PHYTOCHEMICAL SCREENING AND PROXIMATE COMPOSITION OF A WHOLLY COMPOUNDED INDIGENOUS SUPPLEMENTARY FEED FLOUR TO TREAT BURNS.

BY

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CERTIFICATION

This is to certify that OKORO MARO HONEY with matriculation number (BMS1902174) of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry.

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Abstract

Burn injuries significantly challenge patient recovery, often requiring specialized nutritional support to address complications and aid healing. This study analyzed the phytochemical and proximate composition of a fully compounded indigenous supplementary feed flour for burn patients. Maize, unripe plantain, soybean, groundnut, and crayfish were procured, washed, and dried. These ingredients were mixed in specific ratios to produce a compounded flour blend, which was then evaluated for its nutrient composition and phytochemical content. The proximate composition analysis revealed: moisture content (6.56%), dry matter (93.44%), ash (1.73%), fat (4.45%), fiber (0.56%), protein (3.5%), and carbohydrates (16.24%). Phytochemical screening showed saponins and tannins levels at 1805.47 mg/kg and 5157.530 mg/kg, respectively. These results indicate that the feed flour adequately meets the metabolic needs of burn patients, providing essential nutrients along with significant amounts of saponins and tannins, which have anti-inflammatory, antioxidant, and wound-healing properties beneficial for recovery.

CHAPTER ONE

INTRODUCTION.

1.1. Background of Study

A wholly compounded indigenous burn feed is a specialized nutritional product designed to meet the unique dietary needs of individuals recovering from burn injuries. This feed flours are formulated to provide a concentrated source of protein along with other essential nutrients to support the healing process and promote recovery. This specialized nutritional product are an integral part of the comprehensive care and treatment of burn injuries, helping patients regain their strength and return to health.

Phytochemicals are bioactive compounds found in plants that have potential health benefits. Research aims to identify and quantify phytochemicals present in indigenous supplementary burn feed flour.healing in burn patients. Proximate composition analyse determines the nutritional composition of feed flour to include levels of protein, fat, carbohydrates, fiber, moisture, and ash.

Recently, interest in using indigenous plants as supplementary food for humans has grown. These plants are known for their bioactive compounds, which can boost nutritional value and improve health. The composition of supplementary flour varies by region and the plant species available. Despite its common use, there is limited scientific research on the phytochemical and proximate composition of supplementary flour specifically designed for burn treatment. Understanding these compositions is essential for optimizing the use of this supplementary food for burn patients.

1.2. Justification of Study

Burns are a significant public health concern worldwide, and nutritional support plays a crucial role in the recovery process. Indigenous supplementary burn feed flour offers a potentially valuable dietary intervention for burn patients, but its phytochemical and proximate composition may vary widely depending on the ingredients and preparation methods.

Understanding the phytochemical and proximate composition of indigenous burn feed flour is essential for optimizing its nutritional value and therapeutic benefits in burn patient care.Indigenous diets and traditional healing practices are integral to many cultures and communities. Investigating the phytochemical and proximate composition of indigenous supplementary burn feed flour respects cultural diversity and acknowledges the potential therapeutic value of traditional food sources in burn care. Phytochemicals present in indigenous plants have been associated with various health-promoting properties, including antioxidant, anti-inflammatory, and antimicrobial effects.

By identifying phytochemical-rich ingredients in indigenous burn feed formulations, this study seeks to harness their potential therapeutic benefits and enhance the nutritional quality of burn patient diets.

1.3. Statement of the Problem

Burn injuries frequently lead to substantial metabolic alterations and elevated nutritional requirements for optimal healing and recovery in humans. Various supplementary food products have been developed to address these nutritional needs. However, there is a lack of information regarding the phytochemical composition and proximate composition of a fully compounded indigenous supplementary flour intended for burn treatment. Assessing the phytochemical constituents and nutritional profile of this novel indigenous supplementary food product is essential for determining its potential health benefits and suitability for burn patients.

1.4. Aim/Objective of Study

To assess the phytochemicals and proximate composition of the indigenous supplementary burn feed flour.

CHAPTER TWO

Literature Review

According to the World Health Organization (WHO), burns refers to the damage of the skin or other organic tissues caused by the sun, hot liquids, fire, electricity or chemicals . The severity of most burns is based on the size and depth of the burn, extent of tissue damage and the surface area affected.

Burn epidemiology involves studying the incidence, prevalence, causes, outcomes, and risk factors associated with burn injuries.

Burns can be classified into three categories such as:

- Severity based on depth (first degree, second degree)
- Extent (rules of nine)
- Etiology (thermal, chemical, radiation)

SEVERITY BASED ON DEPTH:

Burns are categorized into four primary degrees based on the depth of tissue damage which are

• First-Degree Burns (Superficial Burns)

It affects only the outer layer of the skin (epidermis). Symptoms include redness, minor inflammation, pain, and dryness without blistering. An example of first degree burn is mild sunburn.

•Second-Degree Burns (Partial-Thickness Burns)

This type of burns extend into the second layer of the skin (dermis). It's sub classified into two namely: Superficial Partial-Thickness Burns and Deep Partial-Thickness Burns. Symptoms include red or splotchy skin, blisters, severe pain, and swelling. An example of second degree burn is scald injuries from hot liquid.

•Third-Degree Burns (Full-Thickness Burns):

This type of burn extends through the entire dermis and affects deeper tissues symptoms include waxy white, leathery, or charred black appearance. The burned area may be numb due to nerve

damage. An example of this type of burn includes flames from a fire or prolonged contact with a hot object.

•Fourth-Degree Burns:

This type of burn involves damages that extends beyond the skin into underlying tissues such as muscle, fat, and bone. symptoms include charred appearance, loss of function in the affected area, and may involve life-threatening complications. An example of fourth degree burns is high-voltage electrical burns or severe fire burns.

CLASSIFICATION BY EXTENT:

The extent of a burn is usually determined by the total body surface area (TBSA) affected, typically estimated using the "Rule of Nines" or the Lund and Browder chart:

•Rules of Nine: This is a quick method to estimate TBSA where the body is divided into sections, each representing approximately 9% (or multiples of 9%) of the total surface area.

Head and neck: 9%

Each arm: 9%

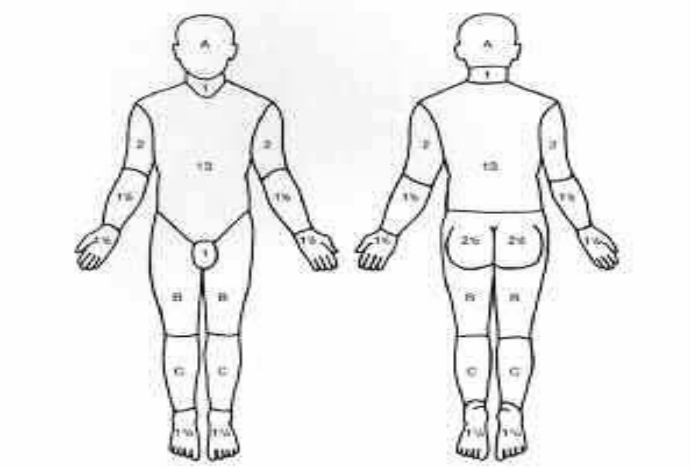
Each leg: 18%

Anterior trunk: 18%

Posterior trunk: 18%

Perineum: 1%

Lund and Browder Chart : It provides a more precise TBSA estimation, particularly in children, by accounting for age-related body proportion differences.



Relative Percentage of Areas Affected by Growth

Age in Years	0	1	5	10	15	Adult
A – ½ of head	9½	8½	6½	5½	4½	3½
B – ½ of one thigh	2¾	3¼	4	4¼	4½	4¾
C – ½ of one leg	2½	2½	2¾	3	3¼	3½

CLASSIFICATION BY ETIOLOGY:

Burns are also categorized based on the causative agent:

• Thermal Burns:

These occur due to exposure to flames, hot liquids, steam, or hot surfaces. Common scenarios include fires, scalds from hot liquids, and contact with hot objects.

• Chemical Burns:

Chemical burns result from contact with corrosive substances such as acids, alkalis, or strong cleaning agents. Industrial accidents, household mishaps, or deliberate acts of violence can lead to chemical burns.

• Electrical Burns:

Electrical burns occur when the body comes into contact with an electrical current. This can happen due to faulty wiring, electrical appliances, lightning strikes, or occupational hazards.

• Radiation Burns:

Exposure to ionizing radiation, such as X-rays or radioactive materials, can cause radiation burns. These burns are commonly seen in medical settings, industrial accidents, or nuclear incidents.

• Friction Burns:

Friction burns occur due to abrasive contact with surfaces, such as road rash from motorcycle accidents or rug burns from falls.

2.1.2 **Risk Factors for Burns:**

Age:

Children and the elderly are at higher risk of burn injuries due to their limited mobility, sensory impairment, or cognitive abilities. Young children are particularly vulnerable to scald burns from hot liquids.

Occupation:

Certain occupations, such as firefighting, cooking, welding, or working with chemicals, pose a higher risk of burn injuries due to exposure to heat, flames, or hazardous materials.

Environment:

Living or working in environments with inadequate fire safety measures increases the risk of burn injuries. Overcrowded housing, lack of smoke alarms, or proximity to industrial facilities can heighten the risk.

Substance Abuse:

Alcohol and drug abuse can impair judgment and coordination, leading to accidents such as cooking fires, falls, or mishandling of flammable materials, increasing the risk of burn injuries.

Medical Conditions:

Individuals with conditions such as epilepsy, diabetes, or peripheral neuropathy may be at increased risk of burns due to impaired sensation, mobility issues, or compromised healing.

Psychological Factors:

Mental health disorders or cognitive impairments can affect an individual's ability to recognize and respond to burn hazards, increasing their susceptibility to injuries.

Poverty:

Socioeconomic factors, such as limited access to safe housing, education, or healthcare, can contribute to a higher incidence of burns in economically disadvantaged communities.

2.1.3 **Pathophysiology of Burn**

Pathophysiology is the study of the functional changes in the body that occur as a result of a disease or pathological condition. It involves understanding how normal physiological processes are altered by disease, including the mechanisms and effects of these changes on the body's systems and organs.

Burn injuries comprises of a complex interplay of cellular, molecular, and systemic responses triggered by exposure to thermal, chemical, electrical, or radiation energy. Knowing the pathophysiological mechanisms of burn injuries is essential for guiding therapeutic interventions and improving patient outcomes.

Pathophysiological Processes involved in Burn Injuries:

Immediate Tissue Damage:

- Thermal burns cause direct tissue destruction through denaturation of proteins, lipid membrane disruption, and cellular dehydration.

- Chemical burns induce tissue injury via corrosive reactions with cellular components, leading to necrosis and inflammation.

- Electrical burns result in tissue damage due to resistive heating and direct cellular injury from high-voltage electrical currents.

- Radiation burns cause DNA damage, cell death, and inflammation in irradiated tissues.

Inflammatory Response:

- Burn injury triggers the release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), leading to local and systemic inflammation.

- Neutrophils infiltrate the injured tissue, releasing reactive oxygen species (ROS) and proteolytic enzymes, exacerbating tissue damage.

- Macrophages play a crucial role in phagocytosing debris and modulating the inflammatory response.(Church, D., et al, 2006)

Vascular Changes:

- Endothelial cell injury and activation lead to increased vascular permeability, edema formation, and microvascular thrombosis.

- Loss of endothelial integrity exacerbates fluid and protein extravasation, contributing to tissue edema and impaired tissue perfusion.

- Systemic vascular changes, including hypovolemia, hypotension, and increased vascular resistance, occur in severe burn injuries.(Pruitt, B. A., & Mason Jr, A. D. (2007).

Cellular Responses:

- Keratinocytes undergo apoptosis or necrosis in response to burn injury, disrupting the epidermal barrier and impairing wound healing.

- Fibroblasts and endothelial cells proliferate to initiate tissue repair and angiogenesis, while myofibroblasts mediate wound contraction.

- Immune cells, including T lymphocytes and dendritic cells, regulate the immune response and contribute to tissue repair and remodeling. (Finnerty, C. C, et al, 2009)

Systemic Effects:

- Burn-induced inflammation and tissue damage can lead to systemic inflammatory response syndrome (SIRS), characterized by fever, tachycardia, and leukocytosis.

- Hypermetabolism, insulin resistance, and catabolism occur in severe burns, contributing to muscle wasting, organ dysfunction, and impaired wound healing.

- Complications such as sepsis, acute respiratory distress syndrome (ARDS), and multiple organ dysfunction syndrome (MODS) may develop in the aftermath of severe burn injuries.(Jeschke, M. G. et al, 2016)

2.1.4 Epidemiology of Burns in West Africa:

Burn injuries are a big health problem in West Africa, affecting different groups of people in various ways. Knowing who is most at risk and why is important for creating effective ways to prevent and treat these injuries. This section looks at burn injuries in West Africa, especially focusing on how they impact children and older adults.

1. Incidence Rates:
2. Burn injuries occur across all age groups in West Africa, with incidence rates varying by demographic factors such as age, gender, and socioeconomic status.
3. Pediatric Predominance:
4. Children under the age of five represent a significant proportion of burn victims in West Africa, accounting for a substantial portion of hospital admissions and mortality related to burns.
5. Gender Disparities:
6. While burn injuries affect both genders, there may be gender disparities in the types and causes of burns, with women and girls often experiencing burns related to cooking and domestic activities.
7. Occupational Hazards:
8. Certain occupational groups, such as agricultural workers, artisans, and informal sector workers, are at higher risk of burn injuries due to occupational hazards such as exposure to heat, chemicals, and electrical sources.
9. **Age-Specific Vulnerabilities:**
10. Pediatric Population:
    a. Vulnerability: Children are particularly susceptible to burn injuries due to their exploratory behavior, limited understanding of danger, and reliance on caregivers for supervision.

b. Causes: Scald burns from hot liquids, contact burns from hot surfaces or objects, and flame burns from open fires are common causes of burns among young children in West Africa.
c. Prevention Strategies: Education campaigns targeting parents and caregivers on burn prevention, safe cooking practices, and child supervision can help reduce the incidence of pediatric burns.

11. Geriatric Population:
    a. Vulnerability: Older adults are at increased risk of burn injuries due to age-related factors such as decreased mobility, sensory impairment, and cognitive decline.
    b. Causes: Scald burns from hot liquids, flame burns from cooking accidents, and electrical burns from faulty appliances are prevalent among the geriatric population.
    c. Prevention Strategies: Home safety assessments, installation of smoke alarms, and modification of cooking environments to reduce hazards can help prevent burn injuries among older adults in West Africa.

2.2. **Burns and Nutritional Needs**

Burn injuries are a serious health risk, damaging the body's tissues and causing complex bodily reactions. It's important to understand how burns affect the body to provide better treatment and improve recovery for patients. Some immediate effects of burns include:

• Tissue Damage:

Burns cause immediate destruction of skin and underlying tissues through thermal, chemical, electrical, or radiation energy. (Klein, M.B. et al, 2011).

• Inflammation:

The release of pro-inflammatory cytokines and recruitment of immune cells initiate an acute inflammatory response, leading to edema, erythema, and pain at the burn site.

• Fluid Loss:

Severe burns disrupt the skin barrier, resulting in significant fluid loss through evaporation and damaged blood vessels, leading to hypovolemia and shock.(Herndon, D. N., 2012)

Systemic Response to Burns:

• Hypermetabolism:

Burn injuries induce a hypermetabolic state characterized by increased oxygen consumption, energy expenditure, and protein catabolism.(Saffle, J. R., 2017).

• Endocrine Dysfunction:

Burn-induced stress triggers hormonal alterations, including elevated cortisol levels, insulin resistance, and decreased thyroid hormone production. (Saffle, J. R., 2017).

• Immunosuppression:

Burn injuries impair immune function, predisposing patients to infections, sepsis, and delayed wound healing.

Impact on Organ Systems:

• Integumentary System:

Burns disrupt the skin barrier, leading to impaired thermoregulation, increased risk of infection, and compromised wound healing.(Herndon, D. N., 2012)

• Cardiovascular System:

Burn-induced hypovolemia and systemic inflammation can result in hemodynamic instability, cardiac dysfunction, and increased risk of thromboembolic events. (Klein, M.B. et al, 2011).

• Respiratory System:

Inhalation injuries from smoke or hot gases can cause airway obstruction, pulmonary edema, and acute respiratory distress syndrome (ARDS).

• Renal System:

Burn-induced hypoperfusion and systemic inflammation may lead to acute kidney injury (AKI) and electrolyte imbalances.(Klein, M.B. et al, 2011).

2.2.1 Nutritional Challenges in Burn Patients

Burn injuries make taking care of patients very challenging, especially when it comes to their nutrition. Burns can cause the body to use up more energy and lose important nutrients quickly. Getting the right nutrition is crucial for helping wounds heal, preventing further problems, and improving recovery. Below are brief notes on the challenges burn patients face:

Hypermetabolism:

Burn injuries induce a hypermetabolic state characterized by increased energy expenditure, oxygen consumption, and protein breakdown, necessitating higher caloric and protein intake to meet metabolic demands. (Williams, F.N. et al, 2009)

Muscle Wasting:

Severe burns lead to muscle protein breakdown, resulting in lean body mass loss, compromised immune function, and delayed wound healing, highlighting the importance of adequate protein intake in burn patients.(Hart, D.W, et al 2002).

Malnutrition:

Burn patients are at risk of developing malnutrition due to metabolic stress, impaired nutrient absorption, and prolonged fasting, exacerbating the catabolic state and impairing recovery.

2.2.2 Energy Requirements Across the Phases of Burn Recovery

Nutritional support is vital for burn patients' recovery, as their nutritional and energy needs change during different stages of healing. Knowing these changing needs helps healthcare providers manage treatment and improve outcomes. This section discusses the nutritional needs at each stage of burn recovery and provides proven strategies for giving the best nutrition.

• Acute Phase:

Immediate Post-Injury Period (0-72 hours):

- Energy Requirements: During the acute phase, energy needs are significantly elevated due to the hypermetabolic response, with resting energy expenditure (REE) typically doubling or even tripling normal levels. (Pereira, C. et al, 2005).

- Protein Requirements: High-protein intake is crucial to support wound healing, minimize muscle catabolism, and attenuate the hypermetabolic response. Protein requirements may range from 1.5 to 2.5 grams per kilogram of body weight per day.(Pereira, C. et al, 2005).

Fluid Replacement and Maintaining Stable Blood Flow:

- Adequate fluid resuscitation is essential to maintain hemodynamic stability, prevent hypovolemia, and optimize tissue perfusion. Balanced crystalloid solutions are commonly used for initial resuscitation, with adjustments based on urinary output and clinical parameters.(Pereira, C. et al, 2005).

• Subacute Phase:

Early Wound Healing (3-14 days):

- Energy Requirements: Energy needs remain elevated during the subacute phase to support ongoing wound healing, tissue repair, and immune function. A combination of carbohydrates, fats, and proteins is necessary to meet increased metabolic demands.

- Micronutrient Support: Vitamins and minerals, including vitamin C, zinc, and selenium, are essential for collagen synthesis, antioxidant defense, and immune function. Supplementation may be indicated to support wound healing.(Pereira, C. et al, 2005).

Enteral Nutrition Initiation:

- Early enteral nutrition within 24-48 hours post-injury is recommended to mitigate the catabolic response, preserve lean body mass, and promote gut integrity. Tube feeding or oral supplements may be utilized if oral intake is inadequate.(Berger, M. M. et al, 2012).

• Rehabilitation Phase:

Advanced Wound Healing (14 days to several months):

- Energy Requirements: As wound healing progresses and the hypermetabolic response attenuates, energy needs may gradually decrease but remain higher than baseline due to ongoing tissue repair and rehabilitation efforts.

- Protein Redistribution: While protein requirements may decrease compared to the acute phase, maintaining adequate protein intake is crucial to support scar maturation, wound remodeling, and functional recovery.(Pereira, C. et al, 2005).

2.3. Overview/Composition of a Wholly Compounded Indigenous Feed .

A wholly compounded indigenous feed for burn patients is a specialized nutritional product designed to meet the unique dietary needs of individuals recovering from burn injuries. Creating an indigenous feed supplement for burn patients is important because it matches their cultural food preferences and is more affordable. Using ingredients that are readily available in the area makes the supplement fit better with the patients' usual diets, making them more likely to use it. Plus, local ingredients cost less than imported ones, which is very important where resources are limited.

Also, indigenous feed supplements also make use of nutrient-rich ingredients found nearby, which provide the essential proteins, vitamins, and minerals needed for wound healing and tissue repair. This not only supports the local economy but also ensures the ingredients are fresher and of higher quality. By customizing the supplement to meet the specific nutritional needs of the local population, it can more effectively aid in the recovery of burn patients. The indigenous feed consists of the following ingredients:

• Maize

• Soyabean

• Unripe Plantain

• Groundnut

• Crayfish

• Dry fish

• Eggs

• Garri

• **Maize (Zea mays):**

- Source: Maize, also known as corn, is a cereal grain.

- Purpose: It provides carbohydrates for energy and fiber for digestion.

- Nutritional content: it contains carbohydrates, fiber, vitamins (such as vitamin B6), and minerals (such as magnesium). Carbohydrates provide a readily available source of energy, which is crucial for supporting the increased metabolic demands during the recovery process. Fiber aids in digestion and may help prevent constipation, a common issue during recovery.

• **Soybean (Glycine max)**

- Source: Soybeans are legumes

- Purpose: It's an excellent source of protein, essential for tissue repair and growth.

- Nutritional content: It contains protein, fiber, healthy fats (such as omega-3 fatty acids), vitamins (especially B vitamins), and minerals (such as iron and calcium). Protein is essential for tissue repair and wound healing. Additionally, soybeans provide a complete source of protein, containing all the essential amino acids needed for cellular repair and regeneration. Omega-3 fatty acids have anti-inflammatory properties that may help reduce inflammation associated with burns.

• **Unripe Plantain (Musa paradisiaca):**

- Source: Unripe plantains are the immature fruits of the plantain tree.

- Purpose: Provides complex carbohydrates for sustained energy and dietary fiber for digestive health.

- Nutritional content: It contains complex carbohydrates, fiber, vitamins (especially vitamin C and vitamin A), and minerals (such as potassium). Complex carbohydrates provide sustained energy, while fiber aids in digestion and helps maintain gastrointestinal health. Potassium is important for fluid balance and muscle function, which can be disrupted in burn patients.

• **Groundnut (Arachis hypogaea):**

- Source: Groundnuts, commonly known as peanuts, are legumes.

- Purpose: It's a good source of protein, healthy fats, and essential nutrients for tissue repair and immune support.

- Nutritional content: It contains protein, healthy fats (such as monounsaturated fats), vitamins (especially vitamin E), and minerals (such as magnesium and phosphorus). Protein from groundnuts supports tissue repair and muscle recovery. Vitamin E is an antioxidant that may help protect cells from damage caused by inflammation and oxidative stress, which are common in burn injuries.

• **Crayfish (Cambaridae):**

- Source: Crayfish are small freshwater crustaceans.

- Purpose: Adds flavor and provides additional protein and essential nutrients.

- Nutritional content: It contains protein, vitamins (especially B vitamins), and minerals (such as calcium and iron). Protein from crayfish supports tissue repair and wound healing. Iron is important for oxygen transport in the blood and may help prevent anemia, which can occur due to blood loss or decreased red blood cell production in burn patients.

• **Dry Catfish (Clarias gariepinus)**

- Source: It's found in fresh or muddy waters

- Purpose: It adds flavor and provides protein, omega-3 fatty acids, and essential nutrients.

- Nutritional content: It contains protein, healthy fats (especially omega-3 fatty acids), vitamins (such as vitamin D), and minerals (such as calcium and phosphorus). Protein from dry fish

supports tissue repair and wound healing. Omega-3 fatty acids have anti-inflammatory properties and may help reduce inflammation and pain associated with burns.

• **Eggs:**

- Source: Eggs are produced from poultry birds, such as chickens.

- Purpose: An excellent source of high-quality protein, essential for tissue repair and growth.

- Nutritional content: It contains protein, healthy fats, vitamins (especially vitamin B12 and vitamin D), and minerals (such as iron and zinc). Protein from eggs supports tissue repair and muscle recovery. Vitamin B12 is important for nerve function and may help alleviate neuropathic pain, which can occur in burn patients. Vitamin D is crucial for bone health and may aid in bone healing, which can be compromised in severe burns.

2.4 Overview on Phytochemical Screening and Proximate Composition Anslyse

Phytochemical screening is the process of identifying and characterizing the chemical compounds in plants. These compounds, known as phytochemicals, can include a variety of substances such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolics (J. B. Harborne,1973).The screening typically involves both qualitative and quantitative methods to detect and measure these compounds.

The main steps in phytochemical screening generally include:

• Extraction

• Preliminary Tests

• Advanced Analysis

Phytochemical screening plays a crucial role in various fields, including pharmacognosy, medicine, agriculture, and food science. Here are some key reasons why phytochemical screening is important:

• Discovery of New Drugs:

Phytochemical screening is fundamental in the search for new drugs. Many modern medicines have been derived from plant compounds discovered through such screening. For instance, the

anticancer drug paclitaxel (Taxol) and the antimalarial drug artemisinin were both identified through phytochemical analysis of plants. By identifying bioactive compounds, researchers can develop new pharmaceuticals to treat a range of diseases.

•Nutritional Analysis:

Plants are a significant source of essential nutrients and phytochemicals that contribute to human health. Screening helps in identifying vitamins, antioxidants, and other beneficial compounds in fruits, vegetables, and other plant-based foods. This information is vital for developing dietary supplements and functional foods that promote health and prevent disease.

•Food Safety and Quality Control:

The food industry uses phytochemical screening to ensure the safety and quality of food products. Detecting potentially harmful compounds, such as natural toxins or contaminants, ensures that food products are safe for consumption. Additionally, screening can help in verifying the authenticity and purity of food ingredients and supplements.

Methodology of Phytochemical Screening

• Extraction:

The process typically begins with the extraction of phytochemicals from plant material using solvents like water, ethanol, methanol, or chloroform. The choice of solvent depends on the polarity of the compounds being targeted.(R. S. Chhikara et al. 2016).

• Preliminary Phytochemical Tests:

These tests are simple qualitative assays used to detect the presence of different classes of phytochemicals:

Alkaloids: It is detected using Dragendorff's or Mayer's reagent.

Flavonoids: It is identified through the use of sodium hydroxide or ferric chloride tests.

Tannins: It is detected with ferric chloride or gelatin tests.

Saponins: It is identified by observing frothing in water.

Terpenoids: It is detected using Salkowski's test.

Phenolics: It is identified through ferric chloride tests.

(R. S. Chhikara et al. 2016).

• Advanced Analytical Techniques

Once preliminary tests indicate the presence of certain phytochemicals, advanced techniques are used for further analysis:

Chromatography: Techniques like High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Thin Layer Chromatography (TLC) are employed to separate and identify compounds (N. S. Kumar et al, 2013).

Spectroscopy: Methods like UV-Vis, Infrared (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry (MS) provide detailed information on the molecular structure and composition of phytochemicals (N. S. Kumar et al, 2013).

2.4.2. Proximate Composition Analyse

Proximate composition analysis is the process of determining the basic nutritional components of food and other biological materials. It typically includes the quantification of moisture, ash (mineral content), protein, fat (lipids), fiber, and carbohydrates. These components are essential for understanding the nutritional value and quality of food products, as well as for various applications in food science, nutrition, and agriculture. (Nielsen, S. S., 1998).

Components of Proximate Composition Analysis

• Moisture Content: The amount of water present in the sample. Moisture content affects the shelf life, texture, and weight of food products.

• Ash Content: The total mineral content of the sample, determined by incinerating the sample and weighing the residue. It indicates the presence of inorganic substances.

• Protein Content: The amount of protein present, usually determined by measuring the nitrogen content of the sample using methods like the Kjeldahl or Dumas method and converting it to protein content using a conversion factor.

• Fat Content: The total lipids present in the sample, typically extracted using solvents such as ether (Soxhlet extraction) and measured gravimetrically.

• Fiber Content: The indigestible part of the sample, often measured as crude fiber, which includes cellulose and lignin. It is important for assessing the dietary fiber content.

Importance of Proximate Composition Analysis

• Nutritional Labeling: It provides essential information for food labeling, helping consumers make informed dietary choices. (AOAC International, 2016).

• Quality Control: It ensures consistency and quality of food products by monitoring their nutritional composition. (AOAC International, 2016).

• Research and Development: It assists in the development of new food products and the improvement of existing ones by understanding their nutritional profile.

• Regulatory Compliance: It helps manufacturers comply with nutritional standards and regulations set by authorities. (AOAC International, 2016).

• Health and Diet Planning: It provides valuable data for dietitians and health professionals to plan balanced diets and manage nutritional disorders.(Nielsen, S. S., 1998).

CHAPTER THREE

MATERIALS AND METHODS

3.1 **APPARATUS**

The following materials were used during the research study:

- Maize
- Soyabean
- Unripe Plantain
- Groundnut
- Crayfish
- Dry fish
- Eggs
- Measuring Cups
- Hand Gloves
- Sponge
- Soap
- Ziploc bags
- Stainless flat plate
- Cooking pots
- Kitchen towels
- Kitchen knives
- Kitchen trays
- Kitchen Sieves

Masking tape

• Whitman filter paper

• Silica dish

• Cotton wool

• Thimble

• Beaker

• Soxhlet apparatus

3.2 Equipments:

• Digital pH meter

• Electric oven

• Gas cylinder

• Electric blender

• Digital scale

• Desiccator

• Muffle Furnace

• Condenser

• Spectrophotometer

3.3. Chemical and Reagents used :

• Vanillin

• Ethanol

• Sulphuric Acid

• Sodium Carbonate

• Methano

• Distilled Water

• Sodium Hydroxide

• Copper Sulphate

• Sodium Sulphate

• Folin-Denate Reagent

• Selenium Tablets

• Boric Acid

• Tetraoxosulphate(IV) acid

3.4 PREPARATION OF FOOD SAMPLES

The constituents of the feed listed were processed into flour/powder form and were incorporated together to form the final product. The process each constituent of the indigenous feed went through are described below:

• Maize (Zea mays):

The maize was purchased at Uselu Market, Benin City. It was soaked overnight, then rinsed and boiled with 3.75 liters of water for 20 minutes using a gas stove. The initial weight was recorded as 5.39 kg. After cooling for a few minutes, the maize was placed in a hot air oven set at 80°C for 24 hours, after which the weight was 2.323 kg. The maize was then returned to the hot air oven for an additional 24 hours, and the weight was measured again at 2.261 kg. It was subjected to a further 3 hours in the hot air oven, reaching a constant weight. Finally, the maize was packed into Ziploc bags, taken back to Uselu Market for grinding, allowed to cool for 5 to 10 minutes, and then repacked into fresh Ziploc bags.

• Soybean (Glycine max):

The soybeans were purchased at Uselu Market, Benin City, with an initial weight of 2.236 kg. They were soaked overnight, then peeled and rinsed with 3.75 liters of water for 20 minutes on a gas stove, resulting in a weight of 4.128 kg. After cooling and draining for 5 to 10 minutes, the

soybeans were placed in a hot air oven set at 80°C for 24 hours, reducing the weight to 1.661 kg. They were then kept in the oven for another 14 hours, maintaining the same weight of 1.661 kg. Following an additional 3 hours in the hot air oven, the weight remained constant. The soybeans were packed into Ziploc bags and taken to Uselu Market for grinding. After grinding, they were allowed to cool for 5 to 10 minutes and then repacked into fresh Ziploc bags.

• Unripe Plantain (Musa paradisiaca):

Unripe plantains were purchased from Uselu Market in Benin City, Nigeria, with an initial weight of approximately 6.8 kg. The plantains were manually peeled using a kitchen knife and submerged in water one by one until all were peeled. The plantains were then sliced longitudinally to a thickness of about 12 mm. After peeling, the total weight was recorded at 4.1 kg. The slices were washed with distilled water and boiled with 1.875 liters of water on a gas stove for 20 minutes.

After boiling, the plantain slices were sieved for 5-10 minutes to allow proper drainage, and the weight was recorded at 6.2 kg. The slices were then dried in a hot air oven at 80°C for 24 hours, reducing the weight to 1.74 kg (a reduction of 4.46 kg). The drying process continued for another 15 hours, reducing the weight to 1.559 kg, indicating a moisture content reduction of 0.181 kg (181 g). The slices were dried for an additional 3 hours, resulting in a further 3 g reduction in moisture content, and then placed back in the oven for another 3 hours, at which point the weight remained constant. In total, the unripe plantains were dried for 45 hours at 80°C. The dried plantains were taken to Uselu Market for grinding, allowed to cool for a few minutes, and then packed into Ziploc bags.

• Crayfish (Cambaridae):

The crayfish were purchased at Uselu Market in Benin City, with an initial weight of 236 g. They were boiled with 1.5 liters of water for 20 minutes on a gas stove. After boiling, the crayfish were drained, and their weight increased to 520 g. They were then placed in a hot air oven at 80°C for 24 hours, resulting in a constant weight of 137 g.

3.5. Phytochemical Screening

The final product was taken to the laboratory to test for the following phytochemicals saponin and tannin. The procedures below were carried out on in the lab:

• Estimation of total saponin content:

Estimation of total saponins content was determined by the method described by Kicel, and Olszewska (2015) based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800mg of vanillin in 10Ml of 99.5% ethanol) was added. Then 2.5mL of 72% Sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°C for 10min. After 10min, it was cooled in ice-cold water and the absorbance was read at 570nm. 0- 25 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly to test samples. Total Saponin content was calculated from the mathematical expression:

Total Saponin = SE ×V × D÷W

Where SE is the Saponin equivalent obtained from the calibration curve, V is the volume of the sample, D is the dilution factor, and W is the mass in grams of the sample.

• Estimation of tannin content:

This was done following the method adopted by Kavitha and Indira (2016).

Exactly 0.20 mL of sample was added to 20 mL of 50% methanol and placed in a water bath at 77˚C - 80˚C for one hour and shaken. The extract was quantitatively filtered using a double-layered Whatman No.1 filter paper and 20 mL of distilled water, 2.5 mL Folin-Denis reagent, and 10 mL 17% Na2CO3 were added and mixed. The mixture was allowed to stand for 20 min. A series of standard tannic acid solutions were prepared in methanol and their absorbance as well as samples was read after colour development on a UV/ Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from the mathematical expression:

Total Tannin Content =TAE ×V × D÷W

Where TEA is the Tannic acid equivalent obtained from the calibration curve, V is the volume of the sample, D is the dilution factor, and W is the mass in grams of the sample.

3.6. Proximate Composition Analyse :

The final product was taken to the laboratory to assess it's composition. The method used partitioned nutrients in feed into 6 components: water, ash, crude protein, ether extract, crude fibre and nitrogen free extract (NFE).

• **Moisture Determination**:

Moisture is determined by the loss in weight that occurs when a sample is dried to a constant weight in an oven. About 2g of a feed sample is weighed into a silica dish previously dried and weighed. The sample is then dried in an oven for 650C for 36 hours, cool in a desiccator and weigh. The drying and weighing continues until a constant weight is achieved.

$$\%\text{Moisture} = \frac{\text{wt of sample} + \text{dish before drying} - \text{wt of sample} + \text{dish after drying}}{\text{Wt of sample taken}} X100$$

Since the water content of feed varied very widely, ingredients and feed are usually compared for

their nutrient content on moisture free or dry matter (DM) basis.

%DM = 100 - %Moisture.

• **Ash:**

Ash is the inorganic residue obtained by burning off the organic matter of feedstuff at 400-600C in muffle furnace for 4hrs. 2g of the sample is weighed into a pre-heated crucible. The crucible is placed into muffle furnace at 400-6000C for 4hrs or until whitish-grey ash is obtained. The crucible is then placed in the desiccator and weighed.

$$\%\text{Ash} = \frac{\text{wt of crucible} + \text{ash} - \text{wt of crucible}}{\text{wt of sample}} X100$$

• Ether Extract

The ether extract of a feed represents the fat and oil in the feed. Soxhlet apparatus is the equipment used for the determination of ether extract. It consist of 3 major components

1. An extractor: comprising the thimble which holds the sample

2. Condenser: for cooling and condensing the ether vapour

3. 250 ml flask

Procedure: about 150ml of an anhydrous diethyl ether (petroleum ether) of boiling point of 40 - 600°c is placed in the flask. 2-5 g of the sample is weighed into a thimble and the thimble is plugged with cotton wool. The thimble with content is placed into the extractor; the ether in the flask is then heated. As the ether vapour reaches the condenser through the side arm of the extractor, it condenses to liquid form and drop back into the sample in the thimble, the ether soluble substances are dissolved and are carried into solution through the siphon tube back into the flask. The extraction continues for at least 4 hrs. The thimble is removed and most of the solvent is distilled from the flask into the extractor. The flask is then disconnected and placed in an oven at 650C for 4 hrs, cool in desiccator and weighed.

$$\%\text{Ether extract} = \frac{\text{wt of flask} + \text{extract} - \text{tare wt of flask}}{\text{wt of sample}} X\ 100$$

• Crude Fibre

The organic residue left after sequential extraction of feed with ether can be used to determine the crude fibre, however if a fresh sample is used, the fat in it could be extracted by adding petroleum ether, stir, allow it to settle and decant. Do this three times. The fat-free material is then transferred into a flask/beaker and 200 mls of pre-heated 1.25 % H_2SO_4 is added and the solution is gently boiled for about 30 mins, maintaining constant volume of acid by the addition of hot water. The Buckner flask funnel fitted with whatman filter is pre-heated by pouring hot water into the funnel. The boiled acid sample mixture is then filtered hot through the funnel under sufficient suction. The residue is then washed several times with boiling water (until the residue is neutral to litmus paper) and transferred back into the beaker. Then 200 mls of pre-heated 1.25 % Na_2SO_4 is added and boiled for another 30mins. Filter under suction and wash thoroughly with hot water and twice with ethanol. The residue is dried at 650C for about 24 hrs

and weighed. The residue is transferred into a crucible and placed in muffle furnace (400-6000C) and ash for 4hrs, then cool in desiccator and weigh.

$$\% \text{ Crude fibre} = \frac{\text{Dry wt of residue before ashing} - \text{wt of residue after ashing}}{\text{wt of sample}} X\ 100$$

•Crude Protein

Crude protein is determined by measuring the nitrogen content of the feed and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16 % nitrogen. Crude protein is determined by kjeldahl method. The method involves: Digestion, Distillation and Titration.

Digestion: weigh about 2 g of the sample into kjeldahl flask and add 25 mls of concentrated sulphuric acid, 0.5 g of copper sulphate, 5 g of sodium sulphate and a speck of selenium tablet. Apply heat in a fume cupboard slowly at first to prevent undue frothing, continue to digest for 45 mins until the digesta become clear pale green. Leave until completely cool and rapidly add 100 mls of distilled water. Rinse the digestion flask 2-3 times and add the rinsing to the bulk.

Distillation: Markham distillation apparatus is used for distillation. Steam up the distillation apparatus and add about 10 mls of the digest into the apparatus via a funnel and allow it to boil. Add 10 mls of sodium hydroxide from the measuring cylinder so that ammonia is not lost. Distil into 50 mls of 2 % boric acid containing screened methyl red indicator.

Titration: the alkaline ammonium borate formed is titrated directly with 0.1 N HCl. The titre value which is the volume of acid used is recorded. The volume of acid used is fitted into the formula which becomes

$$\%N = \frac{14 \text{ x VA x } 0.1 \text{ x} X\text{w x} 100}{1000\text{x}100}$$

VA = volume of acid used w= weight of sample

% crude protein = % N x 6.25

• Nitrogen Free Extract (NFE):

NFE is determined by mathematical calculation. It is obtained by subtracting the sum of percentages of all the nutrients already determined from 100.

% NFE = 100 - (% Moisture + % CF + % CP + % EE + % Ash)

NFE represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in the sample.

CHAPTER FOUR

4.0 RESULTS

Table 4.1: Phytochemical Analysis of Maize Blend

S/N	PARAMETERS	UNITS	MAIZE BLEND (R1)	MAIZE BLEND (R2)
1	Total Saponin	mg/kg	1805.471	1517.530
2	Total Tannin	mg/kg	5157.530	5125.396

Table 4.2. Proximate Composition Analyse of Maize Blend

Sample	Moisture Content	Dm	Ash	Fat	Fiber	Protein	Carbohydrates
Maize Blend (R1)	6.56	93.44	1.73	4.45	0.56	3.5	16.24
Maize Blend (R2)	5.47	94.53	1.56	4.27	0.56	3.5	14.8
Maize Blend (R3)	6.45	93.55	1.62	5.43	0.73	5.57	19.07

Chapter Five

Discussion

5.1 Phytochemical Screening

The phytochemical screening indicated that the feed flour contains substantial levels of saponins and tannins.

Saponins are recognized for their antimicrobial characteristics and their capacity to improve nutrient absorption by diminishing ammonia production in the gut .With lots of saponins in both rations, there's a good chance they can help calm inflammation, fight infections, and protect against damage caused by things like burns. Saponins also seem to help the body make collagen and repair tissues, which is important for healing wounds. The elevated saponin content in the first ration (1805.47 mg/kg) compared to the second (1517.530 mg/kg) suggests a potentially greater influence on nutrient absorption and gut health, yet also poses a higher risk of anti-nutritional effects if consumed excessively.

Tannins, known for their antioxidant properties and protective effects against certain pathogens, Tannins can tighten tissues and decrease fluid loss from burns. They also fight germs and reduce swelling, helping to prevent infections and create the right conditions for wounds to get better. Both rations have relatively high tannin levels, with the first ration containing a slightly higher concentration (5157.530 mg/kg) compared to the second ration (5125.396 mg/kg). Although this minor difference is unlikely to significantly affect the overall nutritional value.

5.2 Proximate Composition Analyse

The moisture content of the first ration (6.56%) is slightly higher than that of the second ration (5.47%), indicating that the second ration may have a longer shelf life due to its lower moisture content. The dry matter content follows this trend, with the second ration having a higher dry matter percentage (94.53%) compared to the first ration (93.44%).

Ash content, which represents the total mineral content, is slightly higher in the first ration (1.73%) compared to the second ration (1.56%). This indicates a marginally higher mineral content in the first ration, which could contribute to the overall nutritional value of the feed.

The fat content is relatively similar between the two rations, with the first ration containing 4.45% and the second ration containing 4.27%. This similarity suggests that both rations provide a comparable energy contribution from fats.

Fiber content is identical in both rations (0.56%), indicating that both feeds have a low fiber content, which is typical for feed formulations intended for high digestibility and nutrient absorption.

Protein content is also identical in both rations (3.5%), suggesting that both formulations provide the same level of protein, which is crucial for growth and repair in animals.

Carbohydrate content shows a slight difference, with the first ration having a higher carbohydrate content (16.24%) compared to the second ration (14.8%). This difference might influence the energy density of the feeds, with the first ration potentially providing slightly more energy from carbohydrates

Conclusion

This study successfully developed an indigenous supplementary feed flour for burn patients using maize, groundnut, soyabean, unripe plantain, crayfish, dry fish, and eggs, processed at a constant rate and temperature. The phytochemical and proximate composition analyses revealed a balanced nutritional profile, highlighting the feed's potential as a specialized dietary intervention to significantly enhance recovery outcomes for burn patients.

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