Research Project Title

PROTOTYPE DEVELOPMENT OF A MODULAR AND HIGHLY SENSITIVE DNA/RNA EXTRACTION AND PURIFICATION KIT FOR DOWNSTREAM APPLICATIONS IN MOLECULAR BIOLOGY

Proposed Budget ₦ 48,633,986.00

EXECUTIVE SUMMARY

Nucleic acids, both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) contain information about an organism which can be used to identify, isolate and characterize it. Molecular assays such as the polymerase chain reaction (PCR), next generation sequencing (NGS), microarray, gene cloning etc. usually rely on high quality nucleic acids as a starting material, and have diverse applications in the life sciences. These include, isolation of disease-causing organisms, cancer research, paternity testing, improvement of seed varieties in agriculture, biotechnology for production of generic drugs and so on. For instance, the PCR, NGS and bioinformatics used to isolate the novel SARS-Cov-2 virus pathogen responsible for Covid-19 pandemic utilized high quality nucleic acid. Few foreign companies, notably Qiagen, Thermo fisher Scientific, Zymo Research and New England Biolabs (NEB) dominate the production of DNA/RNA kits, which they hold several patents that are guarded as industrial secrets. In Nigeria we do not produce these kits, and must import them, when they are needed for research in our universities, diagnosis of diseases in our hospitals, improvement of crop varieties in our agricultural institutes, and identification of pathogens in our environment. If we can build the capacity to produce DNA/RNA isolation kits, we can save and earn foreign exchange by exporting these kits. The aim of our research is to develop prototype kits, for the isolation and purification of high-quality nucleic acids from diverse samples, using silica spin columns for applications in molecular biology. Three in-house lysis (extraction) buffers will be prepared from chaotropic salts – guanidine thiocyanate (GuSCN), guanidine hydrochloride (GuHCl) and sodium chloride (NaCl) at various pH, and ionic concentrations. The lysis buffers will be known as PuriNA buffers I, II, and III. PuriNA is derived from “purified Nucleic Acids”. Silica spin columns with 6, 7, 8, and 9 membrane layers will be sourced from original equipment manufacturers on https://alibaba.com based on a pre designed template. Each lysis buffer will be applied to bacteria, virus, fungi, plant tissue, animal tissue, water and soil samples to extract DNA and RNA at various pH, temperature, and time of incubation. The extract will be bound to the 6, 7, 8, and 9 - layer silica spin column using in-house prepared PuriNA binding buffers, 1, 2, and 3. The columns will be washed with in-house PuriNA column wash buffers 1, 2, and 3. The washed DNA and RNA from the silica spin column will be eluted using PuriNA elution buffers 1, 2, and 3 each with different composition and pH. The purity of DNA and RNA will be measured with a spectrophotometer. The ratio of OD 260/280, and 260/230 for the PuriNA buffers and a Qiagen reference kit will be compared by multivariate analysis. The integrity of both extractions will be determined by PCR, and agarose gel electrophoresis of DNA and RNA. The concentration of the nucleic acids will be determined using OD readings and dilution factor of the nucleic acid. Based on analyzed, results, a set of protocols will be complied for specific extraction of DNA or RNA from a sample using a silica column, lysis, binding, washing and elution buffers under different pH conditions. The in-house PuriNA buffers will be optimized in validation trials to match or surpass the purity and concentration of nucleic acids obtained using the Qiagen reference kits. A minimum of 10 prototypes and protocols (kits) will be developed for the extraction of DNA and RNA in different samples. The project is expected to last for a period of 18 months, at the expected budget of 48,633,986 Naira only.

Keywords: Nucleic acids, DNA, RNA, Purification

GENERAL BACKGROUND OF RESEARCH PROJECT

Introduction (provide general background of the problem and justification leading to the proposed research project)

Nucleic acids, both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) contain information about an organism which can be used to identify, isolate and characterize it. Molecular assays such as the polymerase chain reaction (PCR), next generation sequencing (NGS), microarray, etc usually rely on high quality nucleic acids as a starting material, and have diverse applications in the life sciences. These include, isolation of disease-causing organisms, cancer research, paternity testing, improvement of seed varieties in agriculture, biotechnology for production of generic drugs and so on. For instance, the PCR, NGS and bioinformatics used to isolate the novel SARS-Cov-2 virus pathogen responsible for Covid-19 pandemic utilized high quality nucleic acid. Few foreign companies, notably Qiagen, Thermo Fisher Scientific, and Zymo Research and New England Biolabs (NEB) dominate the production of DNA/RNA kits, which they hold several patents that are guarded as industrial secrets.

Despite the fact that DNA and RNA extraction kits are needed in our hospitals for diagnosis and treatment of infectious diseases, in universities and research institutions for advanced biomedical or agricultural research, there are no industries in Nigeria that have indigenous capacity to produce DNA or RNA extraction and purification kits. These kits are expensive, but whenever they are needed for use, Nigerians have to source for foreign exchange to import them. The problem became very acute during the protracted era of COVID-19, when the kits were in high demand globally, but were in short supply, particularly in countries that lack the technical know-how to produce them. If we can develop local skills to produce high quality DNA/RNA extraction kits in Nigeria, it can save our scares foreign exchange. Moreover, if we can produce high quality nucleic acid extraction kits that compete favourably with similar kits produced by market leaders in the sector, we can export them to other countries and earn foreign exchange. Besides the startup scientific industry that will emerge and provide jobs for the unemployed Nigerians, it will also improve our technological ability to produce useable scientific products for both local and international consumption.

The use of silica spin column for nucleic acid extraction has gained universal acceptance among scientists because it is fast, cost effective, minimizes the use of toxic reagents and requires few steps to obtain high purity grade nucleic acid (Hidayat and Prasetyo, 2021). It is our reason for proposing this research project.

Aim and Specific Objectives of the Research Project

The goal of this research is to develop prototypes for DNA and RNA extraction and purification with silica spin columns from different sources like bacteria, virus, fungi, animal and plant tissues, water and soil as environmental sources for applications in molecular biology.

Specific Objectives

1. Composition, testing and validation of inhouse prepared lysis buffers, under varying conditions (temperature, pH and ionic concentrations) for nucleic acid extraction and purification.

2. Composition testing and validation of inhouse prepared buffers for the removal of PCR inhibitors from animal, plant tissues, bacteria, virus, and environmental samples.

3. Composition, testing and validation of suitable buffers and conditions for binding of high molecular weight nucleic acids to silica spin column for purification of high-quality nucleic acids from specific sample types.

4. Preparation testing and validation of inhouse buffers for washing nucleic acids bound to the silica spin column for specific samples.

5. Preparation testing and validation of inhouse buffers for the elution of bound nucleic acid from the silica spin column.

6. Development of a modular prototype kit for nucleic acids extraction from specific samples.

Statement of the problem (why does the research need to be conducted and the problem to address)

Nigeria does not produce DNA and RNA extraction and purification kits that are used in the country to do research in the universities, research institutes, and some hospitals. During the first global wave of Covid-19 pandemic, DNA and RNA extraction and purification kits needed for the diagnosis of SARS-Cov-2 virus by PCR, were in high demand, but limited in supply globally. Because of the lack of indigenous capacity to produce these kits in Nigeria, we had to rely on remnants of supplies from countries that had the technical ability to produce them, only after their citizen were adequately served for the diagnosis of SARS-Cov-2 virus. This delayed timely and accurate diagnosis of the virus, leading to some avoidable loss of human lives to the SARS-Cov-2 virus in Nigeria. Moreover, the unit cost of diagnosis was high, due to inadequate supply of reliable diagnostic kits.

Thus, to close this technological gap, we propose to develop prototypes of a modular and highly sensitive DNA and RNA extraction and purification kits, using silica spin columns. The viability of the project is based on our preliminary data which supports the realization of this goal. the Departments of Zoology, Chemistry, Microbiology and Biochemistry at the Joseph Sarwuan Tarka University Makurdi (formerly, University of Agriculture Makurdi) will collaborate on the project.

The goal is to develop prototypes for DNA and RNA extraction and purification using virus, bacteria, fungi, animal and plant tissues, and environmental sample sources which contain humic acids, and polyphenolic compounds as inhibitors. The focus of the project will be on silica spin columns, which use reagents that are non-toxic, compared to other nucleic acids extraction methods; are relatively cost effective, less time consuming, and do not require a huge outlay of purification equipment. In addition, the project will utilize three chaotropic salts – guanidine thiocyanate (GuSCN), guanidine hydrochloride (GuHCl), and sodium chloride (NaCl). These salts have been referenced as potent agents for nucleic acids extraction and purification as they possess the dual properties of cell lysis, and the ability to promote reversible binding of nucleic acids to silica spin columns (Vandeventer *et al.*, 2012).

Research Questions

The questions to be addressed in the course of the project are:

1. Which specific compounds, at which concentration, and condition (temperature and pH) is suitable for preparation of lysis buffers that yield high quality nucleic acids from animal and plant tissues, bacteria, virus, and environmental sample lysates?

2. Which compound preparation, at which concentrations, are suitable for the removal of PCR inhibitors from sample lysates?

3. Which compound preparations at what concentration, and condition can be used to bind specific lysates to silica spin column?

4. which compound preparations can be used as suitable buffers for washing nucleic acids bound to the silica spin column?

5. which buffer preparations can be used to elute high quality nucleic acids bound to silica spin column?

6. Which specific prototype kits for DNA and RNA extraction equals, or outperform the existing commercially available kits for nucleic acids extraction and purification?

Conceptual Framework of the study (classify, identify and define the central concepts of ideas underlying the study)

The theoretical framework that underpins this research is that under certain conditions of pH, temperature and ionic concentration of chaotropic salts-based buffers, DNA or RNA present in a lysate will bind to silica (Vandeventer, *et al*., 2012). The ability to promote binding of nucleic acids to silica has been observed among some chaotropic salts such as guanidine thiocyanate (GuSCN), guanidine hydrochloride (GuHCl) and sodium chloride (NaCl) at certain ionic concentrations (Zhou et al., 2018; Zainabadi *et al,.* 2019). The chaotropic salts also possess the ability to disrupt cell membrane of living organisms, thus releasing nucleic acids that reside within the cell into a lysate solution, therefore they are key components of lysis buffers.

When nucleic acids selectively bind to silica membrane columns, it allows cell debris, proteins, fats and carbohydrates in the lysate to the removed by washing with a combination of specific buffers and alcohol leading to the purification of nucleic acids. The DNA or RNA is eluted from the silica column by rehydration of the column. The appropriate condition of pH, temperature, and ionic strength of buffers, under which high quality DNA or RNA can be purified vary between the two nucleic acids, and with different sources of samples. Thus, an extraction protocol for a urine sample for instance may not work for a stool sample. The conditions need to be investigated, and a standard protocol developed for a given source of sample.

Research Outputs and Outcomes (provide the expected outputs and outcomes of the project)

It is expected that this project will lead to the development of prototypes of a modular and highly sensitive nucleic acid extraction kits for DNA and RNA extraction from specific sample types. These can be used in various molecular biology applications. If successfully developed, our kit would be known as PuriNA DNA/RNA purification kit. The name PuriNA is derived from “Purified Nucleic Acid”. Our target product profile (TPP) outlines the specifications and characteristics of the DNA/RNA extraction and purification prototype kits as product that would be the outcome of this research.

Product description:

- **Product name**: PuriNA™ DNA/RNA Extraction and Purification Kit
- **Intended use**: Isolation and purification of high-quality DNA and RNA from various sample types, including bacteria, virus, animal, fungi, plant, water and soil (environmental samples).

Key performance characteristics:

(i). **Yield and purity**:

- DNA and RNA yields should be competitive with leading commercial kits.
- High purity with A260/280 and A260/230 ratios of 1.8 - 1.9 for DNA and 1.9.-2.1 for RNA .
- Minimal contamination with proteins, salts, or other impurities.

(ii). **Sample types**:

- Compatible with diverse sample types, including plant and mammalian cells, and tissues, bacteria, virus, fungi, and environmental samples.
- Ability to isolate nucleic acids even from samples that contain inhibitors that inhibit molecular biology assays.

(ii). Processing time:

- Efficient extraction within 30-60 minutes, depending on sample type and scale.
- Minimize hands-on time to increase user convenience.

(iii). **Kit size and throughput**:

- Offer multiple kit sizes to accommodate different sample volumes and throughput requirements (e.g., mini, midi, maxi, and high-throughput versions).

(iv). **Ease of use:**

- User-friendly protocol with step-by-step instructions suitable for both novice and experienced researchers.
- Minimize the risk of user errors.

(v). **Compatibility:**

- Compatible with various downstream applications, including PCR, qPCR, RT-qPCR, sequencing, cloning and other molecular biology techniques.

(vi). **Safety and environmental impact**:

- Ensure user safety with non-hazardous reagents.
- Promote sustainability by reducing waste and packaging materials.

(vii). **Storage and shelf Life:**

- Long shelf life and stable reagents, ensuring reliability and minimizing product waste.

(viii). **Cost-effectiveness**:

- Competitive pricing to make the kit an economically attractive option for research labs with varying budgets

Quality control:

- Rigorous quality control processes during manufacturing to ensure lot-to-lot consistency and reproducibility.
- Comprehensive quality assurance documentation and support for troubleshooting.

Regulatory compliance:

- Compliant with relevant regulatory standards and guidelines, such as ISO 13485 and CE-IVD, where applicable.

Support and training:

- Comprehensive technical support and customer service to address user inquiries and troubleshoot issues.
- Offer training resources, including video tutorials and technical webinars.

In summary, the PuriNA™ DNA/RNA Extraction and Purification Kit's target would be to compete favourably with existing commercial solutions by providing high yields, purity, ease of use, and versatility while remaining cost-effective. It would offer researchers a reliable tool for DNA and RNA isolation and purification across a wide range of sample types and applications.

Project Impact

If the project is successfully executed, it will produce prototypes of nucleic acid extraction kits from the university. A collaboration between the university and selected manufacturing industries in Nigeria, will lead to the formation of a startup manufacturing industry for the manufacture of the prototype molecular biology kits into commercially available DNA and RNA extraction kits. The kits will be of direct benefits to the academic, medical, biotechnology, agricultural research institutes and the entire scientific community in Nigeria. It would be sold at reduced cost, compared to foreign imported kits. The startup industry will create jobs for social and economic development of Nigeria. The kits can be exported to earn foreign exchange. At the same time, foreign exchange will be saved as there would be no need to source for foreign exchange to import DNA and RNA extraction kits, since they will be produced locally, in Nigeria. Our technological advancement in the area of molecular biology research and production will improve long-term as young academics on the project would acquire useful technical knowledge during the research.

RESEARCH DETAILS

Literature Review

The extraction of DNA or RNA using silica columns usually involves tissue or cell lysis , inhibitor removal, binding, washing, and elution (Thermo Fisher Scientific 2012). Lysis involves the breaking of cell or tissues to release DNA or RNA which are enclosed in the nuclear of cellular membrane. The released nucleic acids, may contain inhibitors from other cellular debris that can interfere with downstream applications such as PCR which has numerous applications in molecular biology. Therefore, inhibitors need to be removed from the lysate (Rajakani, *et al*., 2013). The binding of nucleic acids to silica which is reversable has enabled the development of solid phase extraction and purification of nucleic acids from complex samples by washing the silica column with specific buffers (Zainabadi *et al*., 2019). The composition of buffers used in the nucleic acid extraction and purification process is a trade secret. Some chaotropic salts particularly guanidine thiocyanate (GuSCN) and guanidine hydrochloride (GuHCl) have been reported as powerful cell or tissue lyses agents and therefore are key components of lysis buffers (Pramanick *et al.*, 1976, Scallan *et al*., 2020). In addition, these salts facilitate the reversible binding of DNA or RNA to silica matrices (Weng *et al.,* 2020).

The pH condition under which RNA or DNA bind to silica vary (Vandeventer, *et al.,* 2012). This differential binding can be utilized for selective development of either RNA or DNA purification kits. After washing the column, the bound nucleic acid can be eluted from the column by rehydration with specific buffers at appropriate pH. The quality of nucleic acid is determined by spectrophotometric measurement of the ratio of OD 260/280 = 1.8 for pure DNA and OD 260/280 =2.0 for pure RNA. When the concentration of a nucleic acid from a source is too low for measurements, qPCR can be used to amplify a target gene of interest from the source, and the cycle threshold (Ct) value of the amplification

plotted against the standard curve of a Ct value of a control nucleic acid from a source, of a known concentration. Thus, the concentration of the low nucleic acid be determined.

Cell lysis and nucleic acid extraction methods

There exist a number of methods for the lysis of cells or tissues to extract and purify nucleic acids. A particular method could be very useful depending on the source or type of nucleic acid to be purified. One method for DNA extraction is the salting out method which uses a 0.4 M NaCl as a key component of the lysis buffer. In addition, 10 mM Tris-HCl pH 8.0; 2 mM EDTA pH 8.0); SDS, and proteinase K enzyme, is mixed with the cell or tissue containing the nucleic acid. After overnight incubation at 55-60°C, saturated NaCl at 6 M concentration is added to the lysate to precipitate protein. The resulting supernatant containing the nucleic acid is transferred to another tube and is precipitated with 70% ethanol (Dairawan and Shetty 2020).

The cetyltrimethylammonium bromide (CTAB) lysis and extraction method uses 2% CTAB at alkaline pH as the major component of the lysis buffer (Schenk et al., 2023). In a solution of low ionic strength, the extraction buffer precipitates DNA and acidic polysaccharides from the rest of the cellular components. Solutions with high salt concentrations are then used to remove DNA from the acidic polysaccharides which form a precipitate with CTAB. This method is therefore useful for DNA extraction from plants and bacteria that produce high amounts of polysaccharides (Budelier and Schorr, 1998). By lowering the pH of the medium, RNA can also be recovered from this method.

The sodium dodecyl sulfate (SDS) Proteinase K method is also used to isolate nucleic acids (Edwards *et al*., 1991). SDS is a detergent which disrupts cell membrane, while Proteinase K is a serine protease. For DNA extraction, 20–50 µL of 10–20 mg/mL proteinase K is usually added, Sodium dodecyl sulfate is also added to dissolve the cell membrane and nuclear envelope as well as denature and unfold proteins, exposing them to the protease activity of proteinase K which degrades proteins in the solution without interfering with the nucleic acids (Alexander, 2016). The solution is incubated for 1–18 h at 50–60°C and can then be used to extract DNA using the phenol-chloroform or salting-out method.

The phenol-chloroform-isoamyl alcohol DNA extraction. In this method, cells are first treated with a lysis buffer containing detergents such as sodium dodecyl sulphate (SDS) to dissolve cell membranes and the nuclear envelope. Other components of the lysis buffer can include 10 mM Tris, 1 mM EDTA and 0.1 M NaCl. Phenol-chloroform-isoamyl alcohol (PCIA) reagent, is then added in a ratio of 25:24:1 (McKiernan and Danielson, 2017). Both SDS and phenol denatures the proteins efficiently, while isoamyl alcohol prevents emulsification and enhances precipitation of nucleic acids. Chloroform increases the density of the organic phase, and prevents phenol from inverting into the aqueous phase, and thus preserve DNA from being degraded by phenol (Maniatis *et al*., 1982). The nucleic acid in the solution is concentrated by series of washing with 70 % ethanol. The phenol-chloroform-isoamyl alcohol nucleic acids extraction method produces high quality nucleic acids and is regarded as a 'gold standard' for nucleic acid extraction. However, phenol is poisonous and dangerous to humans' health as well as chloroform. The process also takes a long time to isolate nucleic acids (McKiernan and Danielson, 2017).

The silica spin column method of nucleic acids extraction utilizes chaotropic salts as lysis buffer. Salts like GuSCN or GuHCl, in combination with 0.05 M EDTA, 0.2 M Tris, pH 8.0, 1% SDS and 100 ug/ml of Proteinase K are added to the sample to lyse. However, in lysis buffers that contain GuSCN, SDS detergent is not included as the two chemicals are not compatible (Braakman *et al*., 2015). After incubation at 60°C for an hour or more, the mixture is transferred to a 1.5 ml microcentrifuge tube and

mixed with a binding buffer and alcohol in the ratio of 1:1:1 (ThermoFisher Scientific 2012). The content is then transferred to a silica spin column and centrifuged at high speed to wash the nucleic acid which binds to the column; while other debris is removed as flow through.

After drying the column for a few minutes, the bound nucleic acid is eluted in another tube by rehydrating the silica column with a buffer at appropriate pH for RNA or DNA. The DNA yield for this type of extraction has been reported to be 40% higher than the usual organic solvents-based DNA extraction methods. Recent RNA and DNA extraction kits by commercial companies are based on this method because it's fast, cheap, non-toxic and yield high quality nucleic acids of interest (Zhou *et al*., 2018).

At the height of COVID-19 pandemic when reagent kit for the viral RNA extraction were in short supply globally, a team of scientists at the Cox University Hospital in Ireland, and their academic colleagues formulated a lysis buffer modified from the published work of Chomczynski and Sacchi. (1987) for Sars-Cov-2 RNA extraction. The buffer had a concentration of 4 M GUSCN salt with 3 % Triton-100 detergent that proved excellent for Sars-Cov-2 viral RNA extraction (Scallan *et al*., 2020). Their pharma – colleagues then mass produced the buffer and distributed it worldwide for mass diagnosis of COVID-19 using RT-qPCR. The ThermoFisher Scientific silica-based virus RNA commercial extraction kit currently in the market is based on the 4 M GuSCN buffer by Scallan *et al*. (2020). Further test results of 4.75 M, 5 M, and 6 M GITC concentration by the same authors was not released to the public. However, the authors recommended that further variations of the 4 M GuSCN buffer concentration be tested, in particular formulations with graded increases in % w/v of Triton X-100. In addition, the variable effect on the molarity of Tris-HCl as it affects pH of the lysis buffer and its performance was also recommended for investigation (Scallan *et al.*, 2020). We are interested in following this lead in our proposed Project.

A 4 M GuHCl concentration in lysis buffer for DNA extraction from viruses, bacteria cells, cells of higher organisms, and cellular organelles had earlier been reported (Pramanick *et al*., 1976). Nowadays, GuHCl is widely used in lysis buffers for nucleic acid extraction. It's also incorporated in binding buffers that bind nucleic acids to silica spin columns for purification of DNA/RNA from different sample sources (Weidner *et al*., 2022).

The chemistry of DNA and RNA separation is that optimum pH plays a critical role in the separation process. Under acidic condition (pH 4-6), DNA partitions to the organic phase while RNA remains in the aqueous phase and can be isolated, however, at neutral to alkaline pH (pH 7-8), both nucleic acids remain in the aqueous phase (Perry *et al*., 1972, Brewerman *et al.,* 1972, Chomczynski and sacchi, 1987). Thus, RNA can be isolated by lowering the pH to acidic levels, while DNA can be isolated by raising the pH, and degrading RNA with RNase A, which also partitions to the aqueous phase with DNA.

It was observed during isolation of nucleic acids from Gram-negative bacteria that when there is a competition between RNA and DNA binding to silica, there is a preferential binding of RNA over DNA to the silica matrices (Uyttendaele *et al.* 1996). It was also shown that a pH range of 6.0 - 6.5 enhanced the isolation of RNA, while at range of pH 8.0 - 8.5 large amounts of DNA were obtained from the Gram-negative bacteria (Uyttendaele *et al*. 1996). In evaluating an improved method of nucleic acids extraction with 3 M GuSCN lysis buffer at pH 6.0 – 6.5 containing 2-mercaptoethanol, it was reported that the same buffer was used for lysis, binding and washing of the silica spin columns (Zainabadi *et al*., 2019). However, the binding and washing steps of the silica spin columns had the 2-mercaptoethanol component of the buffer removed. Both RNA and DNA were extracted from the method with yields slightly higher than a Qiagen buffer (Zainabadi *et al*. 2019). The silica based nucleic acid isolation method enables isolation of either RNA or DNA when taking into account the appropriate

conditions of pH of the lysis and purification buffers. This is central to the development of prototypes for DNA/RNA extraction in our current proposed project.

Research Methodology (give detail methodology of the proposed research project)

Study area

The study will be conducted in North Central Nigeria. A total 21 sites would be sampled for data collection in the entire region. Three sites will be sampled from each of the 6 states and the Federal Capital Territory (FCT) - Abuja. The sampling locations have been identified as follows:

(i). Benue State – Makurdi, Otukpo, Zaki-Biam

(ii). Federal Capital Territory – Kogo 1, Kasuwan Dare, and Kubwa.

(iii). Nasarawa State – Lafia, Obi, Keffi.

(iv). Plateau State – Naraguta, Pankshin, Shendam.

(v). Kogi State – Ankpa, Kabba, Lokoja.

(vi). Niger State – Minna, Bida, Kontangora.

(vii). Kwara State – Oro, Ilorin, Jeba.

Field samples will be collected from each of the identified locations for nucleic acid extraction.

Study Subjects

The subjects for this project will include both model organisms maintained in the laboratory that are frequently used in molecular biology, as well as field samples. The following organisms and environmental samples would be experimented with, for the extraction of gDNA (genomic DNA) and total RNA.

(i). *Escherichia coli* (*E. coli*) - bacteria.

(ii). T4 bacteriophage – a harmless virus particle.

(iii). *Aspergillus flavus*- fungi (that contaminates food items).

(iv). Slaughtered animal meat purchased at abattoir from meat vendors – animal tissue

(v). *Azadirachta indica* – plant tissue.

(vi). Environmental samples – water and soil samples.

(vii). Silica spin columns will be sourced at https://alibaba.com from Green Mall Jiangsu Green Union Science Instruments Co. LTD. The company produces and supplies the type of silica spin columns needed for this research. That is, spin columns with 6 layers, 7 layers, 8 layers, and 9 layers of silica membranes at relatively low cost. Preliminary assessment also indicated that these silica spin columns were useful for different types of nucleic acid purification protocols.

Sample Size

The sample size is estimated at 441 samples. Three samples will be replicated at each site in field sampling.

Experimental Design

The experimental design is a randomized complete block design with nested experimental factors (Bagchi, 1991, Culinski and Kageyama, 1996). The main treatments are the types of buffers prepared either for sample lysis, binding of the lysate to silica spin column, washing of the silica spin column, and the elution buffers. The blocks in the design will be the four different layers of silica spin columns, or type of organism from which nucleic acids will be extracted. The nested factors will be the variable values of the buffers pH, the % composition detergents in the buffer, the temperature of incubation of the buffer during sample lysis, and the variable time used to incubate the buffer. All the experimental factors will be randomly assigned to each experimental unit.

***In vitro* cultivation and maintenance of model organisms for DNA and RNA extraction**

Each model organism for use in the experiment will be procured, cultivated and maintained in vitro in the laboratory at the Microbiology laboratory, Joseph Sarwuan Tarka University Makurdi (Formely, University of Agriculture Makurdi).

***In vitro* cultivation of *Escherichia coli* (*E. coli*)**

The *E. coli* ATCC 8739, and 'B' strain stock cultures will be obtained from Vom Veterinary Research Institute and maintained in the laboratory using standard protocol (Son and Taylor, 2021). The model organism will be cultivated in Luria-Bertani broth (10 g tryptone, 5 g yeast extract, 5 g NaCl, volume adjusted to 1000 ml with double-distilled water, autoclaved and stored at room temperature) with antibiotics in capped sterile glass culture tubes. Tubes will be incubated at 37°C in a shaking incubator. Cells for nucleic acid extraction will be pelleted during the log-phase of growth at 18 hours of incubation. The remaining cell cultures will be diluted in 30 % glycerol and stored at stock organism at - 20°C.

***In vitro* cultivation of T4 bacteriophage**

The virus particle T4 bacteriophage infects and multiplies in *E. coli* 'B' strain. It will be also sourced from Vom Veterinary Research Institute, cultured, and maintained in the laboratory following the procedure described by (National Centre for Biotechnology Education, 2016). When received, the tubes containing the T4 bacteriophage preparation will be briefly stored in a freezer at -18 to -20°C using glycerol to stabilize the phage. To multiply, 100 ul of the T4 bacteriophage preparation will be inoculated in 10 ml nutrient or LB broth of host *E. coli* 'B' strain, and incubated for 24 hours at 30°C.

After 24 hours, cell from the culture will be pelleted and 50 µl of the supernatant aliquot into sterile 0.5 ml microtubes plus equal volume of glycerol, the tube will be stored frozen at -18 to -20°C until needed for DNA/RNA extraction.

Culture and maintenance of *Apergillus flavus* (*A. flavus*)

Aspergillus flavus will be grown and maintained in the laboratory in Rose Bengal medium (Beuchat and Cousin, 2001). The medium will be supplemented with chloramphenicol (mycological peptone 5.0 g; glucose 10.0 g; potassium dihydrogen phosphate 1.0 g; magnesium sulfate 0.5 g; Rose Bengal 0.05 g; chloramphenicol 0.1 g; agar-agar 15.5 g. pH 7.2 ± 0.2 at 25 °C) to enhance the selective growth of the fungus.

Collection of slaughtered meat samples from vendors at abattoir

A half kg of beef meat would be purchased from meat vendors at abattoir in a sample location. A sample drill will be used to punch and collect a 5 cm2 area of the meat and preserved into a sterile

sampling bottle containing 96% alcohol for transportation to the laboratory. The sample will be homogenized with a mixer mill in the laboratory by addition of 5 g of meat tissue with 2 ml of TE buffer (10 mM Tris, 1mM EDTA, pH 8.0). The homogenate will be divided into small aliquots in a 1.5 ml microcentrifuge tubes containing RNAlater solution and stored at - 20°C for nucleic acid extraction (Batule *et al*., 2020).

Collection of plant materials

Leaves of *Azadirachta indica* will be collected from three different A. indica trees in designated sampling site. The A. indica which is a medicinal plant has many compound metabolites that can crosslink nucleic acids and inhibit their natural function and therefore would be a good case to test the removal of inhibitors during nucleic acid extraction from its tissue. A leaf disk 0.05-0.1 g will be cut from the plant leaves and briefly homogenized with 0.1-0.15 mm Biospecs beads solution in a Biospecs Bead Beater. The content will be stored in RNALater solution at 4°C for nucleic acid extraction (Hataya, 2021).

Environmental sample collection (water and soil)

Water sampling: three 40 litres of water each, will be collected at each site. An organic matter polluted water source, and an apparently clean water source in the sampling location. The entire collection will be filtered through ultrafiltration and the faucet filter backflushed with ultrapure water. The backflushed water will be further concentrated by centrifugation at 4000 × g for 30 min in a 500 mL conical tube. The pelleted materials will be resuspended with clean nuclease free water sample and the final concentrate transferred to a 15 mL conical tube containing RNAlater solution and stored at 4 °C for nucleic acid extraction (Hill *et al.,* 2015).

Soil sampling: three samples of 5 g of soil will be randomly collected from each sampled location in a farm area. After scrapping the leaves on the top of the soil, a clean sterile spoon will be used to collect 4 inch depth of soil. The soil will be measured and stored in a Ziplock. The collected soil will be mixed and passed through a 2 mm sieve (Morita and Akao, 2021). The Ziplocks will be stored at 4 C for nucleic acid extraction.

Rational composition and preparation of lysis buffer

Three lysis buffer composition of chaotropic salts will be prepared and tested for DNA and RNA extraction under variable conditions. The buffers would be based on a modification of published nucleic acids extraction protocols (Pramanick *et a*l., 1976; Chomczynski and Sacchi, 1987; Scallan *et al*., 2020; Mason and Botella 2020). The modified lysis buffers will be called PuriNA^{TM} Lysis Buffer (PLB). The term is coined from “Purified Nucleic Acid”. The buffers will be designated as PLB-I, PLB-II, and PLB-III. Each of the three lysis buffers will be modified to contain:

(i). A chaotropic salt at high molar concentration to disrupt the cell membrane, degrade proteins, inactivate nucleases (RNase and DNase) and release the nucleic acids intact and maintain the ionic balance in the mixture of the lysis buffer and the cell/tissue component to be broken (lysate). Salts that would be used include GuSCN, GuHCl and NaCl.

(ii). Tris-HCl to stabilize the pH of the lysate and maintain the buffering activities of enzymes in the lysate.

(iii). EDTA to inactivate nucleases in the lysate and prevent the degradation of the released nucleic acids from the nuclear or cell membrane.

(iv). A detergent to solubilize and break the lipid bi-layer of cell membranes that encloses cellular organelles to release the nucleic acids. Two selected detergents, i.e. Triton X-100, and SDS will be used.

(v). An inhibitor removal to remove substances such as polyphenolic compounds, humic acids, tannic acids and other substances in the lysate that can crosslink nucleic acids and inhibit their function. The inhibitor removals will include, activated charcoal, PVP-40, citric acid in the lysis buffers, while BSA will be added in PCR reactions.

PuriNA Lysis Buffer I (PLB-I): will be modified from Scallan *et al*. (2020) which was a previous modification of Chomczynski and Sacchi. (1987), and contained (4 M GuSCN, 55 mM Tris-HCl pH 7.6, 25 mM EDTA, 3% (v/v) Triton-X 100, 0.01% (w/v) Bromophenol blue). In our case, Bromophenol blue which was used in the buffer as a fluorescent dye in qPCR will be removed, and 2% PVP-40 an inhibitor removal component, used in Mason and Botella. (2020), added together with 1% activated charcoal to produce our PLB-I.

4 M GuSCN,

50 mM Tris-HCl pH 6.0,

25 mM EDTA pH 8.0,

2.5% (v/v) Triton-X 100,

2% (v/v) PVP-40,

1.5%(v/v) activated Charcoal.

PuriNA Lysis Buffer II (PLB-II): will be modified from Pramanick *et al*. (1976), and Weidner *et al.* (2022). The original buffer composition had (4 M GuHCl, 0.05 M Tris-HCl, 0.01 M EDTA pH 7.6) while the later contained (4 M GuHCl, 50 mM Tris-HCl pH 6.5, 20 mM EDTA pH 6.5, 1% (v/v) Triton-X 100). Our PLB-II will be composed of:

4 M GuHCl,

50 mM Tris-HCl pH 6.0

25 mM EDTA pH 8.0

3% (v/v) Triton-X 100

2% PVP-40

PuriNA Lysis Buffer III (PLB-III): will be modified from Edwards *et al*. (1991), Alexander (2016) and Mason and Botella (2020). The corresponding composition of these buffers were: (0.5% SDS, 200 mM NaCl, 25 mM EDTA, 200 mM Tris-HCl pH 8.0) and (20 mM NaCl, 20 mM Tris -HCl pH 8.0, 2.5 mM EDTA pH 8.0, 0.05 % (w/v) SDS, 2% (w/v) PVP-40). Our PLB-III would be modified to contain:

200 mM NaCl

50mM Tris -HCl pH 6.0

25 mM EDTA pH 8.0

0.4% SDS

2% (v/v) PVP-40,

1.2% Citric acid.

To prepare each of the buffer, stock solution of high molar salt concentration will made, using the molecular weight (MW) of each salt obtained from the safety data sheet of each compound. The mw of GuSCN = 118.16, GuHCl = 95.53, NaCl = 58.44, Tris = 121.14, HCl = 36.48, SDS = 288.86 among other compounds. The working concentration of the buffer will then be calculated and prepared from the stock solutions.

At the start of the experiment, each of the three lysis buffers will be fully constituted and its aliquots kept on the shelf and assessed weekly to monitor the useful shell life of each of the three lysis buffers.

Experimental variables for manipulation During Extraction of Nucleic Acids with PuriNA Lysis Buffers I, II, and III

The values of these variables will be altered and randomly assigned to experimental units:

(i). molarity of Tris-HCl at graded concentrations from (50, 75, 100, 125, 150) mM.

(ii). the lysis buffers pH at: 6.0, 6.4, 6.8, 7.2, 7.6, 8.0.

(iii). Percentage concentration of detergents for Triton-x 100; (v/v) 2.5%, 3.0% 3.5% 4.0%, 4.5%.

SDS (v/v) 0.4%, 0.6%, 0.8%, 1.0%, 1.2%.

(iv). Layers of silica membrane spin column at: 6, 7, 8, 9 layers.

(v). Incubation temperature -: 50°C, 55°C, 60°C, 65°C, 70°C, 75°C

(vi). incubation time of the lysis buffer -: 10 min, 20 min, 30 min, 40 min, 50 min, 60 min.

(vii). PuriNA Binding Buffers (PBB)-1, 2, and 3.

Three variants of the binding buffer would be produced based on the range of the binding buffer's pH between 6.0-6.5 (Uyttendaele *et al.* 1996, Zainabadi *et al*., 2019). Guanidine thiocyanate, guainidine hydrochloride and sodium chloride all bind nucleic acids to matrices. However, GuSCN is not compatible to SDS. Since SDS is present in one of the buffers, the three binding buffers will be formulated from GuHCl for ease of comparison with the final buffer pH adjusted with glacial acetic acid (HOAc).

PBB-1 will constitute 4.25 M GuHCl, 3.75 M NH4Ac, pH 6.0

PBB-2 will constitute 4.5 M GuHCl, 3.75 M NH4Ac, pH 6.0

PBB-3 will constitute 4.75 GuHCl, 3.75 M NH4Ac , pH 6.0

(viii). PuriNA spin column wash buffer (PWB)

Ethanol is normally used to wash the silica spin column to remove excess salts and unbound nucleic acids. However, the percentage composition of ethanol alone or in combination with other buffers or isoamyl alcohol may improve the recovery of nucleic acids (Zainabadi *et al*., 2019). Three PWB will be prepared and tested.

PWB-1 to contain: 1 M Tris-HCl pH 7.5, 80 % ethanol

PWB-2 will contain: 1 M Tris-HCl pH 8.0, 80 % ethanol

PWB-3 will contain: 1 M Tri-HCl pH 8.5, 80 % ethanol

(ix). PuriNA elution buffer (PEB) the elution buffer will contain the standard TE buffer known to elute nucleic acid. The pH will be varied to determine the elution of which nucleic acid (DNA or RNA) will be eluted, and at what concentration. The elution buffers will contain:

PEB-1: 10 mM Tris-HCl, 1 mM EDTA, final pH 6.5

PEB-2: 10 mM Tris-HCl, 1 mM EDTA final pH 7.5

PEB-3: 10 mM Tris-HCl, 1 mM EDTA final pH 8.5

Fixed Experimental variables

The values of these experimental variables will not be altered but remain constant:

(i). The concentration of the salt – 4 M GuSCN, 4 M GuHCl, 200 mM NaCl.

(ii). Molar concentration of EDTA at 25 mM.

(iii). The pH of EDTA at pH 8.0

(iv). The percentage composition of inhibitor removals specified in each of the three PuriNA lysis buffers.

Nucleic acids extraction from experimental organisms or samples using silica spin column

1. From each sample, that is: E. coli resuspended pellets, T4 Bacteriophage particles, Aspergillus flavus suspension, homogenized animal tissue, water sample resuspended particles, and soil samples, a volume of 100 µl of the sample will be transferred into a labelled 1.5 ml Eppendorf microcentrifuge tube. Each sample will be treated separately.

2. An equal volume: 200 µl of one of the PuriNA lysis buffer (PLB-I, PLB-II, PLB-III) which was randomly assigned to a pre labelled tube will be transferred to the 1.5 ml microcentrifuge tube and vortexed for a few seconds.

3. The tube will be incubated in a heat block at either, 50°C, 55°C, 60°C, 65°C, 70°C, or 75°C, based on random assignment of both temperature and time. The time of incubation will vary from 10 min, 20 min, 30 min, 40 min, 50 min, or 60 min.

4. After the incubation period, 2 ul of RNase A (10 mg/ml) is added and further incubated at 37°C for 30 min (applicable only for DNA extraction).

5. After the incubation period, the 1.5 ml micro centrifuge tube will be removed from the heat block.

6. Then 200 µl of Purina binding buffer (PBB 1, PBB 2, PBB 3) randomly assigned is added, another 200 µl of 96% ethanol is also added and the tube inverted several times.

7. The 600 µl of the content in each tube is transferred to a silica spin column (layers: 6, 7, 8, or 9).

8. The silica spin column is placed in a high-speed micro centrifuge and centrifuged at 13,000 g for 1 minute.

9. The flow through from the silica spin column is discarded.

10. The silica spin column will be washed by the addition of 700 µl of PuriNA column wash buffer (PWB 1, PWB 2, or PWB 3).

11. The silica spin column is returned to the micro centrifuge and centrifuges at 13,000 g for 1 min.

12. The flow through is discarded, and another 500 µl of PWB 1, PWB 2, or PWB 3 added.

13. The silica spin column is centrifuged at 13,000 g for another 1 min.

14. The silica spin column is removed, placed on a new collection tube and spun at 13,000 g for 2 min to dry the column.

15. The spin column is mounted on to a new clean 1.5 ml Eppendorf tube and 100 µl of PuriNA elution buffer (PEB 1, PEB 2, or PEB 3) added in the centre of the silica spin column and incubated at room temperature for 5 min.

16. The spin column is centrifuged at 13,000 g for 1 min and purified nucleic acid (DNA or RNA) collected in the 1.5 ml Eppendorf tube and stored briefly at 4°C or -20°C for molecular biology assays.

Spectrophotometric quantification of purity of nucleic acid extracted from each sample

The purity of DNA or RNA isolated by the PuriNA nucleic acid extraction and purification protocol, and the yield from *E. coli*, T4 Bacteriophage, *A. flavus*, animal tissue, plant tissue, water sample and soil samples will be individually assessed by taking 1 µl of the purified product into a cuvette, loaded on a NanoDrop for 5 seconds. The absorbance value of the sample will be each measured at the wave lengths of 230 nm, 260 nm and 280 nm, and 320 nm. The ratio of 260/280 and 260/230 will be calculated to check nucleic acid purity or contamination from protein, salts, ethanol and other contaminants (Bruijns *et al*., 2022).

The 260/280 of pure RNA =1.9 - 2.1, while 260/280 of pure DNA = 1.8 -1.9 (New Englang Biolabs (2023). The 260/230 of pure RNA = 2.0 – 2.2, while pure DNA = 1.8 -2.5. A significant deviation from these, expected ratio values of 260/230 will indicate contamination from protein, salts, ethanol or other contaminants. The above ratios will be compared with the PuriNA sample extracts and Qiagen positive control extracts.

Determination of DNA yield

The DNA concentration will be estimated by measuring the absorbance at 260nm, adjusting the A260 measurement for turbidity (measured by absorbance at 320nm), multiplying by the dilution factor, and using the relationship that an A260 of 1.0 = 50µg/ml pure dsDNA (double stranded DNA).

Concentration (µg/ml) = (A260 reading – A320 reading) × dilution factor × 50µg/ml

Total yield will be obtained by multiplying the DNA concentration by the final total purified sample volume.

DNA yield (µg) = DNA concentration × total sample volume (ml).

Determination of RNA yield

The concentration of RNA isolated with PuriNA buffers will be determined by the absorbance at 260 nm (A260). An absorbance of 1 unit at 260 nm corresponds to 40 µg of RNA per ml (A260 = 1 = 40 µg/ml). This relationship is valid for measurements in water. Therefore, the isolated RNA will be diluted in water for spectrophotometric quantification.

A hypothetical example of the calculations involved in RNA quantification is shown below. The buffer in which the RNA is diluted is used to calibrate the spectrophotometer to zero. Thus if the volume of RNA eluted from the silica spin column is 100 µl then:

,• Volume of RNA sample = 100 µl

- Dilution = 10 µl of RNA sample + 490 µl distilled water (1/50 dilution)
- Absorbance of diluted sample measured in a 1 ml cuvette (RNase-free): A260 = 0.23
- Concentration of original RNA sample = 40 x A260 x dilution factor = 40 x 0.23 x 50
- RNA concentration: 460 µg/ml
- Total yield = concentration x volume of sample (ml) = 460 µg/ml x 0.1 ml
- RNA yield: 46 µg

Quality and integrity check of RNA and DNA by agarose gel electrophoresis

To assess the integrity of total RNA, an aliquot of RNA isolated by different PuriNA lysis and purification buffers from each sample source will be run on a denaturing agarose gel stained with ethidium bromide (EtBr). That is 2 µl of RNA in denaturing buffer (50 µl formamide, 20 µl formaldehyde, 10 ul 10X MOPS, and 2 µl ethidium bromide) denatured at 70°C for 3 minutes and immediately placed on ice. Then run on 1.2% TBE agarose gel electrophoresis at 90 Volts (Figure 1).

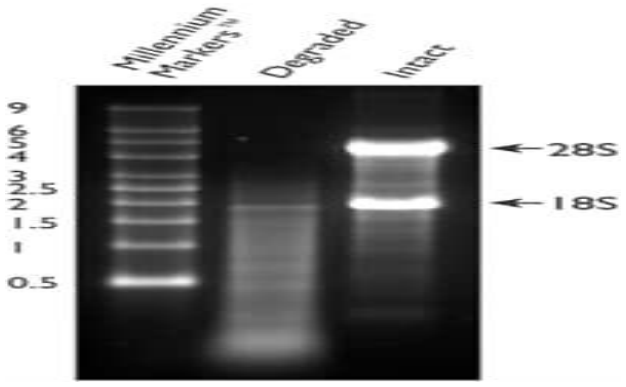

Figure 1. Electrophoresis of degraded and intact total RNA on denaturing agarose gel

(Source: New England Biolabs (NEB) technical handbook 2023).

Left lane = Millennium markers (RNA size marker), middle lane = degraded RNA, right lane = intact RNA.

Intact total RNA run on a denaturing gel will give sharp, clear 28S and 18S rRNA bands as shown in Figure 1 above. The 28S rRNA band intensity would be approximately twice as intense as the 18S rRNA band (Figure 1, right lane). This 2:1 ratio (28S:18S) would give a good indication that the RNA is completely intact. Partially degraded RNA will yield a smeared appearance, will lack the sharp rRNA bands, or will not exhibit the 2:1 ratio of high-quality RNA. Completely degraded RNA will appear as a very low molecular weight smear (Figure 1, middle lane). The RNA size markers will be included on the gel to the allow the size of any bands or smears to be determined. It and would also serve as a good control to ensure the quality of the gel was run properly (Figure 1, left lane).

The integrity of the genomic - gDNA will be assessed by loading approximately 100 ng per sample on a 0.75% agarose gel, separated by gel electrophoresis and comparing the size distribution to a suitable

DNA molecular weight size marker, such as lambda (λ) DNA, digested with *Hind III* restriction enzyme. Typically, for intact gDNA, the majority of the gDNA signal will be larger than the upper band (23,130 bp) of the *Hind III* restriction enzyme digest (Figure 2).

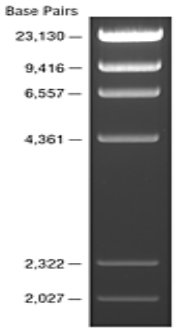

Figure 2. Lambda (λ) DNA Digest with *Hind III* Restriction Enzyme

(Source: New England Biolabs (NEB) technical handbook 2023).

Determination of suitability of nucleic acid extraction and purification by PuriNA protocol for downstream application in molecular biology

The polymerase chain reaction (PCR) has ubiquitous application in molecular biology. Before gene cloning, genome sequencing, restriction digestion of a target gene to determine mutations in a gene, metagenomic analysis of microbial community in an environmental sample, and many other applications are done, PCR is first performed. The product of PCR amplification is then applied to downstream applications in molecular biology. In order to test the success of our PuriNA nucleic acid extraction protocol for universal application in molecular biology, PCR amplification of prokaryotic and eukaryotic genes using degenerate universal primers will be performed with DNA, purified from the PuriNA protocol, while Reverse transcription PCR (RT-PCR) will be conducted for RNA isolated with the PuriNA protocol and compared with Qiagen reference kit.

(i). **Amplification of Prokaryotic genes by PCR**

Prokaryotic genes will be amplified using the primer pair PRO341FB as forward primer and PRO805R as reverse primer, which has very high coverage rate for detection of bacteria and archaea species in the environment (Mazolli et al., 2020).

Primer name	5’ – sequence - 3’	Reference
PRO341FB	CCTACGGGNBGCWSCAG	Mazolli *et al.*(2020)
PRO805R	CCTACGGGNBGCASCAG	Takahashi *et al*.(2014)

The PCR reaction will be optimized by gradient PCR reaction, and amplified to determine if the reaction can produce next generation sequencing (NGS) amplicon. The PCR reaction will follow the NEB Q5 High Fidelity DNA polymerase protocol and would contain the following PCR components:

PCR component	Volume in 25 µl reaction	Final concentration
Nuclease free water	9.75 µl	-
5x Q5 reaction buffer	5.0 µl	1x
10 mM dNTPs	0.5 µl	200 µM
10 µM PRO341FB	1.25 µl	0.5 µM
10 µM PRO805R	1.25 µl	0.5 µM
5x Q5 high GC enhancer	5.0 µl	1x
Q5 high-fidelity polymerase	0.25 µl	0.02 U/ µl
DNA template	2.0 µl	variable

The PCR reaction condition will be: initial denaturation at 98 °C for 30 sec, denaturation at 98 °C for 10 sec, primer annealing at 65°C for 30 sec, 25-35 cycles of extension at 72°C for 30 sec, and final extension at 72°C for 2 min.

The PCR amplicon will be separated on 2 % agarose gel electrophoresis in Tris Borate EDTA (TBE) buffer, stained with ethidium bromide, viewed and photographed on UV-Transilluminator (Analytic Gena) estimation of banding pattern on agarose gel.

(ii) **Amplification of Eukaryotic genes by PCR**

Similarly, degenerate eukaryote-specific small-subunit (SSU) rRNA forward primer- 82FE will be used together with modified "universal" reverse primer -1391RE to selectively amplify eukaryotic SSU rDNA genes. This primer pair has over 98.6% coverage rate for detection of eukaryotic organism species in environmental sample (Dawson and Pace, 2002).

Primer name	5' – sequence - 3'	Reference
F-82FE	GAADCTGYGAAYGGCTC	Dawson and Pace. (2002)
R-1391RE	GGGCGGTGTGTACAARGRG	Dawson and Pace. (2002)

The PCR reaction will be optimized by gradient PCR reaction, and amplified to determine if the reaction can produce next generation sequencing (NGS) amplicon. The PCR reaction will follow the NEB Q5 High Fidelity DNA polymerase protocol and contain the following PCR components:

PCR component	Volume in 25 µl reaction	Final concentration
Nuclease free water	9.75 µl	-
5x Q5 reaction buffer	5.0 µl	1x
10 mM dNTPs	0.5 µl	200 µM
10 µM 82FE	1.25 µl	0.5 µM
10 µM 1391RE	1.25 µl	0.5 µM
5x Q5 high GC enhancer	5.0 µl	1x
Q5 high-fidelity polymerase	0.25 µl	0.02 U/ µl
DNA template	2.0 µl	variable

The PCR reaction condition will be: initial denaturation at 98 °C for 30 sec, denaturation at 98 °C for 10 sec, primer annealing at 60°C for 30 sec, 25-35 cycles of extension at 72°C for 30 sec, and final extension at 72°C for 2 min.

The PCR amplicon will be separated on 2 % agarose gel electrophoresis in Tris Borate EDTA (TBE) buffer, stained with ethidium bromide, viewed and photographed on UV-Transilluminator (Analytic Gena) estimation of banding pattern on agarose gel.

In each case the prokaryotic and eukaryotic gene amplification, nucleic acids extracted and purified by our PuriNA buffers will be run side by side with the commercial reference kit, by Qiagen.

Reverse Transcription PCR for analysis of total isolated RNA by PuriNA purification protocol

The RNA nucleic acid is a single stranded molecule and is not useful for direct PCR unlike DNA which is double stranded. The ability to synthesize a cDNA (complementary DNA) from an RNA template, via reverse transcription, enables researchers to study RNA with the same molecular tools applicable to DNA. The cDNA generated by reverse transcription can be amplified using polymerase chain reaction (PCR). The combination of reverse transcription and PCR (RT-PCR) permits sensitive detection of low abundance RNAs in a sample. The resultant PCR Products can be used in many downstream applications in molecular biology.

The single stranded RNA isolated from the PuriNA purification protocol and the one by a reference New England Biolabs (NEB) RNA purification protocol will be converted to a cDNA (complementary DNA) using the NEB reverse transcription enzyme. RT-PCR will then be performed with the cDNA. For the cDNA strand synthesis, the following components will be mixed for the reaction in a sterile RNase free microfuge tube.

Component	Volume in 20 µl reaction
Total RNA	1.0 µg
Random primer mix (60 µM)	2.0 µl
10 mM dNTPs	1.0 µl
RNA inhibitor murine (40 U/µl)	0.2 µl
5 x Induro RT reaction buffer	4.0 µl
Induro Reverse Transcriptase (200 U/µl)	5.0 µl
Nuclease free water added up to 20 µl	variable

The mixture for the 20 µl cDNA synthesis reaction will be first incubated at 25°C for 2 minutes then raised to 55°C for 10 minutes, follow by inactivation at 95°C for 1 minute (New England Biolabs, 2023). The cDNA product will be stored at -20°C for RT-qPCR which can be used to quantify a specific expression of a gene by determination of the cycle threshold (Ct) value of a gene in parallel with a known standard.

DATA ANALYSIS

Multivariate analysis of data collected on purity and yield (concentration) of DNA and RNA obtained by the PuriNA nucleic acid purification buffers with the type of silica spin column (6, 7, 8, and 9- layers) will be done using principal component analysis (PCA) in R statistics (Nguyen 2020). The purity and concentration of DNA and RNA each determined from the nested variables of pH, incubation

temperature of the lysis buffer, time of incubation and the concentration of detergents in the lysis buffer used on a specific layer of spin column will be analyzed.

Scree plot will be used to describes how much weight is explained by each variable component. Permutation test for eigenvalues will tests if eigenvalues are reliable and will be conducted using Random Resampling. Correlation Plot will be applied to describe the linear relationships between variables when applicable. Bar plots with Bootstrap will be used to describe the effect strength of each variable (Variables Contribution). The analysis of variance (ANOVA) will be used to compare yield and purity of nucleic acids among the PuriNA buffers, the different experimental variables, and the different sources of samples used to extract the nucleic acid. In each case the level of significance will be set as $p \leq 0.05$.

These sets of analysis will identify the most significant variables (pH, temperature, incubation time, or the concentration of detergents) that significantly improves either purity, or concentration of DNA or RNA.

REVALIDATION EXPERIMENTS TO DETERMINE THE MOST SUITABLE VARIABLE CONDITIONS FOR NUCLEIC ACID EXTRACTION PROTOCOL WITH PuriNA BUFFERS

The results of the statistical analysis will be used in revalidation experiments to select the most suitable variable conditions for high quality DNA or RNA extraction with the PuriNA buffers. The extraction will be done alongside a Qiagen reference kit, and improvements made on the buffers by varying the conditions till the yield and purity of RNA or DNA matches of surpasses that of the Qiagen reference kit.

The best conditions will be used to write protocols that describe the extraction and purification of DNA or RNA in a given sample under as set of laboratory apparatus, reagents and supplies as our improved prototypes, for DNA or RNA extraction and purification.

Development of Prototypes of Nucleic Acid Extraction and Purification by PuriNA Buffers

This project will be based on the results of the statistical analysis of data obtained by PuriNA lysis buffers – PLB-I, PLB-II and PLB-III at different protocol conditions of:

(i). The molar concentration of the specific chaotropic salt used in the lysis buffer (GuSCN, GuHCl, and NaCl) at which optimal isolation of DNA or RNA was achieved in a given sample type.

(ii). The molar concentration of Tris-HCl in a given buffer, and the optimum pH at which optimal isolation of DNA or RNA was achieved in a given PuriNA buffer with a specific sample type.

(iii). The type of detergent (Triton-X 100 and SDS) and the percentage concentration in combination with Tris-HCl and chaotropic salt characteristic in (ii) and (i) above.

(iv). The type, and concentration of inhibitor removal that enhanced the combinations of conditions such as detergent, Tris-HCl, and Chaotropic salt in (iii), (ii) and (i) above for a given sample type.

(v). The optimal - incubation temperature of the PuriNA lysis buffer, the time of incubation, the layer of spin column used to purify DNA or RNA, the specific binding buffer, the wash buffer, and the elution buffer for DNA or RNA for a given sample type will be factored in the selection of a particular protocol for the purification of DNA or RNA in a particular type of sample with PuriNA Buffers. At least a total of 10 best prototypes given the various combinations above can be produced from this project.

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Yeates, C., Gillings, MR., Davison, AD., Altavilla, N., and Veal, DA. (1999). Methods for microbial DNA extraction from soil for PCR amplification. *Biological Procedures Online*, 1: 40-47.

Zainabadi, K., Nyunt, M.M., and Plowe. C.V. (2019). An improved nucleic acid extraction method from dried blood spots for amplification of Plasmodium falciparum Kelch 13 for detection of artemisinin resistance. *Malaria Journal,* 18: 192

Zhou, Y., Mason, M.G., Wang, Y., Wee, E., Turni, C., Patrick J. Blackall, P.J., Trau, M., Botella, J.R. (2017). Nucleic acid purification from plants, animals and microbes in under 30 seconds. *PLOS Biology*, 15(11): e2003916.

Zhou, Y., Zhang, Y., He, W., Wang, J., Peng, F., Huang, L., Zhao, S., and Deng, W. (2018). Rapid regeneration and reuse of silica columns from PCR purification and gel extraction kits. *Scientific Reports*, 8: 12870 https://doi.org/10.1038/s41598-018-30316-w

Project Activities and Outputs (give details of the research and their expected output i.e. results to be obtained/produced within the proposed time frame of the project.

1. Collection of necessary equipment and reagent supplies for the project.

2. Collection and maintenance of laboratory organisms – *E. coli* bacteria and T4 bacteriophage and *Aspergillus flavus.*

3. Field sampling to collect other study subjects - animal tissue, *Azadirachta indica* leaves, water sample and soil samples at 3 designated sampling sites in each state of North central Nigeria and Abuja.

4. Preparation of stock and working solutions of our in-house prepared sample lysis buffers known as PuriNA buffers I, II, and III at appropriate concentration of individual chemical constituents, and pH.

5. Preparation of 3 in-house nucleic acid binding buffers to silica column known as PuriNA binding buffers 1, 2 and 3 at appropriate chemical concentration and pH.

6. Preparation of 3 in-house silica column wash buffers known as PuriNA wash buffers 1, 2 and 3.

7. Preparation of 3 in-house DNA or RNA elution buffers known as PuriNA elution buffers 1, 2 and 3.

8. Using 100 µl of each of the PuriNA lysis buffers I, II, and III to incubate with 100 µl preparation of each of the study organisms/subjects at a range of temperatures degree Celsius (°C), and a range of time in minutes. The mixture at this level is known as the lysate. (Note: this step will enable us to determine which of the PuriNA lysis buffer I, II, and III at a given pH, temperature of incubation, and time of incubation will be most appropriate for the isolation of DNA or RNA, in a particular sample type or organism after later steps in the purification of nucleic acids are applied).

9. The lysate in step (8) above from each of the in-house prpared PuriNA lysis buffers I, II, and III will be bound to a silica spin column of 4 types with 6, 7, 8, and 9 layers of silica membrane in the column. The step will be carried out using the three in-house PuriNA binding buffers, 1, 2 and 3. (Note: this step will identify the most appropriate binding buffer to each of the silica spin column for purification of DNA or RNA from a particular sample.

10. The DNA or RNA bound to silica column in step (9) above will be washed with each of the PuriNA wash buffers. This step will identify the most appropriate spin column wash buffer prepared in-house for DNA or RNA isolation.

11. The silica spin column washed in step (10) above is dried at room temperature, and DNA or RNA eluted from it with our in-house PuriNA elution buffers 1, 2, and 3. This will allow us to determine the most appropriate elution buffer for RNA or DNA.

12. The concentration and purity of DNA or RNA will be determined using Nanodrop spectrophotometer. Quality of nucleic acid will be assessed by gel electrophoresis. The suitability of the DNA or RNA isolated for application in molecular biology will be assessed by PCR amplification of prokaryotes and eukaryotes genes, and conversion of RNA to cDNA for amplification by RT-PCR.

13. statistical analysis of data generate in the above steps will enable to compile a set of a number of protocols for isolation and purification of DNA or RNA using a combination of our in-house prepared buffers with silica spin columns under specific conditions of chemical concentration, pH, temperature, time, type of spin column, specific binding, washing, and elution buffers that one can apply to isolate DNA or RNA from a given type of sample.

14. Our protocol will be compared with a similar commercial kit from Qiagen in a validation experiment to match or surpass the quality performance of our in-house prepared buffers, compared to the commercial kits.

Time frame (provide a time frame for major activities of the project)

A Gantt Chart showing the research activities and period of project execution in 18 months after Proposal approval of the project titled: “Prototype development of a modular and highly sensitive DNA/RNA extraction and purification kit for downstream applications in molecular biology”. Is provided below.

Research activities in each quarter (Q) of a year during the period of project execution	**2023**	**2024**				**2025**	
	Q4	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**
Full proposal development and approval	X						
preparation for project implementation		X					
Purchase and delivery of critical equipment		X	X				
Purchase and delivery of critical reagents/supplies		X	X				

Collection and *in vitro* culture of Laboratory organisms (*E. coli ,* T4 bacteriophage and *A. flavus*)		X	X				
Submission of Progress Report I			X				
Preparation of buffer stock solutions			X	X	X	X	
Preparation of working buffer solution for shelf-life estimation			X	X	X	X	
Regular check on Buffer pH, and recording of laboratory data			X	X	X	X	
Extraction and Purification of DNA/RNA from *E. coli* T4 virus and *A. flavus*			X	X			
Collection of field samples from sampling locations			X	X	X		
Extraction of DNA/RNA from meat and plant tissues				X	X		
Extraction of nucleic acids from water and soil samples				X	X		
Optimization of PCR reactions by Gradient PCR				X			
PCR and RT-PCR analysis of Purified nucleic acid				X	X		
Submission of Progress Report II					X		
Statistical analysis of experimental data						X	
Compilation of protocol for DNA or RNA extraction and purification in a given sample (prototype)						X	
Revalidation experiments on identified prototypes						X	
Journal publication of research data and writing of final report						X	X
Writing and submission of final report							X
Total months of project execution = 18 months post proposal approval							

Key performance indicators (clearly state the indicators of each major activity of the project)

The following will form the key performance indicators during this project execution.

1. Timely collection of equipment and reagents critical to the success of the project within 6 months of proposal approval and release of fund.

2. Successful culture and maintenance of laboratory organisms at the designated period.

3. Successful preparation of buffers at the right molarity and pH.

4.Successful extraction and purification of DNA and RNA from the laboratory-maintained organisms.

5.succsseful collection of field samples at the designated sampling areas followed by DNA and RNA extraction from the field samples.

6. Optimization of PCR rations and successful amplification of specific gene targets from out DNA and RNA extracts.

7. Statistical analysis of the data.

8. selection and compilation of protocols for nucleic acid extraction in specific sample sources

9. Timely writing and submission of final report on the project.

Monitoring and evaluation mechanism (state clearly the monitoring and evaluation mechanism you would adopt to achieve the stated objectives)

The monitoring and evaluation mechanism will involve timely or early completion of research activities specified in work plan listed in the Gantt chart. If a task on the Gantt Chart is completed earlier, the next can begin ahead of its scheduled time. The principal investigator will commit to ensuring that scheduled activities are performed on time.

Dissemination Strategies (indicate steps you would take to ensure the project outcomes are brought to the attention of stakeholders)

The project outcomes will be presented at the 41st annual conference of Biochemistry and Molecular Biology Society of Nigeria. Also, findings from the project will be published in a Journal related to nucleic acids research or experimental methods.

RESEARCH TEAM

Position: - Principal Investigator

Name: - Terwase Fabian IKPA

Date of Birth: - 16/04/1970

Sex: - Male

Email: - ikpaft@uam.edu.ng

Qualification: - PhD

Specialization: - Cellular Parasitology

Rank: - Professor

Institution/Organization: - Joseph Sarwuan Tarka University Makurdi

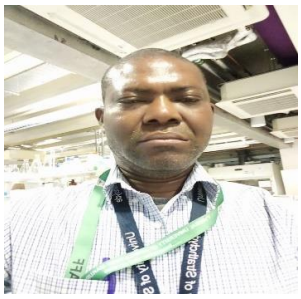
Faculty/Department: - Biological Sciences/Zoology

City/Town: - Makurdi

Phone No: - 08022732614

Postal Address: - Department of Zoology, Joseph Sarwuan Tarka University Makurdi, PMB 2373 Makurdi, Benue State Nigeria.

Position: - Co-Researcher



Name: - John Ogbaji IGOLI

Date of Birth: - 20/11/1964

Sex: - Male

Email: - j.o.igoli@uam.edu.ng

Qualification: - PhD

Specialization: - Natural Product Chemistry

Rank: - Professor

Institution/Organization: - Joseph Sarwuan Tarka University Makurdi

Faculty/Department: - Physical Sciences/Chemistry

City/Town: - Makurdi

Phone No: - 08130991308

Postal Address: - Department of Chemistry, Joseph Sarwuan Tarka University Makurdi, PMB 2373 Makurdi, Benue State Nigeria.

Position: - Co-Researcher

Name: - Grace Mwuese GBERIKON

Date of Birth: - 01/01/1971

Sex: - Female

Email: - gberikon.grace@uam.edu.ng

Qualification: - PhD

Specialization: - Industrial Microbiology

Rank: - Professor

Institution/Organization: - Joseph Sarwuan Tarka University Makurdi

Faculty/Department: - Biological Sciences/Microbiology

City/Town: - Makurdi

Phone No: - 08034519567

Postal Address: - Department of Microbiology, Joseph Sarwuan Tarka University Makurdi, PMB 2373 Makurdi, Benue State Nigeria.

Position: - Co-Researcher

Name: - John Vershima ANYAM

Date of Birth: - 09/06/1980

Sex: - Male

Email: - john.anyam@uam.edu.ng

Qualification: - PhD

Specialization: - Natural Product Chemistry

Rank: - Senior Lecturer

Institution/Organization: - Joseph Sarwuan Tarka University Makurdi

Faculty/Department: - Physical Sciences/Chemistry

City/Town: - Makurdi

Phone No: - 08059081460

Postal Address: - Department of Chemistry, Joseph Sarwuan Tarka University Makurdi, PMB 2373 Makurdi, Benue State Nigeria.

Position: - Research Mentees/Young Academics

Name: - James Agada Okete

Date of Birth: - 27/11/1988

Sex: - Male

Email: - okete.james@uam.edu.ng

Qualification: - PhD

Specialization: - Applied Parasitology

Rank: - Lecturer I

Institution/Organization: - Joseph Sarwuan Tarka University Makurdi

Faculty/Department: - Biological Sciences/Zoology

City/Town: - Makurdi

Phone No: - 09014155119

Postal Address: - Department of Zoology, Joseph Sarwuan Tarka University Makurdi, PMB 2373 Makurdi, Benue State Nigeria.

Position: - Research Mentees/Young Academics

Name: - Samuel Atabo

Date of Birth: - 19/05/1983

Sex: - Male

Email: - samuel.atabo@uam.edu.ng

Qualification: - MSc

Specialization: - Nutritional Biochemistry

Rank: - Lecturer I

Institution/Organization: - Joseph Sarwuan Tarka University Makurdi

Faculty/Department: - Biological Sciences/Biochemistry

City/Town: - Makurdi

Phone No: - 08059457421

Postal Address: - Department of Biochemistry, Joseph Sarwuan Tarka University Makurdi, PMB 2373 Makurdi, Benue State Nigeria.

Position: - Research Mentees/Young Academics

Name: - Etim Esin ETIM

Date of Birth: - 16/01/1980

Sex: - Male

Email: - etim.esin@uam.edu.ng

Qualification: - MSc

Specialization: - Medical Biochemistry

Rank: - Lecturer II

Institution/Organization: - Joseph Sarwuan Tarka University Makurdi

Faculty/Department: - Biological Sciences/Biochemistry

City/Town: - Makurdi

Phone No: - 08160899755

Postal Address: - Department of Biochemistry, Joseph Sarwuan Tarka University Makurdi, PMB 2373 Makurdi, Benue State Nigeria.

Position: - Research Mentees/Young Academics

Name: - Faith Odije OKITA

Date of Birth: - 18/05/1987

Sex: - Female

Email: - fokita@bsum.edu.ng

Qualification: - MSc

Specialization: - Zoology

Rank: - Lecturer II

Institution/Organization: - Benue State University Makurdi

Faculty/Department: - Sciences/Biological Sciences

City/Town: - Makurdi

Phone No: - 07038586569

Postal Address: - Department of Biological Sciences, Benue State University Makurdi.

Position: - Research Mentees/Young Academics

Name: - Joel Ireoluwa YINKA

Date of Birth: - 08/08/1995

Sex: - Male

Email: - joelireoluwa@gmail.com

Qualification: - MSc

Specialization: - Molecular Biology

Rank: - Assistant Lecturer

Institution/Organization: - Joseph Sarwuan Tarka University Makurdi

Faculty/Department: - Biological Sciences/Biochemistry

City/Town: - Makurdi

Phone No: - 07011603713

Postal Address: - Department of Biochemistry, Joseph Sarwuan Tarka University Makurdi, PMB 2373 Makurdi, Benue State Nigeria.

BRIEF PROFILE OF RESEARCH TEAM

Prof. Terwase Fabian Ikpa

is the Principal Investigator (PI) and Professor of Parasitology in the Department of Zoology, Joseph Sarwuan Tarka University Makurdi (JOSTUM). His research interests include investigating the *in vitro* and molecular resistance of malaria parasites to antimalarial drugs, and hybridization in schistosome species. As the Project's PI, he will coordinate the activities of the research group, conduct DNA/RNA extraction and purification assays, and perform statistical analysis of the project data.

Prof. John Ogbaji Igoli

is a co-researcher and Professor of Natural Products and Drug Discovery in the Department of Chemistry JOSTUM. He has expertise in the analysis of bioactive molecules and compounds at molecular and cellular levels. He uses NMR and LC-MS to examine and identify metabolites from biofluids and extracts. In this project, Prof. Igoli will formulate in-house buffers, perform risk assessment of handling chemical and biochemicals, and participate in report writing.

Prof. Grace Mwuese Gberikon

is a co-researcher and Professor of Industrial Microbiology in the Department of Microbiology, at JOSTUM. She has expertise in identification of fermenting strains of microorganisms by using DNA technology to identify genes encoding enzymes for fermentation. In this project, she will culture and maintain model organisms in the laboratory, and participate in DNA/RNA extraction and purification.

Dr. John Anyam

is a co-researcher, and a Senior Lecturer in the Department of Chemistry at JOSTUM. He specializes in natural products and drug discovery, isolation, synthesis, and characterization of bioactive compound. In this project, Dr. Anyam will be responsible for preparing and optimizing biological buffers for DNA/RNA extraction and purification.

Dr. James Agada Okete

Is a mentee and Lecturer I in the Department of Zoology, at JOSTUM. His research interests include molecular diagnosis and epidemiology of parasitic helminths. He is a recipient of Humboldt Research

Hub (HRH)-CERID scholarship and a collaborator with the Humboldt-Bayer Foundations Research Hub. In this project, Dr. Okete will be responsible for field sample collection.

Samuel Atabo

is a mentee and Lecturer I in the Department of Biochemistry, JOSTUM. He is also a PhD student at UNN, with interest in molecular characterization of the impact of nutrition on experimental models. He has published on tropical diseases and ethnopharmacology. He had experience as a data management officer at Alpha Research and Development Centre, Abuja. He is proficient in Microsoft Excel and currently exploring the use of Python and R. In this project, he will participate in data collation and analysis.

Etim Esin Etim

is a mentee and Lecturer II in the Department of Biochemistry, JOSTUM. He is also PhD student at UNN. He is versatile in tissue culture methods using cell lines. His current area of interest is the utilization of plant products in Prostatic hyperplasia. In this project, he will participate in the laboratory maintenance of model organisms.

Faith Odije Okita

is a mentee and Lecturer II at the Benue State University Makurdi. She is about to round up her PhD in Parasitology in the Department of Zoology at JOSTUM. Her research investigated the genetic hybridization of schistosome species in Benue State, using Sanger sequencing and PCR. She completed her benchwork at the Natural History Museum London. In this project, she will perform PCR, and agarose gel electrophoresis.

Joel Ireoluwa Yinka

is a mentee and an Assistant Lecturer in the Department of Biochemistry at JOSTUM. He is also a PhD student with research interest and experienced in molecular biology, bioinformatics, and chemoinformatic. He is proficient in Microsoft office, Python, and R. Joel will participate in RNA/DNA extraction, prepare PCR master mixes, and perform RT-PCR on isolated RNA.

RESEARCH WORK TO DATE

Salimonu, A.L., **Joel, I.Y**., Sanusi, O.S. Olanipekun, Q.A., Haliru, A.M. et al. (2023). Metagenomic analysis of gut microbiota of patients with colorectal cancer at the Federal Medical Centre (FMC), Abeokuta, Ogun State, Nigeria. *Journal of Clinical Oncology*, 41, no. 4_suppl (February, 01 2023) 195-195. doi.10.1200/JCO.2023.41.4_suppl.195

Adeyemi, O.O., Ndodo, N.D., Sulaiman, M.K., Ayansola, O.T., Buhari, O.I.N., Akanbi, O.A., Bolarinwa, O.A., Chukwu, C., **Joel, I.Y.,** et al. (2023) SARS-CoV-2 variants-associated outbreaks of COVID-19 in a tertiary institution, North-Central Nigeria: Implications for epidemic control. *PLoS ONE* 18(1): e0280756. https://doi.org/10.1371/journal.pone.0280756

M.A. Obeid, C.A. Ogah, C.O. Ogah, O.S. Ajala, M.R. Aldea, A.I. Gray, **J.O. Igoli**, V.A. Ferro. (2023). Formulation and evaluation of nanosized hippadine-loaded niosome: Extraction and isolation, physicochemical properties, and in vitro cytotoxicity against human ovarian and skin cancer cell lines. *Journal of Drug Delivery Science and Technology*, doi: https://doi.org/10.1016/j.jddst.2023.104766

Ifeoma C. Ezenyi, Jersley D. Chirawurah, Nekpen Erhunse, Prakhar Agrawal, Dinkar Sahal, **J.O. Igoli**. (2023). Marmesin isolated from Celtis durandii Engl. root bioactive fraction inhibits β-hematin

formation and contributes to antiplasmodial activity. *Journal of Ethnopharmacology*, 317. 116804

Naser F. Al-Tannak, Abdullah Al Ali, Eman Y Santali, C. J. Clements, A. I. Gray, **J. O. Igoli** .(2023). A New Isoflavone from *Lomariopsis guineensis* (Underw.) Alston. *Emirates Journal of Food and Agriculture*. 2023. 35(4): 305-310. doi: 10.9755/ejfa.2023.v35.i4.3034

Ifeoma Ezenyi, Evanka Madan, Jhalak Singhal, Ravi Jain, Amrita Chakrabarti,Gajala Deethamvali Ghousepeer, Ramendra Pati Pandey, Ngozichukwuka **Igoli, John Igoli** and Shailja Singh (2023). Screening of traditional medicinal plant extracts and compounds identifies a potent anti-leishmanial diarylheptanoid from *Siphonochilus aethiopicus*. *Journal of Biomolecular Structure and Dynamics.* P1-15. DOI: 10.1080/07391102.2023.2212779

Adulugba, O.A., Amali, O., Manyi, M.M., **Ikpa, T.F.**, Obisike, V.U. (2022). Genetic diversity and molecular surveillance of antimalarial drug resistance of *Plasmodium falciparum* among hospitals patients in Benue State Nigeria. *Microbiology Research Journal International*, 32(1):1-10.

Ikpa, T.F., Auta, I.K., Ikpa, G.I. (2017). Evidence of inconsistency among laboratory technicians collecting dry blood spots for molecular analysis of falciparum malaria dhfr gene. *Nigerian Journal of Parasitology,* 38(1): 7-13. Available online at https://www.ajol.info/index.php/njpar/article/view/153834

Obisike, V.U., Imandeh, G.N., Amuta, E.U., **Ikpa, T.F.** (2017*). In vitro* studies on the effects of cercariae shedding (Schistosoma parasitosis) on fecundity, hatchability and longevity of *Bulinus globosus*. *Asian Journal of Research in Medical and Pharmaceutical Sciences,* 1(4):1-6. Available online at: http://www.sciencedomain.org/issue/3143

Samuel, N. Dibua, M.E.U. **Ikpa, T.F**. (2015). Screening of fruit pulp extracts of *Picralima nitida* against *in vitro* cultures of *Plasmodium falciparum* and acute oral toxicity in white albino mice. *International Journal of Biological and Chemical Sciences*, 9(1): 430 – 437. Published by the International Formulae Group (IFG). Available online at: http://www.ajol.info/index.php/ijbcs

Ikpa, T.F. Shaa, K.K. and Auta, I.K. (2014). Molecular markers of sulfadoxine-pyrimethamine resistant malaria prior to intermittent preventive treatment among pregnancies in Makurdi, Nigeria. *International Journal of Biological and Chemical Sciences*, 8(5): 1961 – 1968. Published by the International Formulae Group (IFG). Available online at: http://www.ajol.info/index.php/ijbcs

Ikpa, T.F., Ajayi, J.A., Imandeh, G.N., and Usar, J.I. (2010). *In vitro* surveillance of drug resistant falciparum malaria in north central Nigeria. *African Journal of Clinical and Experimental Microbiology,* 11 (2): 111 - 119. Available online at: http://www.ajol.info/index.php/ajcem/issue/view/7195

Ikpa, T.F., Ajayi, J.A., Imandeh, G.N., and Usar, J.I. (2009). The *in vitro* assessment of drug resistant malaria in Makurdi, North central Nigeria. *Science World Journal*, 4 (4): 16 –21. Published by Kaduna State University. Available online at:http://www.scienceworldjournal.org/issue/view/443

ON GOING RESEARCH ALREADY CONCLUDED

Hybridization of *Schistosoma haematobium* with some livestock species of Schistosoma in Makurdi, Benue State.

Previous research grants (provide a short summary of grants and the amount won and managed in the last five years)

No grants received within the last 5 years.

Group research (provide below details about the roles and responsibility of each member)

Prof. Terwase Fabian Ikpa

- Principal investigator (PI) will supervise and coordinate the activities of the research group
- Assemble the necessary equipment and reagents for the project at each level of implementation.
- Interface with the host institution and the researchers.
- Run gradient PCR to optimize PCR reactions.
- perform statistical analysis of the project data and interpret results.
- Develop nucleic acids extraction protocol.
- Write and disseminate research findings.

Prof. John Ogbaji Igoli

- Procure molecular grade chemicals and reagents.
- Review the safety data sheet (SDS) of chemicals as risk assessment to ensure safety to minimize danger due to mixture of incompatible chemicals.
- Weigh and dissolve chemicals to prepare stock solutions for composition of in-house buffers.
- participate in report writing.

Prof. Grace Mwuese Gberikon

- prepare and autoclave culture media for cultivation of *E. coli* and *A. flavus* in the laboratory.
- culture and sub culture to maintain *E. coli* and *A. flavus* in the laboratory at appropriate conditions.
- infect *E. coli* with T4 bacteriophage for the multiplication of the viral particle.

Dr. John Anyam

- Prepare working solutions of DNA/RNA extraction buffers from stock solutions.
- Check and maintain molarity, pH, percentage concentration of specific components of buffers.
- Record and determine the shelf life of working solutions and buffers.
- Operate the UV-transilluminator and capture gel pictures.

Dr. James Agada Okete

- will be responsible for field sample collection of meat, agricultural plant materials, water and soil samples from 6 states and FCT in north central Nigeria.
- Will operate the microcentrifuge during nucleic acids extraction.

Samuel Atabo

- Will operate the nanodrop and obtain optical density (OD) values of purified nucleic acid at 230 nm, 260 nm, and 280 nm wavelengths.
- Compute the ratio of OD 260/280 and 260/230 and record in Excel for each buffer and sample.
- Obtain and keep data on every experiment.
- Clean data for statistical analysis.

Etim Esin Etim

- Will collect field samples from different location in north central Nigeria.
- Micropipette volumes into silica spin columns during extraction of nucleic acids.
- Assist in the laboratory maintenance of microorganisms.

Faith Odije Okita

- Will prepare PCR master mixes, pipette micro volumes into PCR tubes and perform conventional PCR on DNA elutes.
- Cast agarose gels
- Perform gel electrophoresis.

Joel Ireoluwa Yinka

- Will perform reverse transcription of RNA to cDNA
- Prepare PCR master mixes, pipette micro volumes into PCR tubes and perform RT-PCR.
- Cast agarose gels.
- Perform gel electrophoresis.
- Conduct nucleic acids extraction.

THE HOST INSTITUTION

Office and laboratory space (to be provided by host institution)

Each of the Three Professor as well as the senior lecturer on the team has a standard Professorial office and a laboratory for Postgraduate research work attached to the Office.

Equipment and materials to be provided by the host institution

The Vice Chancellor, Joseph Sarwuan Tarka University has guaranteed the use specialized equipment Centre laboratory which has some functional equipment like the thermal cycler and Uv-transilluminator needed for PCR that would be required in this project.

Equipment and materials to be provided by the host institution

The Joseph Sarwuan Tarka University has 3 functional thermal cyclers (PCR) machines which will be used for the Project. There is also a UV- transilluminator for viewing and photographing electrophoresis gel.

Other technical support (expected from host institution).

There is constant availability of electricity on campus produced from a large solar farm located on campus. A water treatment plant is also available on campus, thus water will not be a problem for this research project.

Equipment and materials available in nearby institutions

There is a large volume centrifuge at the Department of Biological Sciences Benue State University Makurdi which is accessible by the research team.

FINANCIAL ASPECTS OF THE RESEARCH PROJECT IMPLEMENTATION

Budget justification

All the equipment, reagents and plastic consumables listed in the budget are strictly meant for this project. No external components were included.

The budget estimates, especially for equipment and consumables was based on the following:

1. The cost of items from reliable suppliers of scientific equipment and reagents included in the budget was based on the cost listed by the suppliers on their web site in dollars'

2. The dollar value of the item was converted to Naira using 1000 Naira per US dollar due to inflation and devaluation of the Naira.

3. The local VAT of 7.5 % was calculated based on the cost of the item.

4. The cost of transportation was calculated at 15 % of the listed value of the item, and converted to Naira.

5. The 7.5% VAT Plus 15% transport cost Plus the listed value of the item now made the total cost of each equipment or reagent estimated in the Budget.

Additional source(s) of funding

There is no additional source of funding for this project.

ATTACHMENTS

1. Commitment Declaration

2. Optional document – RNA electrophoresis

FINANCIAL ASPECTS OF THE RESEARCH PROJECT IMPLEMENTATION

PERSONNEL COST/ALLOWANCE

S/N	ITEM DESCRIPTION	EXPECTED FROM			Total (₦)
		TETFund and NRF (₦)	INSTITUTION (₦)	OTHER (₦)	
1	Prof. TF Ikpa stipends for 18 months	3,330,000	0	0	3,330,000
2	Prof. J.O. Igoli stipends for 12 months.	1,200,000	0	0	1,200,000
3	Prof. G.M. Gberikon stipends for 6 months	600,000	0	0	600,000
4	Dr. J. Anyam stipends for 12 months	960,000	0	0	960,000
5	Dr. J.A. Okete stipends for 9 months	540,000	0	0	540,000
6	Mr. S. Atabo stipends for 9 months	450,000	0	0	450,000
7	Mr. E.E. Etim stipends for 9 months	450,000	0	0	450,000
8	Mrs. F.O. Okita stipends for 9 months	450,000	0	0	450,000
9	Mr. J.I. Yinka stipends for 9 months	450,000	0	0	450,000
10	Miscellaneous personnel services	20,000	0	0	20,000
				Sub-Total	**8,450,000**
				Percentage	**17.37%**

EQUIPMENT (LIST & SPECIFY)

S/N	ITEM DESCRIPTION	EXPECTED FROM			Total (₦)
		TETFund and NRF (₦)	INSTITUTION (₦)	OTHER (₦)	
1	ND-Nanodrop 1000 uv-vis Spectrophotomoter	2,862,470	0	0	2,862,470
2	GETS-9612 Thermal cycler	0	2,850,000	0	2,850,000
3	UV-Transilluminator Gena Analytic	0	1,065,900	0	1,065,900
4	Digital Programmable Shaking Dry bath, shaking incubator	245,000	0	0	245,000
5	BioSpec Mini Bead Beater	833,500	0	0	833,500
6	p2 micropipette	100,000	0	0	100,000
7	p10 micropipette	100,000	0	0	100,000
8	p50 micropipette	100,000	0	0	100,000
9	p1000 micropipette	100,000	0	0	100,000
10	24-hole 2 ml Corning LSE High speed microcentrifuge, Fisher brand, Cat# 10-320-806 - 1 unit	3,800,000	0	0	3,800,000
				Sub-Total	**12,056,870**
				Percentage	**24.79%**

SUPPLIES/CONSUMABLES

S/N	ITEM DESCRIPTION	EXPECTED FROM			Total (₦)
		TETFund and NRF (₦)	INSTITUTION (₦)	OTHER (₦)	
1	Tryptone, Glentham chemicals, Cat # GE6771 - 100 g	466,000	0	0	466,000
2	Yeast extract, Glentham chemicals, Cat# GE4420 - 100 g	404,000	0	0	404,000
3	Mycological Peptone, Fisher chemicals Cat# OXLP0040B - 500 g	594,500	0	0	594,500
4	Potassium dihydrogen phosphate, Fisher brand Chemicals, Cat# AA 1159430 - 500 g	56,000	0	0	56,000
5	Magnesium sulfate, Fisher brand Chemicals, Cat# M63-500 g	288,500	0	0	288,500
6	Rose Bengal + chloramphenicol, Fisher brand chemicals, Cat# R110366 - 10 pack of 10	170,000	0	0	179,000
7	Agar-agar, Fisher brand chemicals, Cat# A360 - 500 g	544,500	0	0	544,500
8	Glycerol, Glentham Chemicals, Cat# GC5551 - 1 L	94,500	0	0	94,500
9	Absolute ethanol (100%) Sigma Chemicals cat# 7148 - 1 L	0	178,000	0	178,000
10	Tris-EDTA pH 8.0, Sigma chemicals, # 93283 - 500 ml	286,500	0	0	286,500
11	0.5 M EDTA pH 8.0, Millipore life sciences, #324506 - 500 ml	303,500	0	0	303,500
12	0.1-0.15 mm Biospecs Bead granules, Biospecs, #11079101 - 4 lb	182,000	0	0	182,000
13	Nuclease free water (RNase, DNase, Endotoxin free) Sigma chemicals, #9811 - 5 L	340,000	0	0	340,000
14	Nuclease free water PEPC treated, Sigma chemicals #9612 - 5 L	320,000	0	0	320,000
15	Guanidine thiocyanate, molecular grade, Fisher brand, Cat# BP221- 1 kg	1,004,000	0	0	1,004,000
16	Guanidine hydrochloride, Fisher brand Chemicals Cat# BP178 - 1 kg	611,500	0	0	611,500
17	Sodium Chloride (NaCl) Fisher brand Chemicals Cat# S271 - 1 kg	0	171,000	0	171,500
18	Tris molecular grade, Glentham chemicals, Cat# GB1923 - 1 kg	200,400	0	0	200,400
19	Sodium dodecyl sulphate (SDS), Glentham chemicals, Cat# GB2962 - 500 g	99,300	0	0	99,300
20	6 M hydrochloric acid (HCl), Glentham chemicals, Cat# GX0143 - 1 L	0	56,900	0	56,900

21	Boric acid, molecular grade, Glentham chemicals, Cat# GX7729 - 1 kg	571,299	0	0	571,299
22	Triton X-100 molecular grade,Sigma chemicals, Cat# T8787 - 50 ml	80,200	0	0	80,200
23	PVP-40, Sigma chemicals, Cat#NC1230803 - 500 ml	147,900	0	0	147,900
24	Activated charcoal, Glentham, chemicals, Cat# GX1921-250 g	55,300	0	0	55,300
25	Citri acid monohydrate ultrapure grade, Gk9002 -250 g	40,200	0	0	40,200
26	Glacial Acetic Acid (ethanoic acid), glentham chemicals cat# GK1129 - 2.5 L	71,100	0	0	71,100
27	Ammonium acetate, glentham chemicals, Cat# GE6258- 100g	179,600	0	0	179,600
28	Agarose, Top vision, Thermo Scientific, Cat# FERR0492-500 g	633,500	0	0	633,500
29	RNA weight size marker -Ribo ruler, Fisher brand, Cat# FERSM 1823 -1	259,000	0	0	259,000
30	RNA stabilization solution, Fisher brand Cat# AM702-500 ml	514,650	0	0	514,650
31	DNA size marker, Fisher brand, Cat#M0372-25 ug	351,000	0	0	351,000
32	Formamide, Glentham chemicals, Cat# GS9663 - 100 ml	74,500	0	0	74,500
33	Formaldehyde Solution 36.5 - 38.0 %, Glentham Chemicals, Cat# GX7106 - 1 L	45,500	0	0	45,500
34	3-(N-Morpholino)propanesulfonic acid (MOPS), Glentham chemicals, Cat# GB2811-100 g	55,300	0	0	55,300
35	Synthesis of PRO341FB Primer, thermo Fisher, -17 nt	150,000	0	0	150,000
36	Synthesis of PRO805 Primer, Thermo Fisher - 17 nt	150,000	0	0	150,000
37	Synthesis of F-82FE Primer, Thermo Fisher	150,000	0	0	150,000
38	Synthesis of R-1391RE Primer, Thermo Fisher - 19 nt	167,647	0	0	167,647
39	100 mM dNTPs (dATP, dCTP, dTTP, dGTP) Mix, New England Biolabs (NEB) Cat# N0447S	228,000	0	0	228,000
40	5 Q High-Fidelty Polymerase New England Biolabs - (NEB) Cat# M0491 -1	586,000	0	0	586,000
41	5 Q High-Fidelty 2X PCR mix, NEB, Cat# M0541 - 1	180,000	0	0	180,000
42	5 Q High GC Enhancer NEB, Cat# B9028	251,000	0	0	251,000

43	25 mM Magnesium Chloride Molecular grade for PCR, NEB Cat# B9021S	78,000	0	0	78,000
44	Bovine serum albumin (BSA) molecula grade, NEB Cat~ B9001s	66,500	0	0	66,500
45	10x Tris Borate EDTA (TBE) salt for electrophoresis , Fisher brand, Cat# BP1333-2 L	258,000	0	0	258,000
46	Reverse Transcriptase Enzyme, (NEB kit Cat# M0681 - 1	540,000	0	0	540,000
47	Ribonuclease A (RNase A) DNase free, Glentham chemicals, Cat# GE9871 - 50 mg	105,700	0	0	105,700
48	Ethidium bromide, Fisher brand, Cat# P117898 - 10 mg/ml	102,000	0	0	102,000
49	0.2 ml PCR tubes, flat cap, non-sterile, 1000/cs Corning tubes, Cat# CLS3704 - 5000 pcs	640,000	0	0	640,000
50	0.5 ml PCR tubes,, Axygen Cat# 14-222-292 - 5000 pcs axygen	556,000	0	0	556,000
51	1.5 ml Eppendorf tubes, Eppendorf Cat# 05-414-206 - 5000 pcs	1,591,000	0	0	1,591,0001
52	0.5-10 µl aerosol barrier comb tips, Fisher brand Cat# 02-100-500, 10 pkt	1,279,720	0	0	1,279.720
53	2-200 µl aerosol barrier comb tips, Fisher brand, Cat# 02-707-51, 4 by 5 pkt	2,104,980	0	0	2,104,980
54	5-1000 µl low binding aerosol barrier comb tips ,Fisher brand, Cat# 02-707-167 - 5 pkt	936,520	0	0	936,520
55	6 layer Silica Spin column, Green Mall Science Co. - 5000 pcs	825,000	0	0	825,000
56	7 layer Silica Spin column, Green Mall Science Co. - 5000 pcs	825,000	0	0	825,000
57	8 layer Silica Spin column, Green Mall Science Co. - 5000 pcs	825,000	0	0	825,000
58	9 layer Silica Spin column, Green Mall Science Co. - 5000 pcs	825,000	0	0	825,000
59	Falcon sterile culture plates , Falcon, Cat#08-772E - 200 pcs	455,000	0	0	455,000
60	17 by 100 mm sterile culture tubes, Globe Scientific, Cat#22-171-706 - 500 pcs	168,000	0	0	168,000
61	Ultrapure water filter catridge, Kamamuta, Cat# MT-KA-QC23 - 80 pcs	400,000	0	0	400,000
62	Sterile water sampling bottles,, Thermo Scientific, cat# 05-719-362 - 250 pcs	442,500	0	0	442,500

63	2 ml spin column waste tubes, 1000/pack Qiagen Cat# ID19211 -2 packs	441,000	0	0	441,000
64	DNeasy power soil kit, 50 samples/kit Qiagen	416,000	0	0	416,000
65	DNeasy power soil total RNA kit, 50 samples/kit Qiagen	479,000	0	0	479,000
				Sub-Total	**25,309,116**
				Percentage	**52.04%**

DATA COLLECTION AND ANALYSIS

S/N	ITEM DESCRIPTION	EXPECTED FROM			Total (₦)
		TETFund and NRF (₦)	INSTITUTION (₦)	OTHER (₦)	
1	Cost of obtaining laboratory adapted E. coli, T4 Bacteriophage, and Aspergillus flavus from Vom Veterinary institute	0	120,000	0	120,000
2	Cost of transportation feeding, and accommodation for 2 researchers to collect field samples at 6 different sites in the FCT and Niger State	600,000	0	0	600,000
3	Cost of transportation feeding, and accommodation for 2 researchers to collect field samples at 6 different sites in Kogi and Kwara State	600,000	0	0	600,000
4	Cost of transportation feeding, and accommodation for 2 researchers to collect field samples at 6 different sites in Nasarawa and Plateau States	600,000	0	0	600,000
5	Cost of transportation, feeding, and accommodation for 3 researchers to collect field samples at 3 different sites in Benue State	180.000	0	0	180,000
6	Payment for statistical analysis of the recorded data	100,000	0	0	100,000
				Sub-Total	**2,200,000**
				Percentage	**4.52%**

TRAVEL

S/N	ITEM DESCRIPTION	EXPECTED FROM			Total (₦)
		TETFund and NRF (₦)	INSTITUTION (₦)	OTHER (₦)	
1	Cost of travelling and registration for the 40th and 41 st conference and Annual general meeting of Nigerian Society of Biochemistry and Molecular Biology.	400,000	0	0	400,000
				Sub-Total	**400,000**
				Percentage	**0.82%**

DISSEMINATION

S/N	ITEM DESCRIPTION	EXPECTED FROM			Total (₦)
		TETFund and NRF (₦)	INSTITUTION (₦)	OTHER (₦)	
1	Paper presentation at the 40th scientific conference and annual general meeting of the Nigerian Society of Biochemistry and Molecular Biology	50,000	0	0	50,000
2	Payment for publication in the Journal of Nigerian Society for Biochemistry and molecular Biology	40,000	0	0	40,000
3	Registration for the Conference of the Nigerian Society for Biochemistry and Molecular Biology	20,000	0	0	20,000
				Sub-Total	**110,000**
				Percentage	**0.23%**

OTHERS/MISCELLANEOUS

S/N	ITEM DESCRIPTION	EXPECTED FROM			Total (₦)
		TETFund and NRF (₦)	INSTITUTION (₦)	OTHER (₦)	
1	Paper for report writing	0	8,000	0	8,000
2	Ink for printing of report	0	10,000	0	10,000
3	Payment for typing of 1st and 2nd Progress Reports and Final Report.	90,000	0	0	90,000
				Sub-Total	**108,000**
				Percentage	**0.22%**

PROPOSED BUDGET COST

GRAND-TOTAL : **₦ 48, 633,986.00**

tetfund
TERTIARY EDUCATION TRUST FUND

COMMITMENTS

The Principal Researcher, the other Researchers and the Institutions involved must commit themselves to the successful completion of the project.

Researcher(s) Declaration
I/we declare that information given in this application form is to the best of my/our knowledge complete and correct.
I/we confirm my/our commitment to the successful implementation of the project. [Strictly for the PI and senior Co-Researchers]

1	Name and Signature of Principal Investigator	Prof. T.F IKPA	12/09/23
2	Name and Signature of Co-Researcher	PROF J.D. IGOLI	12-9-23
3	Name and Signature of Co-Researcher	Prof. G.M. Gberikon	12-09-2023
4	Name and Signature of Co-Researcher	Dr J.V. Anyam	13/09/2023
5	Name and Signature of Co-Researcher	Dr. J.A OKETE	13-09-23
6	Name and Signature of Co-Researcher	Samuel Atabo	12/9/2023
7	Name and Signature of Co-Researcher	Mr. Etim Esin	13-9-2023
8	Name and Signature of Co-Researcher	Mrs Faith Odijie Okita	13/9/23
9	Name and Signature of Co-Researcher	Mr. Joel Ireoluwa Yinka	13-9-2023
10.	Name and Signature of Co-Researcher		

Declaration of the Chairman, Ethics Committee (if required)
I declare that the Ethics Committee has carefully reviewed the research proposal and has approved the procedures for the handling of animal and human specimens.

Name, Title/Official Position, Signature, Date and Stamp of the Chairman, Ethics Committee

Dr.o (Mrs) H.O. Abah 22-9-2023
Chairman, College of Veterinary Medicine Ethical Committee

Declaration of Head of Institution
I declare that the Principal Investigator (PI) (and the other indicated members of the Research Team) 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

Engr. Prof. Isaac N. Itodo 18/09/2023
Vice Chancellor

OFFICE OF THE VICE CHANCELLOR
JOSEPH SARWUAN TARKA
UNIVERSITY MAKURDI