NATIONAL RESEARCH FUND (NRF) TETFUND

RESEARCH PROPOSAL

Principal Investigator
Email:
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0.1 Research Project Category/Thematic Area: Science, Technology and Innovation/Health and Social Welfare

0.2 Project Title: Identification and Development of Potential Drug from Nigerian Medicinal Plants against Coronavirus Disease (COVID-19)

0.3 Executive Summary:

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 has metamorphosed into a global pandemic with millions of cases reported globally leading to over 0.5 million deaths, depression in global economies, and exerting a greater toll in the socio-economic lives of millions of people worldwide.

So far as its entry and pathogenicity is concerned, SARS-CoV-2 enters into cells through binding of its spike proteins with host cell receptors, Angiotensin converting enzyme 2 (ACE2) and leads to down regulation of the ACE2 receptor. This leads to viral entry and replication, as well as severe lung injury. The occurrence of COVID-19 elevated intense attention not only within Nigeria but internationally. The available data suggests that there is no specific treatment in modern medicine. Therefore, there is an urgent need for its management and prevention.

In our preliminary studies, some medicinal plants appear to be effective in treating viral infection by enhancing inhibitory activity. Therefore we hypothesized that there are components in Nigerian medicinal plants with high potential for anti-SARS-CoV-2 activity. We propose to isolate and evaluate the active principles from selected medicinal plants with confirmed antiviral activity and to develop a conventional dosage form that will not only control viral infections but also reduces the associated complications. The proposed research serves to answer several interlinked questions with the prime focus and objective to discover new bioactive compound(s) as well as chemical structural elucidation.

The short term goals of this study is to develop experimental models for evaluating antiviral activity of extracts, fractions and phytochemical constituents from medicinal plants, with a view to providing new pathways leading to the discovery of drug candidates from our indigenous medicinal plants. The long term goal is to develop dosage form that will be used in the treatment/management of COVID-19 and associated infections. This project will use a combined natural product isolation and virtual drug screening approach to uncover and identify several biologically active agents.

The estimated duration of the project is 24 months and will be carried out in four phases; Phytochemical, Biological, *In silico* studies and product development. The first phase will involve the phytochemical screening of the plants to test for the presence of secondary

metabolites; proximate parameters determination; chemical fractionation of plant extracts using different chromatographic techniques to isolate pure compounds, and structure elucidation of the isolated compounds with the use of various spectroscopic techniques. The second phase will involve the safety/toxicity evaluations of the plants extracts and isolated compounds *in vitro* using normal cell lines and *in vivo* using rodents. This will be followed by *in vitro* antiviral activity screening against SARS-CoV. The third phase will apply a combined *in silico* methods (virtual drug screening) to screen molecules isolated from Nigerian medicinal plants to identify novel drug candidates against SARS-CoV-2. Phase 4 which is product development and optimization will utilize state-of-the-art nanotechnology equipment to enhance drug likeness of most potent/active compound(s).

At the end of the study, it is expected that a scientifically proven indigenous medicine obtained from Nigerian medicinal plants for the management/treatment of COVID-19 would be presented and this will pave new insights for the discovery and development of anti-infective agents from our indigenous medicinal plants. Major results will be patented with priority for technological transfer and then published to protect intellectual property for possible industrial application. This project will have direct implications for policy design and execution to control the global emergence, a challenge that transcends national and international borders. The estimated budget of the project is Forty-seven million two hundred and forty-four thousand five hundred Naira (₦47, 244,500.00).

0.4 Keywords: Antiviral activity; COVID-19; Herbal remedies; Medicinal plants

0.5 Project Duration: Twenty-four (24) Months (From January 2021 to December 2022)

1.0 GENERAL BACKGROUND OF THE RESEARCH PROJECT

1.1 Background and Problem Statement:

The novel coronavirus disease 2019 (COVID-19), resulted in an outbreak of pathogenic viral pneumonia in Wuhan, Hubei Province, China, as reported to the World Health Organization (WHO) in December 2019. The subsequent spread has led to a global pandemic (officially declared by the WHO on March 11, 2020 (WHO, 2020). COVID-19 disease appears to be a spectrum of clinical presentations ranging from asymptomatic to severe respiratory failure. Initial case analysis from China through mid-February 2020 found 14% of cases were associated with severe disease and 5% of cases were critical (i.e., respiratory failure, septic shock, and/or multiple organ dysfunction or failure) (Wu *et al*., 2020). A more extensive metaanalysis found a slightly higher severe disease percentage (20.3%), (Rodriguez-Morales *et al*., 2020). The disease case fatality rate (CFR) varies depending on region, population demographics, and healthcare capabilities; for instance, in Italy an overall CFR of 7.2% is estimated, in part driven by the higher proportion of individuals of advanced age compared to China (Onder *et al*., 2020). Based on global data, the CFR from COVID-19 based on confirmed cases is estimated to be ∼6.9% (Dong *et al*., 2020).

The COVID-19 pandemic is ravaging the entire globe with over 500 thousand deaths and up to 12 million positive cases. As of July 08, 2020, the ongoing COVID-2019 pandemic has swept through 217 countries/territories and infected more than 11 million individuals (WHO COVID-19 Situation Report-170), posing an unprecedented threat to international health and the global economy. According to the Nigerian Centre for Disease Control (NCDC), a total number of confirmed cases in Nigeria as at 8th July 2020 were 30,249 with 684 deaths (NCDC COVID-19 Report). The management of the disease condition using known and established orthodox

drugs are basically to provide supportive therapy. Some drugs like hydroxychloroquine, azithromycin and Remdesivir have been used to treat the disease. Some of these antiviral agents might produce toxic side-effects. The development of viral resistance toward antiviral agents enhances the need for new effective compounds against viral infections. Thus, new antiviral agents exhibiting different mechanisms of action are urgently needed.

The use of natural products including medicinal plants has become more and more important in primary health care especially in developing countries. Millions of people use herbal medicine for the management of disease conditions. WHO 2014 supports the use of traditional medicine for the management of diseases provided they are proven to be efficacious and safe. It has been observed that the use of traditional herbal medicines, especially in the tropics and African countries for the management of COVID-19, is on the increase (the case of Madagascar). Therefore, it is necessary to look inwards for the search of novel and effective herbal medicine for the effective management of COVID-19.

However, there is a lack of adequate research on the development of anti-coronaviruses agents from such natural products. Such agents are not only important to combat coronaviruses, but also play an important role to prevent a viral attack. Some natural products have been found to exhibit their antiviral activity through the inhibition of viral replication (Moghadamtousi *et al.,* 2015; Oliveira *et al.,* 2017). Therefore, we hypothesized that there are components in Nigerian medicinal plants with high potential for anti-SARS-CoV-2 activity.

Some plants e.g. *Annona muricata*, *Persea americana*, *Lycoris radiate*, *Azadirachta indica*, *Macaranga barteri* and *Andrographis paniculata* have been reported to have inhibitory activity against several pathogenic viruses, including other respiratory viral infections (Parida *et al*., 2002; Li *et al*., 2005; Falodun *et al*., 2014; Ogbole *et al*., 2018; Silva *et al.,* 2020). Also, several individual essential oil components of Garlic (*Allium sativum*), Ginger (*Zingibber officinale*) have been screened for antiviral activity (Thuy *et al*., 2020; Silva *et al.,* 2020). Recently in our preliminary study we found that *Annona muricata* leaf has proven useful in the successful management of COVID-19 and associated symptoms (Unpublished data). For this reason, research for the evaluation of potentially active compounds from active medicinal plant extracts will be designed to overcome COVID-19. The active compound(s) will be traced for its ability to inhibit the infection process of COVID-19 *in silico* with molecular docking simulations. This study will be conducted using molecular docking analysis of the major component of the plants that exhibit antiviral activity with known SARS-CoV-2 protein target (3CL-pro) and Angiotensin Converting Enzyme 2 (ACE2) - the host cell receptor for SARSCoV-2 which play critical role in establishing the infection. The goal of the research is to identify bioactive molecules from Nigerian indigenous medicinal plants with high potential for use against SARSCoV-2, the causative virus for the newly emerged COVID-19, and develop these molecule(s) for preclinical and phase '0' clinical trial.

1.2 Research objectives: The general objective of the research is to identify potential drug(s) against COVID-19 from Nigerian medicinal plants using a combined approach of natural product isolation and virtual drug screening, and develop highly potent molecule(s) for preclinical and phase '0' clinical trial.

The specific objectives are to: -

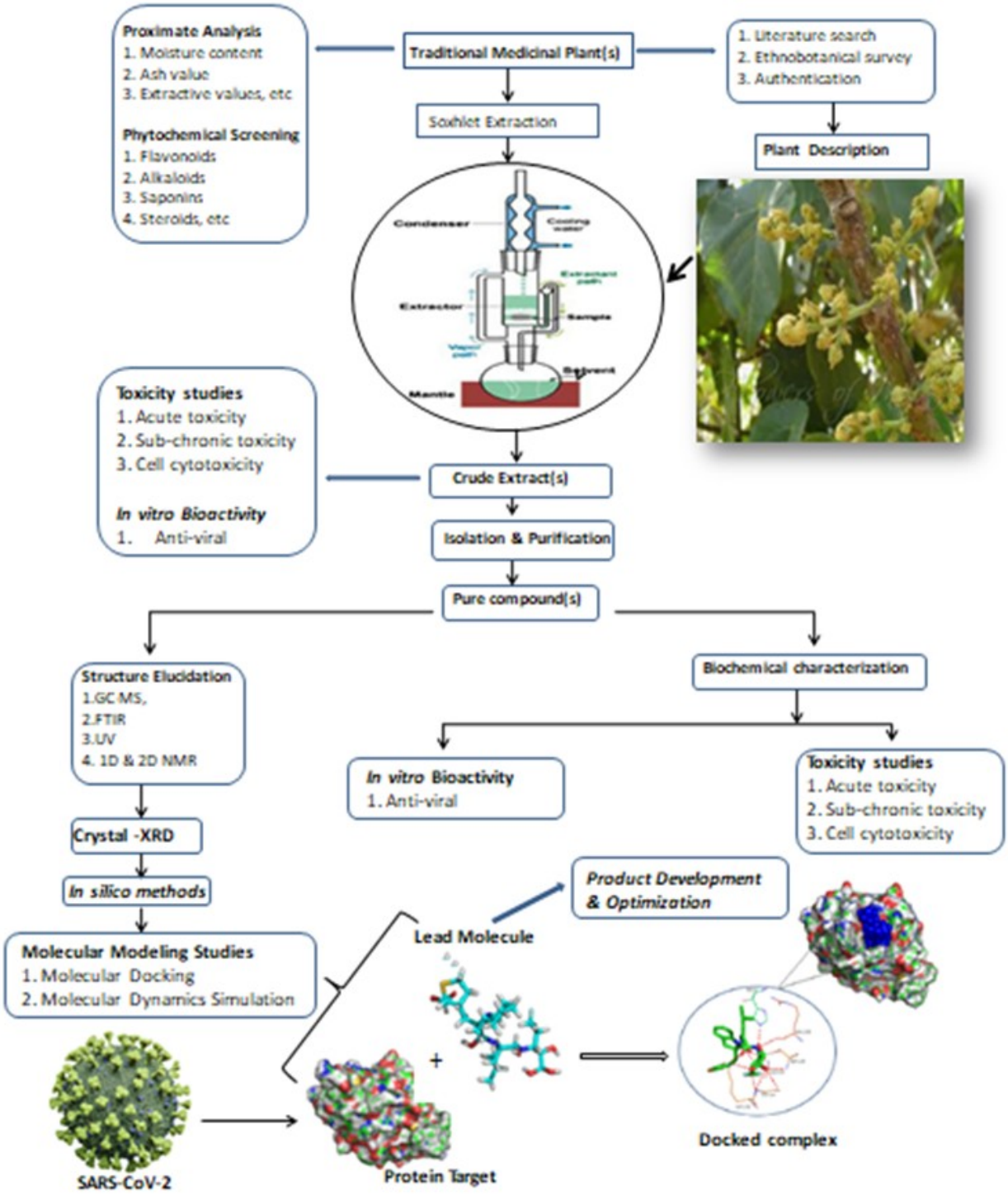
i. screen for the phyto-constituents and proximate parameters of selected medicinal plants with known or reported antiviral activity.
ii. evaluate the *in vivo* toxicity profile and *in vitro* cytotoxicity of the plant extracts. iii. confirm the antiviral activity of the plant extracts.
iv. isolate and elucidate the chemical structures of the potentially active antiviral compounds from the plants.
v. evaluate the *in vivo* toxicity profile and *in vitro* cytotoxicity of the isolated compounds.

vi. evaluate potentially highly active compounds for *in vitro* inhibitory activity against SARS-CoV.
vii. explore the molecular interactions of the compounds with potent antiviral activity against SARS-CoV-2 major protease (3CLpro) and the human host cell receptor – Angiotensin-Converting Enzyme 2 protein *in silico*.
viii. optimize highly potent compound(s) as candidate drug(s)

1.3 Statement of the Problem

COVID-19 is one of the most alarming diseases in the globe right now. Many people have been infected with the novel coronavirus (SARS-CoV-2), and the death toll has reached hundreds of thousands globally and has been increasing. As of July 08, 2020, the on-going coronavirus disease 2019 (COVID-2019) pandemic has swept through 217 countries/territories and infected more than 11 million individuals globally (WHO COVID-19 Situation). The current management of COVID-19 is mainly supportive, although, the medical literature has reported numerous potential therapies, including immune-modulatory agents, antiviral therapy, and convalescent plasma transfusion. Given the pandemic spread of the disease and the resulting global economic loss and the lack of specific treatment for patients with SARS-CoV-2 infection, the need to developing alternative agents to contain the virus is imperative. Natural products have proved to be an important source of lead molecules. From our survey, many extracts and compounds of plant origin have been reported to have antiviral activity and some Traditional Medical Practitioners have achieved success with the use of medicinal plants in the management of COVID-19, this has raised optimism about the future of phyto-antiviral agents, especially of plants origin. With the great diversity of plants growing in Nigeria and their healing properties offers interesting possibilities of finding novel antiviral compounds of natural origin for the prevention and treatment of the COVID-19. It will therefore be proper to obtain new drugs from these plants.

1.4 Conceptual framework of the Study: Active antiviral compound(s) isolated from Nigerian medicinal plants will be traced for its ability to inhibit the infection process of COVID-19 *in silico* with molecular docking simulations. This study will be conducted using molecular docking analysis of the major component of the plants that exhibit antiviral activity with known SARS-CoV-2 protein target (3CL-pro) and Angiotensin-Converting Enzyme 2 (ACE2) – the host cell receptor for SARS-CoV-2 which play a critical role in establishing the infection (Scheme 1).



Scheme 1: Work scheme for the extraction, isolation and spectroscopic characterization of antiviral compounds from Nigerian medicinal plants and *in silico* screening.

1.5 Project Goals Short-term:

a) Establish a resource for the acquisition, screening, and preclinical development of new COVID-19 agents, including identification of active compounds from herbal medicines and natural products.

Long-term

a) Provide additional resources for the design, development, and preclinical testing of inhibitory compounds by the COVID-19 research community.
b) Transition drugs with demonstrated efficacy into implementation within national control programs, in partnership with local authorities, international development programmes, and other relevant entities.
c) Identify commercial partners for new drugs and evaluate additional drug candidates in clinical development programmes.

1.6 Project Impact

Social benefits

a) Nigerian medicinal plants with potent antiviral activity would have been identified and validated.
b) This study provides relevant and reliable information about drug efficacy in the study area and is therefore beneficial for participants.

Economic benefits

c) The study will provide an answer to the question which drug will be most valuable in treatment of COVID-19 patients.
d) Local personnel and students can be trained during the project and in this way the community will benefit further from increased knowledge based and experience.

Technological benefits

e) The discovery and development of antiviral agents from indigenous medicinal plants for the management of COVID-19 infection.

2.0 RESEARCH DETAILS

2.1 Literature Review

Coronaviruses are a family of enveloped viruses with a positive sense, single-stranded RNA genome that infects animal species and humans. Among coronavirus, members are those responsible for the common cold, severe acute respiratory syndrome coronavirus (SARS), Middle East respiratory syndrome-related coronavirus (MERS), and the recently emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, the causative pathogen of the disease COVID-19) (Andersen *et al*., 2020). Coronaviruses primarily cause respiratory and intestinal infections in animals and humans (Fields *et al*., 2013). Discovered in the 1960s, they were originally thought to be only responsible for mild disease, with strains such as HCoV 229E and HCoV OC43 responsible for the common cold (Geller *et al*., 2012). That changed in 2003 with the SARS pandemic and in 2012 with the outbreak of MERS, both zoonotic infections that resulted in mortality rates greater than 10% and 35%, respectively (Song, 2019). Both coronaviruses likely emerged from native bat populations, which maintain a broad diversity of coronaviruses, and were transmitted through an intermediate host to humans. Loss

of natural habitat and increased exposure to new hosts are likely responsible for the increased frequency of zoonotic infections originating from bats (Menachery *et al*., 2017; Omrani *et al*., 2015). Evidence also supports that the novel coronavirus which emerged in the Wuhan region of China in late 2019 also originated from bats (Zhou *et al*., 2020).

This novel coronavirus, SARS-CoV-2, resulted in an outbreak of pathogenic viral pneumonia in Wuhan, Hubei Province, China, as reported to the World Health Organization (WHO) in December 2019. The subsequent spread has led to a global pandemic (officially declared by the WHO on March 11, 2020 (WHO, 2020). COVID-19 disease appears to be a spectrum of clinical presentations ranging from asymptomatic to severe respiratory failure. Common symptomology at the onset of illness are fever, cough, and general myalgia, with less common symptoms including sputum production, headache, and diarrhoea (Chen *et al*., 2020; Huang *et al*., 2020; Li *et al*., 2020). Initial case analysis from China through mid-February 2020 found 14% of cases were associated with severe disease (dyspnea, respiratory frequency ≥ 30/min, blood oxygen saturation ≤ 93%, the partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300, and/or lung infiltrates > 50% within 24 - 48 hours), and 5% of cases were critical (i.e., respiratory failure, septic shock, and/or multiple organ dysfunction or failure) (Wu *et al*., 2020). A more extensive meta-analysis found a slightly higher severe disease percentage (20.3%), (Rodriguez-Morales *et al*., 2020). The disease case fatality rate (CFR) varies depending on region, population demographics, and healthcare capabilities; for instance, in Italy an overall CFR of 7.2% is estimated, in part driven by the higher proportion of individuals of advanced age compared to China (Onder *et al*., 2020). Based on global data, the CFR from COVID-19 based on confirmed cases is estimated to be ∼6.9% (Dong *et al*., 2020).

Presently, the COVID-19 pandemic is ravaging the entire globe and the management of the disease condition using known and established orthodox drugs are basically to provide supportive therapy. Some drugs like hydroxychloroquine, azithromycin and Remdesivir have been used to treat the disease. Remdesivir's antiviral activity, sterically interacting with the viral RdRp to induce delayed chain termination, has been demonstrated *in vitro* against multiple coronaviruses (SARS, MERS, contemporary human CoV and bat-CoVs (Sheahan *et al*., 2017). Some of these antiviral agents might produce toxic side-effects. The development of viral resistance toward antiviral agents enhances the need for new effective compounds against viral infections. Thus, new antiviral agents exhibiting different mechanisms of action are urgently needed.

The use of natural products including medicinal plants has become more and more important in primary health care especially in developing countries. Millions of people use herbal medicine for the management of disease conditions. WHO 2014 supports the use of traditional medicine for the management of diseases provided they are proven to be efficacious and safe. It has been observed that the use of traditional herbal medicines, especially in the tropics and African countries for the management of COVID-19, is on the increase (the case of Madagascar). Therefore, it is necessary to look inwards for the search of novel and effective herbal medicine for the effective management of COVID-19, cannot be over-emphasized. Natural products serve as chemical scaffolds for derivatization to come up with novel compounds with improved pharmacological features. Surveys of the National Cancer Institute, USA, repeatedly demonstrated that three-quarters of drugs for all diseases worldwide during the past half-century were in one way or another based on natural resources (Newman and Cragg, 2016). Hence, chemical scaffolds from natural sources are indispensable for drug development. Nature provides a vast library of chemicals to explore and develop drugs for the treatment of various ailments including viral diseases (Denaro *et al.,* 2019). To date, a good number of herbal medicines or their constituents have shown potential antiviral activity (Lin *et al.,* 2014). However, there is a lack of adequate research on the development of anticoronaviruses agents from such natural products. Such agents are not only important to combat coronaviruses, but also play an important role to prevent a viral attack. Some natural

products have been found to exhibit their antiviral activity through the inhibition of viral replication (Moghadamtousi *et al.,* 2015; Oliveira *et al.,* 2017). Some plants e.g. *Annona muricata*, *Persea americana*, *Lycoris radiate*, *Azadirachta indica*, *Macaranga barteri* and *Andrographis paniculata* have been reported to have inhibitory activity against several pathogenic viruses, including other respiratory viral infections (Parida *et al*., 2002; Li *et al*., 2005; Falodun *et al*., 2014; Ogbole *et al*., 2018; Silva *et al.,* 2020). Also, several individual essential oil components of Garlic (*Allium sativum*), Ginger (*Zingibber officinale*) have been screened for antiviral activity (Thuy *et al*., 2020; Silva *et al.,* 2020). Recently, *Annona muricata* leaf has proven useful in the successful management of COVID-19 and associated symptoms (Personal communication).

This project could produce one of such new drugs whose efficacy could enhance the socio-economic conditions of residents in our locality and in these regions. Given the rich vegetation available in the rural areas, obtaining COVID-19 remedy from a plant growing in these areas will not only increase its acceptance but it will also greatly boost morale and pave way for further rural development.

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2.2 Research Methodology

Chemicals, Equipment and Software

All analytical-grade chemicals (solvents and reagents) to be used for this study will be obtained from Sigma-Aldrich Co. they will be used without further purification. The major equipment to be used includes soxhlet apparatus, melting point apparatus, refrigerator, centrifuge, Haematology analyzer, polarimeter, Rotary Evaporator, Freeze Dryer, Oven, Furnace, Ultraviolet-Visible (UV-VIS) spectrophotometer, Atomic Absorption Spectrophotometer (AAS), Flame Photometer, Fourier Transform Infrared (FTIR) spectrophotometer, Nuclear Magnetic Resonance (NMR) spectrophotometer, Gas Chromatography-Mass Spectrometer (GC-MS), High-Performance Liquid Chromatography (HPLC), Microplate reader, Scanning Electron Microscope (SEM), X-Ray Diffractometer (XRD), Zeta-potential Analyzer (ZPA), Differential Scanning Calorimeter (DSC), Karl Fisher Moisture Titrator (KFT), AutoDock VINA and SAS Visual Analytics Software.

Phytochemical Studies

Plant collection, authentication and drying

Selected plant materials based on the scientific validated antiviral activity will be collected from different localities in Nigeria. The plant materials will be identified and authenticated in the Forestry Research Institute of Nigeria (FRIN), Ibadan. The plant materials will be air-dried, powdered and stored in an air-tight container until ready for use.

Phytochemical screening

Simple chemical tests to detect the presence of secondary metabolites such as alkaloids, tannins, saponins, terpenoids, steroids, anthraquinones, flavonoids and other phenolic compounds will be done according to standard methods (Sofowora, 1982; Evans, 2002).

Proximate analysis of crude herbal materials

The proximate analysis will be carried out to determine the quantative and proximate parameters of the crude plant materials. This will be done according to standard procedures (AOAC, 1984; British Pharmacopoeia 1986). Parameters to be investigated includes; water loss on drying, ash content, extraneous matter, mineral content, and limit tests for heavy metals.

Extraction and isolation

The powdered plant materials (2 - 3 kg) will be extracted separately with methanol by soxhlet extraction. The extracts will be concentrated using a rotary evaporator at reduced pressure. The crude extracts will be subjected to silica gel vacuum liquid chromatography (VLC) using organic solvents in order of increasing polarity [hexane, ethyl acetate, ethyl acetate and methanol in suitable proportions].

The Thin-layer chromatographic (TLC) profile of the VLC fractions will be determined using a commercially available precoated F_{254} silica gel plates. Selected fractions based on their yield and TLC profile will be separated by silica gel column chromatography using appropriate eluting solvents. The column fractions will be monitored by thin-layer chromatography. The plates will be visualised under UV lamp (254 nm and 366 nm), sprayed with appropriate spray reagents and dried with hot air from a handheld dryer.

Semi-purified column fractions will be purified by repeated silica gel column chromatography, and where necessary, Sephadex LH-20 column chromatography, preparative thin-layer chromatography (PTLC) and recycling preparative high-performance liquid chromatography (RP-HPLC). Some semi-purified fractions will also be subjected to gas chromatographic-mass spectrometric (GC-MS) analysis to identify metabolites present.

Characterisation of isolated compounds

Characterization of the pure isolated compounds will be done using various spectroscopic techniques including one and two-dimensional Nuclear magnetic resonance (^{1}H-NMR, ^{13}CNMR, DEPT, COSY, TOCSY, HSQS, NOESY and HMBC) spectroscopy, High-resolution mass spectrometry, Ultraviolet and Infra-red spectrophotometry. Physical characteristics such as melting point and optical rotation will also be determined.

Biological Investigations

Toxicity profile of the plant extracts and isolated compounds

The toxicity profile viz-a-viz the acute, sub-chronic as well as the cytotoxicity of the plant extracts and the isolated compounds will be carried out to predict their safety when consumed.

Experimental animals

Adult Swiss albino mice and Sprague Dawley rats of either sex will be obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals will be kept under a 12-hour light/dark cycle in clean and well-maintained cages for two weeks to acclimatize to the laboratory environment. The animals will be fed with standard rodent pellets, and allowed access to water ad libitum.

Acute toxicity screening

The acute toxicity screening will be carried out according to Lorke's method (Lorke, 1983). Nine adult mice of either sex divided into three groups of three animals per group will be used in the phase. The different plants extracts and compounds will be administered orally at doses of 10, 100, and 1000 mg/kg to groups I, II, and III, respectively. In the second phase of the study, another three mice divided into three groups of a mouse each will be used. The plants extracts and compounds will be administered at doses of 1600, 2900, and 5000 mg/kg to each group, respectively. General symptoms of toxicity and mortality in each group will be observed within 24 hours and then for another 14 days for delayed toxicity. This will be replicated for each plant and or plant species.

Sub-chronic toxicity studies

Chronic toxicity studies will be carried for 28 days. Animals (rats) will be randomly allotted into 4 groups (control and 3 tests groups) of 5 rats per group.

Group A: animals will receive distilled water

Groups B: animals will receive 100 mg/kg of the extracts or compounds.

Groups C: animals will receive 400 mg/kg of the extracts or compounds.

Groups D: animals will receive 800 mg/kg of the extracts or compounds.

The extracts or compounds will be administered daily to the animals for 28 days. During the experimental period, the animals will be monitored for general and behavioural signs of toxicity, body weight changes and mortality for the duration of the experiment. At the end of the 28-day treatment period, animals will be sacrificed under chloroform anaesthesia. Blood samples will be collected by cardiac puncture into heparinized sample bottles. Internal organs (liver, kidney, heart, spleen and lungs) will be collected, weighed and stored in 20 mL sample bottles containing 10% formalin. The organs will be processed for histopathological studies. From the blood samples collected, the following haematological parameters will be determined: red blood cell count (RBC), packed cell volume (PCV), haemoglobin concentration (Hb), and platelet count (PLT), total white blood cell (WBC) counts using Automated Haematology Analyzer. The blood in the heparinized tubes will be spun in a centrifuge and the following biochemical parameters will also be determined in the serum: alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, bicarbonate, total proteins, albumin, total and conjugated bilirubin.

Evaluation of the cytotoxicity and antiviral activity Cell cultures and virus

Mardin-Darby bovine kidney (MDBK) cells, MA104 cells, and HEp-2 cells will be cultivated in Minimal Eagle's Medium (MEM) containing penicillin (100U/ml), streptomycin (100µg/ml), and fungizon (2.5 µg/ml); and supplemented with 10% horse serum. The viral strains bovine viral diarrhoea virus (BVDV), human respiratory syncytial virus (HRSV), and human rotavirus (HRV) will be purchase from culture collection bank. Viral stocks will be prepared as follows: BVDV in MDBK, HRSV in HEp-2, and HRV in MA104 cells will be stored at -70ºC.

Cytotoxicity evaluation

The cytotoxicity of the respective plant extracts and isolated compounds will be evaluated by the 3-4,5-dimethythiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay will be conducted according to Da Silva et al. (2006) and Mosmann (1983). Cells without the plant extracts will be used as a control. The concentration of the plant extracts that decreased the viability of 50% of the cells will be defined as the 50% cytotoxic concentration (CC_{50}). The optical density of the samples will be measured using an ELISA Spectra Count reader at a wavelength of 550 nm. All results will be calculated from the mean of three independent biological experiments performed in triplicate. The CC_{50} values will be estimated from concentration-effect curves after linear regression as described in Da Silva et al. (2006).

Antiviral activity

The antiviral activity of the plant extracts and isolated compounds will be measured using the MTT assay as described by Da Silva *et al* (2006). The antiviral assays will be performed at 24 h (MDBK and HEp-2 cells) or 48 h (MA104) after seeding, using confluent cell monolayers cultured in 96-well plates with MEM and 10% horse serum. The plant extracts will be included in different time points as follows:

Treatment I -The plant extracts or compounds will be in contact with the cell before and, also, after virus inoculation. Cells will be incubated with the plant extracts diluted serial as described by Da Silva et al. (2006), for a period of 1h before virus inoculation. The plant extracts will be removed and, each well will be inoculated with 100TCID50/ml doses of virus. Virus and cells will be maintained in contact for 2 h at 37ºC, in order to allow the virus adsorption to occur. The inoculum will be replaced by fresh medium containing the plant extracts.

Treatment II - The plant extracts or compounds will be added only after the removal of the virus. The virus inoculation protocol will be the same as for treatment I.

Treatment III - Plant extracts or compounds will be added to the cells and incubated for 1h before virus inoculation. The virus inoculation procedure will be conducted as described for treatment I. After the virus removal the inoculum will be replaced by fresh medium without plant extracts.

For all the treatments, the MTT procedure will be performed 72 hours later according protocol described Da Silva *et al*. (2006). The concentration that reduced the absorbance of infected cells to 50% when compared to cell and virus controls will be considered the effective concentration (EC_{50}). The EC_{50} will be calculated according the following equation: $[(A – B) / (C – B) \times 100]$, where A is the control sample absorbance, B is the cell control absorbance, and C is the virus control absorbance. The selectivity index (SI) will be calculated using the CC_{50} and EC_{50} data and applying the formula $SI = CC_{50}/EC_{50}$.

In vitro inhibitory activity against SARS-CoV

The aim of this study is to investigate whether a panel of the pure candidate antiviral compound(s) isolated from Nigerian medicinal plants exhibit *in vitro* anti-SARS-CoV activity. A cell-based assay utilizing cytopathic endpoints (CPE) will be set up using Vero E6 cells to screen candidate antiviral compound(s) (isolated from Nigerian medicinal plants). SARS-CoV has been shown to infect Vero E6 cells, an African green monkey kidney cell line (Ksiazek *et al.,* 2003), and this remains the only *in vitro* model of SARS-CoV infection. The initial screen will be followed by a plaque reduction assay to determine the 50% effective concentration (EC_{50}) of compound(s) showing positive results. These experiments will allow rapid screening of candidate antiviral compound(s), enabling with *in vitro* evidence of activity to move into clinical studies.

Selection of candidate antiviral compound(s) from plants

To identify a pharmacologic agent with potential for use in the management/or treatment of COVID-19, a collection of the antiviral compounds (isolated from Nigerian medicinal plants) will be screened against SARS-CoV, the etiologic agent of the atypical pneumonia. A positive control will be set-up to investigate a wide spectrum of potential molecular targets, to cover the selected pharmacologic range of commercially available antiviral agents, including agents not expected to be active against coronaviruses.

SARS-CoV construction and infection

Vero E6 cells (American Type Culture Collection) will be propagated in 75 cm^2 cell culture flasks in growth medium consisting of medium 199 (Sigma,) supplemented with 10% fetal calf serum. *SARS coronavirus (*strain *2003VA2774)*; an isolate from a SARS patient in Singapore which has been previously sequenced (Ruan *et al.,* 2003) will be purchased. Briefly, 2 mL of stock virus (SARS corona virus) will be added to a confluent monolayer of Vero E6 cells and incubated at 37°C in 5% CO_2 for 1 hour; 13 mL of medium 199 supplemented with 5% fetal calf serum will be added. The cultures will be incubated at 37°C in 5% CO_2, and the supernatant

will be harvested after 48 hour; in >75% of cultures, inhibition of cytopathic endpoints CPE (3+) in each well will be observed with an inverted microscope. The supernatant will be clarified at 2,500 rpm and then divided into aliquots, placed in cryovials, and stored at -80°C until use.

Virus handling and titration assay

All virus culture and assays will be carried out in the biosafety level-3 laboratory at the Lahor Medical Research Laboratory, according to the conditions set out in Biosafety in Microbiological and Biomedical Laboratories (Richmond and McKinney 1999). Virus titer in the frozen culture supernatant will be determined by using a plaque assay. Briefly, 100 µL of virus in 10-fold serial dilution will be added, in triplicates, to a monolayer of Vero E6 cells in a 24-well plate. After 1 h of incubation at 37°C in 5% CO_2, the viral inoculum will be aspirated, and 1 mL of carboxymethylcellulose (CMC) overlay with medium 199, supplemented with 5% fetal calf serum, will be added to each well. After 4 days of incubation, the cells will be fixed with 10% formalin and stained with 2% crystal violet. The plaques will be counted visually, and the virus titer in plaque-forming units per mL (PFU/mL) will be calculated. The concentration of candidate antiviral compound(s) that inhibits 50% of plaques in each well 50% inhibitory concentration (IC_{50}) will be determined. All assays will be carried out in triplicate on three different independent biological experiments.

Cytopathic Endpoint (CPE) assay

The protocol will be adapted with little modification from Al-Jabri *et al*. (1996), and all candidate antiviral compound(s) will be tested in triplicate on three different independent biological experiments. Briefly, 100 µL of serial 10-fold dilutions of the candidate compound(s) will be incubated with 100 µL of Vero E6 cells, in a 96-well plate. The incubation period will be 1 h at 37°C in 5% CO_2. Ten microlitres of virus at a concentration of 10,000 PFU/well will be added to each of the test wells. The plates will be incubated at 37°C in 5% CO_2 for 72 hours and observed daily for CPE. To determine cytotoxicity, 100 µL of serial 10fold dilutions of the candidate compound(s) will be incubated with 100 µL of Vero E6 cells, in a 96-well plate, without viral challenge. The plates will be incubated at 37°C in 5% CO_2 for 72 hours and examined for toxicity effects by using an inverted microscope.

In silico studies

The compound(s) which showed most potent antiviral activity based on their inhibitory activity against SARS-CoV *In vitro* will be subjected to virtual screening using advanced molecular docking and dynamic simulation tools. The molecular docking modelling will be used to predict and describe the interaction of the compounds with the main viral protease (3 CL pro) in SARS-CoV-2 responsible for viral replication and the host cell receptor protein – Angiotensin Converting Enzyme 2 (ACE2) – a cell surface receptor protein that play critical role in establishing the infection in humans.

The molecular docking modelling will be carried out as follows:

- *Selection of proteins in protein data bank:* The biological target; 3CLpro (a major protease of SARS-CoV-2) crystal structure (PDB ID: 6LUY2F) and ACE2 protein crystal structure will be retrieved from the Worldwide Protein Data Bank (PDB) repository. The active sites of the proteins will be determined based on the cocrystallized ligand positions within a radius of 4.5 Å and the presence of important amino acids.

- *Preparation of proteins:* Prior to docking, water molecules. During preparation, the missing hydrogens will be added, and partial charges will be assigned using appropriate force field. Hydrogens and heavy atoms will be optimized by restrained minimization.
- *Ligand preparation for docking:* The 2D (two-dimensional) chemical structure (flat structure) of the compounds will be converted to the 3D (three-dimensional) chemical structure by ChemBioOffice 2018 software. The 3D molecular structures of the compounds will be optimized for docking using SYBYL-X 1.1 software.
- *ADME Properties Prediction:* The ADME properties, and drug likeness of selected compounds will be determined *in silico* in terms of distribution, absorption, metabolism, and excretion.
- *Redocking of protein-ligand (compound) cocrystal structures:* The redocking of protein-ligand complex cocrystal structures aims to assess the suitability of docking parameters. The process will be carried out as follows; separation of compounds from homogenized complexes in proteins, and redocking using Sybyl-X 1.1 software.
- *Molecules docking into protein:* Molecular docking of compounds into prepared proteins will be done using the AutoDock software. Clinically established anti-viral drugs will be selected as presumable standard. The test compounds will be subjected to an automated and comprising molecular docking campaign by using the AutoDock VINA algorithm PyRx algorithm (blind docking mode) and the high-performance supercomputer MOGON II.
- *Docking results analysis (Evaluation of docking score (DS)):* Analysis of the interactions between the ligands (compounds) and targeted proteins (3CLpro and ACE2), and performance of interaction on 2D and 3D planes using AutoDock software. Various interactions, such as van der Waals interactions, hydrogen bonds, cation−π bonds, π−π bonds, and ionic interactions, and the interaction distance between amino acids and the active sites of compounds will be plotted.

Product Development and Optimization

Most potent and highly promising compound(s) from the *in silico* studies will undergo product development which will utilize the state-of-the-art nanotechnology equipment such as Scanning Electron Microscope (SEM), X-Ray Diffractometer (XRD), Zeta-potential Analyzer (ZPA), Differential Scanning Calorimeter (DSC), Karl Fisher Moisture Titrator (KFT) at the newly established Ultra-modern Nanomedicine Centre of the National Institute for Pharmaceutical Research and Development (NIPRD). The optimized product will undergo phase "0" clinical trials at the Universities of Abuja and Benin Teaching Hospitals.

Statistical analysis

The statistical package "SAS Visual Analytics" will be used for data analysis. Data will be expressed Mean ± S. E. M. of replicate measurements. Statistical significance will be calculated by one-way analysis of variance (ANOVA), and, where applicable, differences between means will be estimated by Duncan's multiple range test.

Preliminary Data

Extensive literature search on medicinal plants with antiviral activity has been conducted. Information on the traditional/herbal remedies for viral infection used by the local communities in selected sites within the six geopolitical zones of Nigeria has been collected. Relevant ethnomedicinal data were collected based on an oral interview from Traditional Medical Practitioners (TMPs), herb sellers, and the local communities.

Some medicinal plants have been collected, dried, and their phytochemical compositions as well as proximate parameters determined.

The acute toxicity screening of two of the collected medicinal plants has been executed. Preliminary results recorded no death in mice at all the doses tested up to 5000 mg /kg after 24 h. No sign of toxicity was observed in all the tested groups and all the mice survived 14 days post treatment observation. The results of the toxicity studies show that these plants parts up to a dose of 5 g/kg is not lethal to the experimental animals and there were no physical sign of toxicity. This suggests that the plants parts could be relatively safe when consumed.

2.3 Research Activity/Output indicators:

s/no	Research Activity	Output indicators
1	Oral interviews and field trips to herbal homes	o Provides knowledge on the ethnobotany/ethnopharmacology of antiviral medicinal plants in Nigeria o Provision of scientific based information on the selected plants o Drying and pulverization of plant materials
2	Protocol Development	Optimization of all experimental methods and protocol
3	Phytochemical screening of plant extracts	The secondary metabolites present in the various plants would be known

4	Isolation and characterization of phyto-constituents	○ Potentially active antiviral compounds would be isolated with their structures known ○ New active natural antiviral agent(s) will be discovered
5	Biological investigation	○ Safety and toxicity profile of selected medicinal plants ○ Validation for the use of the plant extracts for the treatment of viral infections.
6	*In silico* studies	○ Preparation of target proteins and 3D modeling of ligands and proteins ○ Molecular docking and dynamics
7	Product Development	○ Potent compound(s) from the *in silico* studies will be revalidated for product development using the stateof-the-art nanotechnology equipment.

2.4 Time Frame:

Project Title:		**Identification and Development of Potential Drug from Nigerian Medicinal Plants against Coronavirus Disease (COVID-19)**																							
Lead Investigator:		**Prof. Abiodun Falodun**																							
Project Duration:	**24 Months**	**2021**												**2022**											
Project Start	Jan-2021	1st Quarter			2nd Quarter			3rd Quarter			4th Quarter			1st Quarter			2nd Quarter			3rd Quarter			4th Quarter		
	Month:	Ja	Fe	Ma	Ap	Ma	Ju	Ju	Au	Se	Oc	No	De	Ja	Fe	Ma	Ap	Ma	Ju	Ju	Au	Se	Oc	No	De
TASK																									
Phytochemical studies																									
Field trips for plant collection																									
Identification/authentication of plant materials																									
Drying and pulverization of plant materials																									
Phytochemical screening																									
Proximate analysis																									
Preparation of plant extracts																									
Fractionation of extracts																									
Isolation of compounds																									
Characterization of isolated compounds																									
Biological investigation																									
Acute toxicity screening																									
Sub-chronic toxicity screening																									
In vitro Antiviral and Cytotoxicity screening																									
In vitro assay against SARS CoV																									
***In silico* studies**																									

Preparation of target proteins																								
3D modeling of ligands and proteins																								
Molecular docking and dynamic simulation																								
Product Development																								
Statistical analysis and Report writing																								

2.5 Activity Indicators:

s/no	Work package	Activity
1	Protocol Development and Procurement of laboratory materials	○ All collaborators, technical and field assistants will be informed about the objectives of the project ○ Workshop to develop efficacy protocols for Phytochemical, Biological and *In silico* studies ○ Purchase of lab equipment and consumables
2	Phytochemical studies	○ Field trips for plant collection ○ Identification/authentication of plant materials and preparation of plant materials ○ Phytochemical screening ○ Isolation of compounds ○ Characterization of isolated compounds
3	Biological investigation	○ Acute toxicity screening ○ Sub-chronic toxicity screening ○ *In vitro* Antiviral and Cytotoxicity screening ○ *In vitro* assay against SARS CoV
4	*In silico* studies	○ Preparation of target proteins ○ 3D modeling of ligands and proteins ○ Molecular docking and dynamics
5	Monitoring and Reporting	To develop multi-institutional monitoring plans for monitoring all aspects of the study at the participating institutions.
6	Data Management and Analysis	To establish data management support capabilities
7	Communication within the network	To develop methods to ensure rapid communication of information within the network
8	Reporting	Reporting of progress on specific time bound
9	Knowledge sharing and Dissemination	○ Seminars to discuss progress of the research work for an effective implementation and evidence-based data ○ National and International seminars/ conferences ○ ISI peer reviewed publications on high impact journals

2.6 Study Location: The study will be coordinated by the Principal Investigator (PI) and the project office will be housed at the Department of Pharmaceutical Chemistry, University of Benin, Benin City, Nigeria. The Department will provide office space, meeting venue, laboratories for analyses and administrative support for the project. It will also provide equipment and facilities for biological analyses, in addition to providing space for storage of consumables. The day to day project operations will be carried out by a project manager in close collaboration with the principal investigator.

The project will become the base workload of the Natural Product Research Laboratory of the Department of Pharmaceutics Chemistry, University of Benin. The lab team is consisted from natural product chemistry, pharmaceutical chemistry and microbiology experts and Ph.D. students plus collaborating computational chemistry expert. The laboratory is equipped with all the instrumentation needed for extraction, separation of natural product metabolites and analytical techniques. The Principal Investigator will ensure regular meeting and assessment of result to support and train junior researchers. Regular correspondence with collaborators will take place to discuss progress and results. An infrastructure of collaboration with experts in National Institute for Pharmaceutical Research and Development (NIPRD), Nigerian Institute for Medical Research (NIMR), Lahor Research Laboratory, Benin City and the International Centre for Chemical and Biological sciences (ICCBS), University of Karachi, Pakistan has been established.

Lahor Research Laboratory is equipped with facilities for Cellular Microbiology and Molecular Biology while the International Centre for Chemical and Biological sciences (ICCBS) is equiped with the state-of-the-art spectrophotometers including the superconducting Nuclear Magnetic Resonance (NMR) spectrometers, X-ray diffractometer, and High resolution mass spectrometers that will be needed for spectroscopic analyses for structure elucidation of isolated compounds. Product optimization and development will utilize the state-of-the-art nanotechnology equipment domicile in the Ultra-modern Nanomedicine Centre of the National Institute for Pharmaceutical Research and Development (NIPRD). All the team members will be coordinated and managed by the PI. The whole team will be having monthly meetings for monitoring and evaluation purposes.

2.7 Data Management and Analysis:

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval from the Principle Investigators (PI). Data will be double entered in created computer access database and computer data will be protected by password available to data manager and the Principle Investigators. After comparison of double entered files for validation, data will be exported to SPSS and STATA for statistical analysis.

Investigators will be bound by the terms and conditions of the study that will include a data release policy. This will include sharing data through publications via peer reviewed literature and accessible databases through web server system.

2.8 Ethical and Environmental Considerations:

The Principle Investigators would ensure that the research is conducted ethically, irrespective of the given context and community actors and/or demographic involved. Ethical approval has been obtained from the Faculty of Pharmacy Ethical Committee on the Use of Experimental Animals, University of Benin, Benin City, Nigeria (Approval No. EC/FP/020/12). Experimental animals will be handled according to the Swiss Academy of Medical Sciences and Swiss Academy of Sciences Ethical Principles and Guidelines for Experiments on Animals

which is adopted by the Faculty of Pharmacy Ethics committee on the use of experimental animals.

2.9 Monitoring and Evaluation Mechanism: An online-secured system (encrypted) backed up in the research office at the University of Benin, Benin City will ensure data are well secured and confidential. Data would be reviewed and updated at every monthly progress meeting for proper monitoring and evaluation. A monitoring and evaluation (M & E) plan is a document that outlines how an implementation research project is monitored and evaluated. This links strategic information obtained from various data collection systems to decisions about how to improve the project on an on-going basis. The monitoring and evaluation process of this proposed project will be between the research project team and the project proponent (TETFUND, NIPRD, NIMR). It is suggested, however, that M & E be conducted after every major task/activity of the project.

This research will adopt the steps below for the monitoring and evaluation of the proposed project:
Step 1: Identification of Research Goals and Objectives.
Step 2: Definition of Indicators.
Step 3: Definition of Data Collection Methods and Timeline.
Step 4: Identification of M&E Roles and Responsibilities.
Step 5: Creation of an Analysis Plan and Reporting Templates. Step
6: Planning for Dissemination and Donor Reporting**.**

2.10 Dissemination Strategies:
On completion of the study, findings will be reported to the academic community directly, by speaking at meetings, seminars, conferences or symposia. Results will also be communicated to the Federal Ministry of Health and WHO. Results generated from the study will be published in peer-reviewed National and International scientific journals.

3.0 COMPOSITION OF THE RESEARCH TEAM AND COLLABORATION PROFILE

3.1 Composition of the Research Team:

A. Principal Researcher:

Name and Position of the Principal Researcher:

Contact Address:

Email: **Tel:**

B.1 Research Partner:

Name and Position of the Researcher:

Contact Address:

E-mail Address: **Tel:**

B.2 Research Partner:

Name and Position of the Researcher:

Postal Address

E-mail: **Tel:**

B.3 Research Partner:

Name and Position of the Researcher:

Postal Address:

E-mail: **Tel:**

B.4 Research Partner:

Name and Position of the Researcher:

Postal Address:
Email: **Tel:**

B.5 Research Partner:

Name and Position of the Researcher:

Postal Address:

Email: **Tel:**

B.6 Research Partner:

Name and Position of the Researcher:

Contact Address:

Email: **Tel:**

B.7 Research Partner:

Name and Position of the Researcher:

Contact Address:

Email: **Tel:**

B.8 Research Partner:

Name and Position of the Researcher:

Postal Address:

Email: **Tel:**

C.1 Research Mentee/Young Academic:

Name and Position of the Researcher:

Postal Address:

Email: **Tel:**

C.2 Research Mentee:
Name and Position of the Researcher:

Postal Address:

Email: **Tel:**

3.2 Research Work to Date

1. **Abiodun Falodun**, MI Qadir and M Iqbal Choudhary (2009). Isolation and characterization of xanthine oxidase inhibitory constituents of *Pyrenacantha staudtii. Acta Pharmaceutica Sinica* 44(4): 390 - 394.

2. Engel N, Oppermann C, **Falodun A** and Kragl U. (2011). Proliferative effects of five traditional Nigerian medicinal plant extracts on human breast and bone cancer cell lines. *Journal of Ethnopharmacology* 137: 1003 – 1010.
3. **Falodun A**, Qiu Sheng-Xiang, G. Parkinson and S. Gibbons. (2012). Isolation and Characterization of a new Anticancer Diterpenoid from *Jatropha gossypifolia. Pharmaceutical Chemistry Journal* 45, 10.
4. **Abiodun Falodun,** Nadja Engel, Udo Kragl, Barbara Nebe and Peter Langer. (2012). Novel anticancer alkene lactone from *Persea americana***.** *Pharmaceutical Biology* **1**-7**.**
5. **Abiodun Falodun,** Udo Kragl, Serge-Mitherand Tengho Touem, Alexander Villinger Thomas Fahrenwaldt and Peter Langer. (2012) A Novel Anticancer Diterpenoid from *Jatropha gossypifolia. Natural Products Communication* 7: 1 - 2.

6. **Abiodun Falodun**, Engel Nadja, Oppermann Christina, Iftikhar Ali, Villinger Alexander, Kragl Udo, Nebe Barbara and Langer Peter. (2013). Isolation of Trimethylammoniumyl - acetate monohydrate from *Cola lepidota* seeds: antiproliferative activity of extracts and fractions. *Journal of Herbs Spices and Medicinal Plants* 19 (4): 329 - 339.

7. **Abiodun Falodun**, Vincent Imeije, Osayemwenre Erharuyi, Ahomafor Joy, Peter Langer, Melissa Jacobs, Shabanna Khan, Mohammed Abaldry and Mark Hamann. (2014). Isolation of antileishmanial, Antimalarial and Antimicrobial metabolites from *Jatropha multifida*. *Asian Pacific Journal of Tropical Biomedicine* 4(5):374-378.
8. Nadja Engel Lutz, **Abiodun Falodun**, Juilane Kuhn, Udo Kragl, Peter Langer and Barbara Nebe. (2014). Pro-apoptotic and anti-adhesive effect of four African Plant extracts on the breast cancer cell line MCF-7. *BMC Complementary and Alternative Medicine* 334(14): 1-13.
9. **Falodun A**, Imieje V, Falodun EJ, Ahomafor J, Onyekaba T, Cox Dan, Abaldry M and Hamann MT. (2014). Antihepatitis C activity of extracts of selected medicinal plants. African Journal of Pharmaceutical Research and Development 6(1): 6 - 10.
10. Osayemwenre Erharuyi, Achyut Adhikari, **Abiodun Falodun**, Aimas Jabeen, Muhammad Ahmmad, Rehan Imad, M. Iqbal Choudhary. (2017). Cytotoxic, antiinflammatory, and leishmanicidal activities of Diterpenes isolated from the roots of *Caesalpinia pulcherrima*. *Planta Medica* 83: 100-110.
11. Anna Adamus, Katharina Peer, Iftikhar Ali, Jan Lise, **Abiodun Falodun**, Marcus Frank, Guido Seitz, Nadja Engel. (2019). *Berberis orthobotrys* – A promising herbal anti-tumorigenic candidate for the treatment of paediatric alveolar rhabdomyosarcoma. *Journal of Ethnopharmacology* 229: 262-271.
12. Franziska Bendrath, **Abiodun Falodun**, Zharylkasyn A, Abilov, Alexander Villinger and Peter Langer. (2014). Regioselective Synthesis of Pyrazoles and Isoxazoles with Cyclopropanated side-chain. Journal of Heterocyclic Chemistry 51(3): 835-840.
13. Osayemwenre Erharuyi, Achyut Adhikari, **Abiodun Falodun**, Rehan Imad, M. Iqbal Choudhary. (2016). Derivatization of Cassane diterpenoids from *Caesalpinia pulcherrima* (L.) Sw. and evaluation of their cytotoxic and leishmanicidal activities. Tetrahedron Letters 57, 20: 2201-2206.
14. Wolf-Diethard Pfeiffer, Klaus-Dieter Ahlers, **Abiodun Falodun**, Alexander Villinger and Peter Langer. (2014). Synthesis and Spectroscopic Characterization of Arylated Selenoureas. Phosphorus, Sulfur, Silicon and the Related Elements 189(3):324 - 332.

On-going Research

1. Acute toxicity and genotoxicity studies of some Nigerian medicinal plants

2. Antioxidant, antimicrobial and Antimalarial activity screening of *Chromolaena odorata*

3. Isolation and Characterisation of potential anti-ulcer metabolites from the stem bark extract of *Pentaclethra macrophylla*

3.3 Previous Research Grants:

Professor Abiodun Falodun

a) Isolation and characterization of anticancer chemical constituents from *Jatropha multifida* and *Persea americana* extracts: Anti-proliferative and pro-apoptotic activities against breast cancer cell lines funded by TETFUND; value ₦1.95 million (2014-2015). Completed.
b) Phytochemical studies and antimalarial activities of some Nigerian Medicinal plants against *Plasmodium falciparum* and *Plasmodium berghei.* USAID (2014-2015) funded project 30,000 USD. Completed.

Dr. Etinosa Igbinosa

a) Molecular characterization of clinically important bacterial pathogens from environmental, veterinary and agricultural sources in southern region of Nigeria TWAS Research Grant Award Research Grant No.: 14-091RG/BIO/AF/AC_I - UNESCO FR: 324028575 (2014-2016). [USD 17,730] Principal Investigator. Completed.
b) Novel antimicrobial and anticancer related secondary metabolites of aquatic actinomycetes diversity of near-shore marine and estuarine environments in the Niger Delta of Nigeria International Foundation of Science (IFS), Stockholm, Sweden. Agreement F/5081-2: (2013-2016) [Amount: USD10, 512] Principal Investigator. Completed.

3.4 Group Research:

The project involves a team from different backgrounds who are specialists in the different disciplines. Each team member has expertise that contributes to different aspects of the project, thereby strengthening and building the capacity of the team and of the project. Most of the investigators of this study are members of the Natural Product Research Group of the University of Benin. Consequently, a strong working professional relationship already exists for the group members. The project will take on two Masters level students as a form of capacity building. The students will be co-opted during the initial stages of the project when they are developing their research proposals. These students will assist in project activities including data collection and organising meetings and workshops. The qualifications, specialisation and roles of members of the research team is presented in the table below.

S/N	**Name**	**Highest Qualification/Rank**	**Area of Specialization**	**Roles**
1	Abiodun Falodun (**Principal Investigator**)	PhD/Professor	Natural Products and Drug Discovery	Project design, Data management and Project monitoring
2	Etinosa O. Igbinosa	PhD/Associate Professor	Molecular & Cellular Microbiology	Biological evaluation (cytotoxicity and antiviral activity) and molecular docking
3	Osayemwenre Erharuyi	PhD/Senior Lecturer	Natural Products and Drug Discovery	Isolation and characterization of bioactive phytoconstituents
4	Vincent Imieje	MSc/Lecturer I	Natural Products and Drug Discovery	Collection & processing of plants materials; phytochemical screening; fractionation & characterization.
5	Sylvester Aghahowa	PhD/Senior Lecturer	Ethnopharmacology and Pharmacogenetics	Preclinical studies (acute and Sub-chronic toxicity evaluation of various medicinal plant extract/pure isolated compounds)
6	Dennis Agbonlahor	PhD/Professor	Medical Microbiology	Antiviral screening
7	Aliyu M. Musa	PhD/Professor	Natural Product Chemistry	Spectroscopic analysis and Structure elucidation
8	John O. Igoli	PhD/Professor	Natural Product Chemistry	Spectroscopic analysis and Structure elucidation

9	Kennedy O. Ogbeide	PhD/Lecturer I	Natural Product Chemistry	Phytochemical screening, proximate analysis, chemical fractionation and isolation of pure and potentially active compounds, and structure elucidation of the isolated compounds
10	Irene Oseghale	MSc/Lecturer II	Natural Product Chemistry	Phytochemical screening, proximate/mineral/heavy metal analysis, acute/sub-
				chronic toxicity profiling and chemical fractionation of plant materials.
11	Oghenovo Ukato	MSc/Technologist I	Biochemical Pharmacology	Acute and Sub-chronic toxicity evaluation of various medicinal plant extract/pure isolated compounds)

4.0 FINANCIAL ASPECTS OF THE PROJECT

4.1 Project Budget:

DESCRIPTION OF ITEM	**EXPECTED FROM**		**OTHER**	**TOTAL (₦)**
	TETFund NRF	**INSTITUTION**		
1.0 Personnel Costs/Allowances				
1.1 Principal Researcher (40,000.00/Month ×24)	960,000.00			960,000.00
1.2 Team Members (23,000.00/Month × 24) x 10 members	5,520,000.00			5,520,000.00
1.3 Research Assistants (10,000/Month × 24) x 2 assistants	480,000.00			480,000.00
1.3 Technical Support (10,000/Month × 24)	240,000.00			240,000.00
Sub-Total (Not >20% of budget)				**7,200,000.00**
2.0 Equipment (List & Specify)				
2.1 Reverse Phase HPLC: ECS28 P Compact preparative system 250 ml, 800 nm. [250;800;7L].	10,880,000.00			10,880,000.00
2.5 Biobase Ultra-low -86°C Freezer model BDF-86V158, 158L capacity.	1,324,000.00			1,324,000.00
Sub-Total (Not > 25% of budget)				**12,204,000.00**
3.0 Supplies/Consumables				

3.1 Solvents for the extraction and isolation of compounds from the medicinal plants (Methanol, n-Hexane, Ethyl acetate, Dichloromethane, Chloroform, Acetone, Acetonitrile and Water	6,184,000.00	500,550.00		6,684,550.00
3.2 Silica gel (200-400 mesh (Merck) for column chromatography) 20 kg @ 25000/kg	750,000.00			750,000.00
3.3 Sephadex LH-20 (300 g @ 900,000/100 g	2,700,000.00			2,700,000.00
3.4 TLC Plates (Analytical) (Aluminium backed 0.5 mm SiO_2 coated plates (Merck)) 20 packs @ 55,000/pack	1,100,000.00			1,100,000.00
3.5 Preparative TLC (Glass plates L x W 20 cm x 20 cm, silica gel 60 matrix,	2,000,000.00			2,000,000.00

binder, polymeric, fluorescent indicator) 10 packs @ 200,000/pack				
3.6 Laboratory reagents and PPEs	600,000.00	300,000.00		900,000.00
3.7 Glass wares and other apparatus		1,000,000.00		1,000,000.00
3.8 sample bottles (heparinized and Plain)	21,000.00	25,500.00		46,500.00
3.9 Albino mice (Wistar stain) for acute toxicity screening (120 mice @ 500/mouse)	60,000.00			60,000.00
3.10 Albino rats (Wistar stain) for subchronic toxicity screening (200 rats @ 1000/rat)	200,000.00			200,000.00
3.11 Pelleted rodent chew (Ewu feeds and flour mill) (10 bags @ 5000/bag)	50,000.00			50,000.00
3.12 Culture Media for cell culture for cytotoxicity *in vitro* antiviral screening	1,975,000.00			1,975,000.00
3.13 Cell lines (American Type Culture Collection (ATCC, Manassas VA, USA)	2,720,000.00			2,720,000.00
Sub-Total				**20,186,050.00**
4.0 Data Collection & Analysis				
4.1 Molecular Docking software (AutoDock VINA)	1,665,000.00			1,665,000.00
4.2 Biochemical Analysis (~200 samples @ 2000/sample)	400,000.00			400,000.00
4.3 Histopathological Analysis (~100 samples @ 3000/sample)	300,000.00			300,000.00

4.4 Spectroscopic Analyses of isolated compounds (NMR and MS) (~40 samples @ 25000/sample)	1,000,000.00			1,000,000.00
Sub-Total				**3,365,000.00**
5.0 Travels				
5.1 Field trips for plant collection including stipend for local collectors	500,000.00			500,000.00
Sub-Total				**500,000.00**
6.0 Dissemination				
6.1 Manuscripts Publication in National/International Journals	200,000.00			200,000.00
6.2 Seminars/Conferences/Workshops	400,000.00	200,000.00		600,000.00
Sub-Total (Not >3%)				**800,000.00**
7.0 Others/Miscellaneous (Specify)				
7.1 Stationeries and computer consumables	100,000.00	55,500.00		155,500.00
7.2 Internet services and Recharge cards	200,000.00	200,000.00		400,000.00
7.3 Fuelling/Electricity		200,000.00		200,000.00
7.3 Note books, printing and photocopying	50,000.00	55,000.00		105,000.00
Sub-Total				**860,500.00**
TOTAL DIRECT COST	**42,579,000.00**	**2,536,550.00**		**45,115,550.00**
INDIRECT COST (5% of TETFund Component of Direct Cost) to Institution	**2,128,950**			
GRAND TOTAL				**47, 244,500.00**

4.2 Budget Justification:

This project is estimated to cost a total of ₦47,244,500.00 (Forty-seven million, two hundred and forty-four thousand, five hundred Naira only) as indicated in the budget table for the 24-month project. Details are provided below:

The sum of ₦960,000.00 is being requested to cover for the Principal Researcher's cost and allowances while the sum of ₦5,520,000.00 is being requested for as cost and allowances for the ten team members @ ₦552,000/member for the 24 months project duration. The sum of ₦480,000 is being requested to cover the cost and allowances of two research assistants and ₦240,000 for one technical support staff. Summarily, the sum of N7,200,000.00 will cover all personnel cost and allowances.

The sum of ₦12,204,000.00 is being requested to cover major equipment (Reverse phase preparative HPLC for the purification of isolated compounds, and -86^{o}C freezer for preservation/storage of biological samples). These equipment are currently not available in the University of Benin, and need to be domicile in the research location for the timely and successful execution of the project. The sum of ₦20,186,050.00 is being budgeted for solvents, reagents and consumables with ₦1,826,050.00 contribution from the University of Benin. We estimate the sum of ₦3,365,500.00 for data collection, analyses of samples and software while ₦500,000.00 is being estimated for field trips for plant sample collection including stipend for local plant collectors for their assistance in plant collection, and herbarium specimen preparation. For the dissemination of research findings, we are requesting the sum of ₦800,000.00 for manuscripts preparation, publication, seminars, conferences and workshops with ₦200,000.00 contribution from the University of Benin. We estimate the sum of ₦860,500.00 as miscellaneous cost for stationeries, data for internet, fuelling, recharge cards and mobilization for research team members with N510,500.00 contribution from University of Benin. Finally, we are requesting the sum of ₦2,128,950.00 which is 5% of the direct cost of N42,579,000.00 as indirect cost for the University of Benin.

4.3 Additional Source(s) of funding: None

5.0 COMMITMENTS

5.1 Researcher(s) Declaration

We declare that information given in this application form is to the best of our knowledge complete and correct.

We confirm our commitment to the successful implementation of the project.

Name and Signature of **Principal Researcher**	**Professor Abiodun Falodun**
Name and Signature of **Research Partner**	**Dr. Etinosa O Igbinosa**
Name and Signature of **Research Partner**	**Dr. Osayemwenre Erharuyi**

Name and Signature of **Research Partner**	**Pharm. Vincent O. Imieje**
Name and Signature of **Research Partner**	**Dr. Sylvester Aghahowa**
Name and Signature of **Research Partner**	**Prof. Dennis Agbonlahor**
Name and Signature of **Research Partner**	**Prof. Aliyu Muhammed Musa**
Name and Signature of **Research Partner**	**Prof. John O. Igoli**
Name and Signature of **Research Partner**	**Dr. Kennedy O. Ogbeide**
Name and Signature of **Research Partner**	**Dr. Irene O. Oseghale**
Name and Signature of **Research Partner**	**Ogheneovo Ukato**

5.2. Declaration of Head of Institution

I declare that the applicant(s) is/are staff member(s) of my institution and that my institution will support and provide space for the successful conduct of the research. I endorse the project and confirm my institutional commitment to the successful implementation of the TETFund NRF grant.

Name, Title/Official Position, Signature, Date and Stamp of Head of Institution

Professor Lillian I. Salami
Vice Chancellor, University of Benin, Benin City, Nigeria.