1 daily application for 15 days or Placebo	NIPRIFAN™ or Placebo
NIPRIFAN™ (2nd high dose) Cohort 2 daily application for 15 days or Placebo	NIPRIFAN™ or Placebo
NIPRIFAN™ (3rd high dose) Cohort 3 daily application for 15 days or Placebo	NIPRIFAN™ or Placebo

Inclusion Criteria

Part A - Healthy Volunteers:

- Written informed consent obtained prior to any required study-related procedure
- Healthy female or male subject aged 18 to 65 years
- Willing to use medically effective methods of birth control
- Females of reproductive potential must have a negative serum pregnancy test at screening and negative serum or urine pregnancy test prior to first study drug application on Day 1
- Non-smoker (no nicotine products for at least 6 months prior to screening)
- BMI ≥18.5 kg/m^2 and ≤32.0 kg/m^2 with minimum weight of 60 kg
- Used medications or skin emollients within 2 weeks prior to Day 1 unless approved by investigator and sponsor

Part B- Subjects with active atopic dermatitis (AD):

- Written informed consent obtained prior to any required study-related procedure
- Confirmed diagnosis of active atopic dermatitis (AD)
- History of AD for at least 6 months prior to Day 1 with an investigator global
- global assessment ≥3 and body surface area covered with 1-10 % AD
- Pruritus score (NRS) ≥ 5 at screening and NRS ≥7 on Day 1

Exclusion Criteria

Part A

- Pregnant or breast-feeding women
- Known allergy or hypersensitivity to any ingredient of NIPRIFAN® or to herbal components of NIPRIFAN®
- Skin disease that may interfere with study assessments
- Febrile illness within 6 days prior to Day 1, history of cancer within 5 years of Day 1, major surgery within 8 weeks prior to Day1, known immunodeficiencies, positive for hepatitis B or C or HIV infection
- Significant medical/surgical history or condition or current physical/laboratory/ECG/ vitals signs abnormality that might compromise the subject
- Corrected QT duration ≥450 milliseconds or other significant ECG abnormality
- Significant drug or alcohol abuse or mental illness in 2 years prior to Day 1
- Use of medications or skin emollients within 2 weeks prior to Day 1, unless specifically approved by the investigator and sponsor.

Part B

- Pregnant or breast-feeding women
- Known allergy or hypersensitivity to any ingredient of NIPRIFAN® or to herbal components of NIPRIFAN®
- Skin disease that may interfere with study assessments
- Febrile illness within 6 days prior to Day 1, history of cancer within 5 years of Day 1, major surgery within 8 weeks prior to Day1, known immunodeficiencies, positive for hepatitis B or C or HIV infection
- Significant medical/surgical history or condition or current physical/laboratory/ECG/ vitals signs abnormality that might compromise the subject
- Corrected QT duration ≥450 milliseconds or other significant ECG abnormality
- Significant drug or alcohol abuse or mental illness in 2 years prior to Day 1
- Evidence of infected atopic dermatitis
- Use of the following medications within 1 week prior to Day 1; Doxepin, hydroxyzine, or diphenhydramine, urea-containing topical products
- Use of systemic antibiotics, topical medicated treatments, or any systemic treatments that could affect AD within 2 weeks prior to Day 1.
- Receipt of phototherapy or laser treatment, including UV-B phototherapy, excimer laser, or psoralen-UV-A treatment within 4 weeks prior to Day 1.

Sample Size Determination

A total of 42 patients will be recruited and interviewed for this study. The required sample size was calculated using the formula described by Whitley & Ball[10] according to the following equation:

$n = Z^2P(1-d)/d^2$

Where: Z = 1.96, which corresponds to a 95 % confidence interval, P = 0.15; estimated prevalence rate of 15 %, d = 0.05; margin of error/precision of 5 %.

Study Procedure

A total of 42 healthy adult volunteers will be enrolled, balanced for age and sex. The study will be conducted in two sequential parts: Single Ascending Dose (SAD) and Multiple Ascending Dose (MAD).

Single Ascending Dose (SAD)

Four (4) cohorts of eight (8) healthy volunteers each will be recruited. Within each cohort, participants will be randomized in a blinded manner to receive either NIPRIFAN™ (n = 6) or placebo (n = 2). Four escalating dose levels of the investigational product will be evaluated. Safety and tolerability data from each cohort will be reviewed prior to dose escalation.

Multiple Ascending Dose (MAD)

Three (3) cohorts of eight (8) adult volunteers with mild to moderate atopic dermatitis will be recruited. Within each cohort, participants will be randomized in a blinded manner to receive either NIPRIFAN™ (n = 6) or placebo (n = 2). Three escalating dose levels of the investigational product will be evaluated, with safety review between cohorts.

Replacement of Participants

Additional volunteers will be enrolled if participants withdraw consent for reasons unrelated to safety. Replacement will also be allowed if an entire cohort requires repetition.

Study Intervention and Assessments

NIPRIFAN™ ointment will be applied in a measured quantity to the outer upper arm and gently rubbed into intact skin. Dosing will follow the Single Ascending Dose (SAD) and Multiple Ascending Dose (MAD) schedules as outlined in the study procedure. Safety will be closely monitored throughout both the SAD and MAD phases. Assessments will include:

- Recording of all adverse events and serious adverse events.
- Clinical laboratory investigations and pathology tests.
- Measurement of vital signs (blood pressure, heart rate, respiratory rate, temperature).
- Electrocardiogram (ECG) monitoring at scheduled time points.
- Physical examinations at each follow-up visit.

Pharmacokinetics

For the Single Ascending Dose (SAD), blood samples will be collected at pre-dose, and at 30 minutes, 1, 2, 4, 8, 12 and 24 hours post-dose, and at 36 hours post-dose on Day 2. For Multiple Ascending Dose (MAD), blood samples will be collected at pre-dose and at 30 minutes, 1, 2, 4, 8, 12, and 24 hours on Days 1 and 7; at pre-dose on Days 2–6; and at 36 hours after the final dose on Day 7.

Participant Health Diary

An appropriate health diary has been designed which will be completed daily by each participant for one month prior to the administration of the NIPRIFAN^{TM}. The participants are also expected to continue to complete the health diary for the duration of the clinical evaluation, which will last for 6 weeks.

Follow-Up Schedule

Participants will be followed up at 24 hours, 72 hours, one week, and then weekly for four weeks after administration. At each visit, a physical examination will be conducted, and any side effects or adverse events will be documented.

End-of-Study Investigations

At the end of six weeks, baseline investigations will be repeated, including:

- Urinalysis, urea and creatinine, random blood sugar.
- Full blood count with differential.
- Serum electrolytes.
- Liver function tests.
- Immunomodulatory and anti-inflammatory markers.
- ECG.
- Chest X-ray.

Pre-Entry Tests

Within 7 days prior to dosing (and as close to Day 1 as possible), the following assessments will be performed to establish patient eligibility and baseline values including;

- Medical history, complete physical examination
- Measurement of height, weight, and Body Mass Index (BMI)
- Vital signs assessment (blood pressure, pulse, respiratory rate, and temperature, ECG, chest X-ray
- Blood chemistry including electrolytes (Na, K, Cl, CO_2), renal function tests (BUN, creatinine), liver function tests (albumin, alkaline phosphatase, LDH, ALT, AST, total and direct bilirubin), glucose, cholesterol, serum iron, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR)

- Urinalysis: pH, Gram stain, color, glucose, protein, specific gravity, and cellular components.
- Parasitology: ova and parasites.
- Mycology: skin scrapings for fungal microscopy/culture.
- Dermatological Evaluation (for AD participants):
- Investigator's Global Assessment (IGA) score ≥ 3 and body surface area (BSA) involvement: 1–10%.
- Pruritus Numerical Rating Scale (NRS): ≥ 5 at screening and ≥ 7 on Day 1.
- Wood's lamp assessment of skin lesions to support diagnosis.
- HIV screening (may be performed earlier than 7 days if necessary).
- Serum or urine pregnancy test for females of reproductive potential.
- Confirmation of appropriate contraception use in female participants.
- Day 1 (Pre-dosing): Repeat measurement of vital signs immediately prior to administration of the investigational product.

Tests During Study, Recording

From Day 1 until the end of the treatment course (as outlined in the schedule of visits), the following evaluations will be performed and documented:

- Vital signs recorded immediately prior to dosing, and at 2 and 4 hours post-dose on Day 1.
- Weight, height, and vital signs will be measured at every study visit.
- A repeat 12-lead ECG will be performed within the first 4 weeks of the study.
- Hematology: Complete blood count with differential at baseline and at each visit.
- Blood chemistry: Sodium, calcium, potassium, chloride, bicarbonate (CO_2), BUN, creatinine, albumin, total protein, alkaline phosphatase, LDH, ALT, AST, total and direct bilirubin, glucose, and cholesterol — performed at baseline and repeated at each visit.
- Stool microscopy: Conducted at baseline and after completion of clinical evaluation.
- Dermatological Assessments (for participants with AD): Investigator's Global Assessment (IGA) score ≥ 3 with 1–10% body surface area (BSA) involvement.
- Pruritus Numerical Rating Scale (NRS) ≥ 5 at screening, and reassessed at Day 1 and Day 15.

- Wood's lamp examination of skin lesions to support diagnosis and monitor disease progression.

Parameters to be evaluated in the NIPRIFAN™ Phase I Clinical Trial

These include:

- Demographic and baseline characteristics: collection of participants' age, sex, weight, medical history, and other relevant baseline characteristics
- Vital signs: measurement of blood pressure, heart rate, respiratory rate, and body temperature at defined study time points
- Laboratory assessments: comprehensive laboratory investigations including hematology, blood chemistry, and urinalysis to monitor systemic safety
- Electrocardiogram (ECG): Assessment of cardiac rhythm and electrical activity to detect potential cardiac effects
- Safety and tolerability: critical observation documentation of adverse events (AEs) and serious adverse events (SAEs)
- Pharmacokinetics (PK): evaluation of absorption, distribution, metabolism, and excretion (ADME) of NIPRIFAN™ using validated pharmacokinetic markers
- Efficacy markers: reduction in lesion size and pruritus intensity will be assessed as exploratory endpoints
- Maximum Tolerated Dose (MTD): the highest dose of NIPRIFAN™ that can be safely administered will be determined.

Ethical Approval and Consent

This study will be conducted in strict compliance with ethical principles governing human research, including the Declaration of Helsinki and Good Clinical Practice (GCP) guidelines. The full study protocol, informed consent documents, and related materials will be submitted to the National Health Research Ethics Committee (NHREC), Federal Ministry of Health, Nigeria for review and approval. Informed consent will be obtained from all participants prior to their inclusion in the study. The evaluation procedure will be explained to each subject and a written consent will be signed or thumb-printed before enrolment for the evaluation. Subjects may withdraw voluntarily at any point of evaluation. The confidentiality, safety, and rights of participants will be strictly protected throughout the trial. Protocol amendments where applicable will be submitted to NHREC as required.

Adverse Events and Serious Adverse Events

Adverse events are any undesirable medical occurrence experienced by a participant during the study or within a reasonable period thereafter, regardless of whether the event is considered related to administration of the investigational product. All adverse events will be documented, monitored, and assessed for severity, duration, and relationship to treatment.

Serious adverse events are those events resulting in death, life-threatening conditions, hospitalization, persistent disability, or congenital anomaly. All such events will be reported appropriately to ensure timely communication and to safeguard the safety of participants across study sites.

Data Collection Forms

The following data collection forms for this clinical trial:

- Informed consent form
- Schedule of visits
- Demographic and personal information form
- Medical and prior history form
- Physical examination/vital signs, and symptom monitoring form
- Cardiac assessment form (ECG, Chest X-Ray)
- Hematology monitoring form
- Clinical chemistry monitoring form
- Microbiology monitoring form
- Adverse events and concomitant medication form
- Discontinuation/termination form

Table 3: Response Outcome Measures

Primary Outcome Measures	
Local tolerability (Primary safety)	Incidence and severity of application-site reactions (erythema, edema, scaling, vesiculation, ulceration), % participants with ≥1 reaction; max severity per participant
Overall adverse events (safety)	Number and proportion of participants with any adverse experience; number of serious adverse experiences
Secondary Outcome Measures	

Systemic safety	Change from baseline in CBC (WBC, Hb, platelets) and liver enzymes (ALT, AST); proportion with clinically significant abnormal results
Vital signs	Change from baseline in BP, HR, temperature
Mycological cure/fungal burden	Proportion with negative microscopy and/or culture at end of treatment; quantitative fungal load change
Clinical response of lesion	Change in lesion size (cm^2), scaling score, and Investigator Global Assessment (IGA)
Exploratory Outcome Measures	
Patient-reported symptom relief	Change in pruritus/burning VAS (0–100 mm); patient global satisfaction
Treatment acceptability/adherence	% doses applied (diary), ease of use, willingness to continue

DATA INTERPRETATION

Data generated from this project will be analyzed systematically across all stages. Data from formulation and scale-up studies will be analyzed analyzed using descriptive statistics (mean, standard deviation, and percentage variation) to assess batch-to-batch uniformity and reproducibility. Data from stability studies will be analyzed using graphical trend analysis, analysis of variance (ANOVA), and regression models to detect significant changes over time. Quantitative data (vital signs, laboratory parameters) obtained from the clinical trials will be summarized using descriptive statistics, while categorical data (adverse events, skin reactions) will be expressed as frequencies and percentages. Appropriate comparisons between baseline and post-treatment measures will be conducted using paired statistical tests while Chi-square test will be applied for categorical comparisons. All summed scores will be assessed for normality using Shapiro-Wilk test for normality. Statistical significance will be set at $p < 0.05$, and 95 % confidence intervals will be reported. All results will be compared with Pharmacopeial and internal benchmark specifications where applicable.

PRELIMINARY DATA

Preliminary data of microbial on efficacy of different batches of the extract of *Mitracarpus villosus* shows the ethylacetate extract has significant extended activity against *Trichophyton rubrum* and *Trichophyton schoeleenii* for 7 days. It also showed suppressive activity against *Aspergillus niger* and *Aspergillus fumigatus* for 72 hours. Other types of extracts including water, methanol and hexane extracts showed no inhibition of growth of the selected microorganisms.

TIMELINES WITH MILESTONES

	MONTHS																							
ACTIVITIES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Project Initiation and Team Meeting																								
Preparation of Standard Operating Procedures (SOPs)																								
Procurement of Raw material, Authentication, Procurement of Equipment																								
Raw material Processing and Phytochemical testing																								
Formulation Development and Pilot development, Physicochemical and microbiological analysis																								
Scale-up manufacture and Quality Control testing																								
Stability testing (Accelerated and Real-time)																								
Pre-clinical safety and Dermal testing																								
Regulatory and Ethics submission																								
Site Preparation and Staff Training																								
Phase I Trial Recruitment, Treatment and Follow-up																								
Data Analysis and Report Writing																								
Dissemination of Project Reports to Funding Agency																								

RESEARCH MANUFACTURING ENVIRONMENT

The manufacturing activities for this project will be conducted in the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, a Federal Government institution under the Federal Ministry of Health which is mandated to conduct research, development, and production of pharmaceutical products from indigenous resources. NIPRD is an ISO 17025:2017 certified laboratory, which provides assurance of quality management systems and compliance with global regulatory expectations for pharmaceutical research and production. NIPRD has qualified and experienced technical staff with different areas of specialization and expertise and a Research Clinic with experts where clinical trials had been conducted in the past. In addition, NIPRD has a a Drug Manufacturing Unit (DMU) that is fairly equipped for small- and medium-scale formulation, scale-up, and pilot production of pharmaceutical dosage forms and can be optimized to accelerate the translation of research findings into viable products such as NIPRIFAN^{TM}. Conducting the project within NIPRD's research manufacturing environment guarantees enhances the credibility of the project and lays a foundation for future scale-up and regulatory submission of such herbal products.

EXPECTED OUTCOMES AND BENEFITS

Upon successful completion of this project, a GMP-compatible pilot manufacturing process, validated quality control methods, and stability data supporting a defined shelf-life for a herbal antifungal ointment would have been developed. A Phase I clinical trial in patients with superficial fungal infections will provide critical human safety and tolerability evidence, and efficacy indications for subsequent clinical trials and eventual market authorization. In addition, this project will produce a regulatory dossier, lay the foundation for commercialization, supporting local pharmaceutical manufacturing leading to reduction in dependence on imported topical antifungal products.

DETAILED BUDGET FOR SCALE-UP PRODUCTION OF NIPRIFAN®

Description of Item	**Rate**	**Quantity**	**Total (N)**
Personnel Costs/Allowances			
Principal Investigator	2.5 % of total cost for the period of project	1	7,660,000
Team Members (co-investigators)	7.5 % of total cost for all co-investigators	6	15,320,000
Equipment			
Project laptop	2,500,000	2	5,000,000
External hard disc	200,000	2	400,000
Project printer/photocopier	1,000,000	1	1,000,000
Digital pH meter	300,000	1	300,000
Climatic chamber	15,000,000	1	15,000,000
Viscometer	10,000,000	1	10,000,000
Homogenizer	15,000,000	1	15,000,000
Rotary evaporator	5,000,000	1	5,000,000
Soxhlet extractor apparatus	1,000,000	2	2,000,000
Supplies/Consumables			
Collection of Plant material	400,000	50 kg	400,000
Plant identification, herbarium specimen production and pharmacognostic evaluation	200,000		200,000
Clean-up, processing, drying and milling of raw materials	100,000		100,000
Extraction:			
Solvent; n-hexane	50,000	50 bottles	2,500,000
Solvent; ethyl acetate	50,000	50 bottles	2,500,000
Filtration and storage units for extracts			1,500,000
Microbial Limit Test: Media, Test Reagents, Consumables			5,000,000
Purchase of standard microorganism; *T. Rubrum* ATCC 28188	750,000 each	1	750,000
Purchase of standard microorganism; *C. albicans* ATCC 10231	750,000 each	1	750,000
Purchase of standard microorganism; *M. canis* ATCC 36299	750,000 each	1	750,000
Safety/Toxicity testing			2,000,000
Formulation/Scale-up production			

Materials for Formulation studies (emulsifying wax, white-soft paraffin, liquid paraffin etc)	1,000,000		1,000,000
Materials for scale –up production	7,000,000		7,000,000
Containers for packaging and labelling	3,000,000		3,000,000
PPEs (coat, goggles, shield, gloves, etc)	1,500,000		1,500,000
Stability evaluation			
Stability studies and Determination of shelf life	5,000,000		5,000,000
Data Collection & Analysis			
Research Assistants for plant collection, scale-up production	20,000 daily	10 days x 2 people	400,000
Research Assistants for scale-up production	20,000 daily	120 days x 5 people	9,600,000
Phase 1 Clinical trial			
Study Personnel Cost; Principal Investigators, Co-Investigators, Site-PIs, Lab scientists, Nurses, Dermatologist, Pharmacists, Social workers, and consultant, Administrative Assistants, Clinical Research Assistant, Data Entry personnel			25,000,000
Pre-study Activities: Protocol development/ Review Stakeholders Meetings Ethical Approvals Protocol Training / Facilitators Meeting			 6,000,000 4,000,000 5,500,000 5,500,000
Trial Study: Participants Screening and Enrolment (sample collection, lab tests and examination) Follow-up (sample collection, lab tests and examination) Participant logistic cost (refund of transport and lost man-hour)			 10,000,000 10,000,000 3,000,000
Monitors/ Regulatory compliance: NAFDAC and DSMB			4,000,000
Logistics: Samples and personnel movement, and communication.			5,000,000
Digital entry devices Software and storage			3,500,000 3,000,000
Clinical record materials and stationeries			5,000,000
Others			
Data Analysis			5,000,000
Report writing and Review meetings			5,000,000
Dissemination: Workshop/seminars/Conference			10,000,000
Internet services, Logistics, Stationaries			5,000,000

TOTAL DIRECT COST			204,900,000
INDIRECT COST			10245000
GRAND TOTAL			235,635,000

INVESTIGATOR'S CONTRIBUTIONS

S/No.	Name	Highest Qualification	Area of Specialization	Contribution
1.	Dr. Olubunmi J. Olayemi (Principal Investigator)	Ph.D.	Pharmaceutical Technology, Formulation and Quality control of phytomedicines	Pilot scale production of NIPRIFAN®. General co-ordination of project
2.	Dr. Jemilat Ibrahim (Co-investigator)	Ph.D.	Medicinal Plant Research and Traditional Medicine	Raw material sourcing and extraction
3.	Dr. Mercy I. Aboh (Co-investigator)	Ph.D.	Pharmaceutical Microbiology and Biotechnology	Microbiological studies including microbial limit and efficacy test
4.	Pharm. Ekere E. Kokonne (Co-investigator)	M. Pharm.	Pharmaceutical Technology and Stability evaluation of Phytomedicines	Stability Studies and Determination of Shelf-life
5.	Pharm. Ajeh J. Isaac (Co-investigator)	B. Pharm.	Pharmaceutical Technology	Formulation Studies and Quality Control
6.	Dr. Margaret Ekpenyong (Co-investigator)	MB.BS	Therapeutics and Clinical Trials	Principal investigator /Coordinator of the clinical trials

SOME of NIPRD's PUBLISHED ARTICLES SHOWING EFFECTIVENESS OF *Mitracarpus villosus*

1. Aboh, M. I., Olayinka, B. O., Adeshina, G. O., Oladosu, P. and Ibrahim, K. (2015). I*n vitro* Antifungal Efficacies of Ethyl Acetate Fractions of *Mitracarpus villosus* from Abuja, Nigeria. British Microbiology Research Journal 7(3): 151-158.

2. Aboh, M. I., Olayinka, B. O., Adeshina, G. O. and Ibrahim, K. (2014). Preliminary studies on the antifungal activities of the successive extracts from *Mitracarpus villosus* (Sw.) DC from Abuja, Nigeria. Journal of Microbiology Research, 4(2): 86-91.

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3. Arastehfar, A., Carvalho, A., Houbraken, J., Lombardi, L., Garcia-Rubio, R., Jenks, J. D., … Perlin, D. S. (2021). The quiet and underappreciated rise of drug-resistant invasive fungal pathogens. *Nature Reviews Microbiology, 19*(9), 619–637. https://doi.org/10.1038/s41579-021-00502-w
4. Bongomin, F., Gago, S., Oladele, R. O., & Denning, D. W. (2021). Global and multi-national prevalence of fungal diseases—estimate precision. *Journal of Fungi, 7*(1), 17. https://doi.org/10.3390/jof7010017
5. Taiwo, B. J., Adedayo, B. C., Adenowo, T. K., Akinsanya, A. A., Ajagun-Ogunleye, O., & Olagunju, J. O. (2022). Phytochemical and GC–MS analysis of *Mitracarpus villosus* flowers. *Journal of Biology, Agriculture and Healthcare, 12*(10), 34–45.
6. Binoodha Remina, A., Subramanian, M., Praveen, R., & Sundar, R. (2022). Phytochemical profiling and antifungal activity of *Mitracarpus hirtus* extracts: FTIR and GC–MS analysis. *Journal of Applied Pharmaceutical Science, 12*(7), 45–53. https://doi.org/10.7324/JAPS.2022.120707
7. Arhewoh, I. M. (2015). Formulation and evaluation of herbal antifungal creams from *Mitracarpus villosus*. *Journal of Pharmacy & Bioresources, 12*(2), 99–106.
8. Fawehinmi, A. B., & Oyedeji, O. (2020). Antifungal activity of topical creams formulated with *Mitracarpus villosus* extracts. *International Journal of Biochemistry Research & Review, 29*(3), 1–9. https://doi.org/10.9734/ijbcrr/2020/v29i330171
9. World Health Organization (WHO), 2009. Stability testing of active pharmaceutical ingredients and finished pharmaceutical products; WHO Technical Report Series, No. 953, World Health Organization, Geneva.
10. Whitley, E., & Ball, J. (2002). Statistics review 4: Sample size calculations. Critical Care, 6(4), 335-341. https://doi.org/10.1186/cc1521