PRESERVATIVE EFFICACY OF SOME MEDICINAL PLANT EXTRACTS IN THE SHELF-LIFE OF SOME PROBIOTIC DRINKS

NWAKIRE, EVERESTUS CHIBUIKE

20194198808

(HND – MICROBIOLOGY, B.TECH. – PUBLIC HEALTH)

A THESIS SUBMITTED TO THE

DEPARTMENT OF PUBLIC HEALTH,

SCHOOL OF HEALTH TECHNOLOGY,

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF PUBLIC (MPH) IN PUBLIC HEALTH

AUGUST, 2024

CERTIFICATION

This is to certify that the Thesis on the **Preservative Efficacy of some Medicinal Plants Extracts in the Shelf-Life of some Probiotic Drinks** was carried out by **Nwakire, Everestus Chibuike,** with the registration number 20194198808 from the Department of Public Health Technology, School of Health Technology, the Federal University of Technology Owerri, Imo State.

.....
Dr. U.G. Ekeleme
(Supervisor)

30-09-2024
Date

.....
Dr. (Mrs.) C.O. Akanazu
(Co-Supervisor)

10/09/2024
Date

.....
Dr. C.C. Iwuala
HOD (Public Health Technology)

10/09/2024
Date

.....
Rev. Sr. (Prof.) E.T. Oparaocha
Dean (School of Health Technology)

10/9/2024
Date

.....
Prof. (Mrs.) J.N. Nwosu
Dean (Post Graduate School)

.....
Date

.....
Dr. Olufemi Oludare Aluko
(External Examiner)

30 July, 2024
Date

DEDICATION

This Thesis is fully dedicated to God Almighty God, who is my source of wisdom, strength and inspiration. May His name be praised forever, Amen.

ACKNOWLEDGEMENTS

I sincerely acknowledge the sincere efforts and encouragement given to me by my Supervisors, Dr. U.G. Ekeleme and Dr. (Mrs.) C.O. Akanazu. I am grateful for your patience, concern, support and taking time to read this work and the useful contributions you made at various stages. May God continue to take you to greater heights in Jesus Name, Amen.

I also acknowledge the Head of Department, Public Health, Federal University of Technology, Owerri, Dr. C.C. Iwuala, and the Dean, School of Health Technology (SOHT), Rev. Sr. (Prof.) E.T. Oparaocha.

I am grateful to my dedicated lecturers in the Department of Public Health; Prof. A.N. Amadi, Prof. I.N.S. Dozie, Prof. (Mrs.) E.A. Nwoke, Prof. (Mrs.) S.N.O. Ibe, Dr. (Mrs.) B.O. Nworu, Dr. U.M. Chukwuocha, Dr. C.C. Okereke, Dr. O.G. Udujih, Dr. C.I.C. Ebirim, Dr. (Mrs.) U.W. Dozie, Dr. (Mrs.) S.M. Orji, Dr. (Mrs.) M.O. Okorie, Dr. G.N.U. Iwuoha, Miss J.C. Ezelote, Mrs. Rita Chukwu, Mrs. J.C. Obi, Mrs. S. Mbachu and Miss. V. Akam, for their professional, outstanding, and updated knowledge, which wasimparted to me. God bless you all for your sacrifice.

I appreciate the effort of the non-academic staff of this noble department. May God who rewards good deeds bless you all.

I appreciate my beloved parents, Mr. and Mrs. Joseph Ohakpugwu Nwakire, for their love, encouragement, moral and financial supports throughout this work. May God Almighty continue to keep and protect you all so as to reap the fruits of your labours.

To my siblings, Mr. Tochukwu Charles Nwakire and Mrs. Juliana Uchechukwu Innocent, for their patience, love, moral support which made my academics easy and conducive. May God continue to hold us together with His unending love, Amen.

This page will not be complete without appreciating the love and kind understanding of MY PEACE, Mrs. Amarachi Ursula Everestus, my daughter, Miss Esther Chinyeremudo Everestus and my sons, Master Samuel-Abundance Chizurumoke and Master Excellent Chimezirim Everestus.

To my academic mentors, Dr. T.I. Mbata, Dr. (Mrs.) Ijeoma Joy Ibe, Pastor Decklin Chukwudi Orih, Pastor Vitus Okechukwu Oparah, Mr. Chibuike Martins Nwachuwu and Mrs. Catherine Nwamaka Ononogbo, for their encouragement, prayers, advice and guidelines which made this work a success.

I appreciate all my friends and well-wishers, especially, Mrs. Somtoochukwu Nwakaeze, Mrs. Chukwuemeka Chinyere Uzoamaka, Miss. Rosemary Egbunonu, Mrs. Uzoamaka Ekezie, Nnanyelugo Ebuka and others, who in one way or the other contributed to the success of this work. May God bless you all, Amen.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

This study evaluated the preservative efficacy of some medicinal plant extracts in the shelf-life extension of some probiotic drinks. The study adopted an experimental study design. *Hibiscus sabdariffa* (calyces) and *Cyperus esculentus* tigernut tubers were obtained from Relief Market, Egbu Road, Owerri while medicinal plants, such as; *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides* were collected from the trees at Aboh Mbaise L.G.A, Imo State. Each leaf was aseptically ground and 100 grams extracted using 100 ml by boiling in sterile beakers. Four (4) different concentrations of the extracts (10.0 ml, 30.0 ml, 50.0 ml and 100 ml) were used to preserve the probiotic drinks. Sensory/organoleptic properties, microbial load and physicochemical properties of the probiotic drinks were examined for eight (8) weeks. Results showed the presence of saponins, anthraquinones, phenols, alkaloids, tannins, phlobatannins, anthranoids and cardiac glycosides in the medicinal plants. The temperature of the probiotic drinks remained stable between 26.5 and 27.2 ^{0}C throughout the shelf-life extension properties determination. pH of the probiotic drinks became acidic from week 7 to week 8. The colour and odour were stable throughout the preservation period. Total viable bacterial count ranged from 3.0 x 10^{1}cfu/ml to 8.0 x 10^{2} cfu/ml with probiotic drink preserved with *Moringa oleifera* leaf, 8.0 x 10^{1}cfu/ml to 7.2 x 10^{2} cfu/ml with probiotic drink preserved with *Alstonia bonnie* leaf and 8.0 x 10^{1}cfu/ml to 8.8 x 10^{2} cfu/ml with probiotic drink preserved with *Pterocarpus santalinoides* leaf. There was an increase in the total viable bacterial count from week 6 to week 8, with the control sample (unpreserved zobo drink) having the highest microbial load. Bacterial isolates such as *Pseudomonas, Bacillus, Lactobacillus* and *Leuconostoc* species were detected. Fungal isolates; *Saccharomyces, Kluyveromyces, Aspergillus* and *Rhizopus* species were detected. There was a significant association between the preservative efficacy of the medicinal plants and the shelf-life extension properties of the preserved probiotic drinks. The study concluded that medicinal plants could be used to preserve probiotic drinks, hence, their uses should be adequately exploited in the society.

Keywords: Extracts, Extension, Medicinal Plants, Preservatives, Probiotics, Shelf-Life

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Food is any material obtained from a plant, animal, or fungal source that contains vital elements (carbohydrates, lipids, proteins, vitamins, or minerals) that are taken to give nutritional support to an organism such as man or animals (Lukas *et al.*, 2018). Probiotics are viable microorganisms that have beneficial effects in preventing and treating specific pathologic conditions when ingested. Probiotic foods and drinks include yoghurt, sourdough bread, soft cheese, sour pickles, acidophilus milk, zobo, tigernut, among others (Sirin & Aziz, 2017). Probiotic foods have short shelf-life due to their high nutrient content and are prone to spoilage.

Food deterioration is a process that renders food products unacceptable or undesirable for consumption. Food deterioration occurs when food undergoes microbial, chemical, or physical changes. When these modifications occur, the food products become unacceptably unhealthy for the customer. Also, the nutritional compositions of the foods are reduced due to the actions of the spoilage microorganisms (Benner, 2014). Microbiological food deterioration is caused by the proliferation of bacteria in foods. Enzymes are created during the growth of certain microbes in foods, resulting in undesirable by-products in the food. Chemical food deterioration occurs when distinct food components react with one another or with extra ingredients, changing the food's sensory properties. Oxidation, enzymatic browning, and non-enzymatic browning are examples of this. Physical food spoilage occurs when moist, dehydrated, or dried foods absorb excessive moisture (Argyri *et al.*, 2014).

When these microorganisms reach food products, they grow by utilizing the nutrients and produce metabolites that cause food spoilage (Parlapani *et al.,* 2017). Microbial food spoilage results in food waste and food-borne infections and diseases in society. As a result, efforts have been made to reduce the menace caused by food spoilage microorganisms. One of the efforts has been the use of preservatives in foods to inhibit the growth of microorganisms, thereby extending the shelf-life of the food products.

Some of the preservatives used in the shelf-life extension of foods are synthetic chemicals such as sulfites, sodium benzoate, potassium benzoate, benzene, nitrites, salt, parabens, lactic acid,etc (Bhat *et al.,* 2014). The mechanisms of these chemical compounds range from inhibiting the growth of the microorganisms to inhibiting specific enzymes in the microorganisms. Most of these preservatives have been reported to cause different human health effects. Combination of benzene with vitamin C forms carcinogen. Consumption of sodium benzoate may cause hyperactivity. In a study involving children who consumed artificial colourings and benzoate preservatives, there were increases in hyperactivity among the children (Kumar *et al.,* 2014).

As a result, new eco-friendly methodologies are required to reduce the growth of pathogenic bacteria and prolong the shelf-life of food products without using chemical preservatives. Recently, many researchers have investigated the possible utilization of some plant extracts as effective natural preservatives (Suppakul *et al*., 2016; Clarke *et al*., 2017). The direct application of natural compounds obtained from plants to food and drink preservation is the most common method of bio-preservatives. Recently, there has been a constant increase in the search for alternative and efficient compounds for food preservation aimed at partial or total replacement of chemical antimicrobial additives. This

led to bio-preservation to minimize the application of chemical preservatives to extend food shelf-life (Irkin & Esmer, 2015; Al-Dalali *et al.*, 2019).

Bio-preservatives are a wide range of natural products from plants, animals and microorganisms that can be useful in extending food's shelf-life. The main natural compounds are essential oils derived from plants, enzymes obtained from animal sources, bacteriocins from microbial sources, organic acids, and naturally occurring polymers (chitosan), which have been used in different food industries. The effects of applying bio-preservatives are to reduce or eliminate survival pathogenic bacteria and to increase overall food quality. Several natural biological compounds have antimicrobial properties, behaving as antioxidants, breaking down cellular membranes, and disrupting biosynthetic microorganism's pathways (Liu *et al.*, 2016; Alfonzo *et al.*, 2017; Simas *et al.*, 2017).

The active components are commonly found in essential oil fractions, and it is well established that most of them have a wide spectrum of antimicrobial activity against food-borne pathogens and spoilage microorganisms (Ramadan *et al.*, 2015). The antimicrobial activity of plant essential oils is due to their chemical structure, particularly the presence of hydrophilic functional groups, such as hydroxyl groups of phenolic components and/or lipophilicity of some essential oil components (Hasija *et al.*, 2015).

Simas *et al*. (2017) reported that plants and spices are excellent sources of biologically active compounds with potential antimicrobial activity. Essential oils, secondary metabolites produced by plants, have valuable capability of suppressing growth of a wide variety of food-spoilage and food-borne microorganisms including bacteria, yeasts and moulds. From chemistry point of view, they consist of aromatic and volatile compounds which play an important role in plant defence and possess antimicrobial properties. These

biologically active compounds can be extracted from different parts of plants including flowers, roots, bark, leaves, seeds, peel, fruits, wood, buds and the entire plant (Ben-Hsouna *et al.,* 2017). Several researchers have attempted to apply essential oils as potential bio-preservative to extend shelf-life and improve microbiological quality of dairy products (Liu *et al.,* 2016; Alfonzo *et al.,* 2017).

Medicinal plants contain several phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids, which possess antimicrobial and antioxidant properties (Ekeleme *et al.,* 2017). The crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria (Min *et al.,* 2014; Djenane, 2015; Ramadan *et al.,* 2015). In addition, it has been reported that the extracts from Chinese chives and cassia can effectively reduce the growth of *Escherichia coli* and other bacteria during storage of meat, juices, and milk (Min *et al.,* 2014; Sanchez-Ortega *et al.,* 2014).

In a similar study, Doddanna *et al.* (2014) investigated the effect of some plant extracts on the growth of *Candida albicans*. The results showed that the alcoholic extract of curry leaves effectively inhibited the growth of *C. albicans* after 48 hours. Moreover, Nzeako *et al.* (2016) reported that thyme oil extract could decrease the growth of *C. albicans* and *Pseudomonas aeruginosa*. Understanding the mechanism of antimicrobial action of medicinal plant extracts is the first step in the optimal utilization of these extracts as natural antimicrobial agents to extend the shelf-life and maintain food quality.

1.2 Statement of Problem

The issue of food losses is of high importance in the efforts to combat hunger, raise income and improve food security in the world's poorest countries. Food losses impact

food security for poor people, food quality and safety, economic development and the environment. To ensure food security, many agricultural products are used to produce local beverages, which different individuals patronize due to their cost-effectiveness and availability. These include kunun-zaki, zobo, tigernut milk, soybean milk, etc. Zobo and tigernut milk are among the well-known non-alcoholic beverages that are patronized. However, these drinks have short shelf-life and are prone to spoilage if not refrigerated.

Unfortunately, due to the epileptic power supply in Nigeria and the high cost of refrigerators, most of the sellers of these drinks cannot afford or use the refrigerator. As a result, they depend on ice blocks or, in most cases, prepare small quantities of these drinks not to have remainders after their sales. In the food industry, however, different chemicalsare used to preserve and extend the shelf-life of local beverages. However, some of these preservatives are synthetic chemicals such as sulfites, sodium benzoate, potassium benzoate, benzene, nitrites, salt, parabens, lactic acid, etc and have been reported to cause diversehealth effects in humans. This is the reason that motivated this research. Therefore, this thesis aims to determine the preservative efficacy of some medicinal plant extracts in the shelf-life extension of some probiotic drinks.

1.3 Objectives of the Study

1.3.1 General objective

The aim of this study was to evaluate the preservative efficacy of selected medicinal plant extracts in the shelf-life extension of some probiotic drinks.

1.3.2 Specific objectives

1. To determine the phytochemical constituents of the leaf extracts of the selected medicinal plants.

2. To determine the microbial load of probiotic drinks produced from calyces and tigernut after production.

3. To preserve the produced probiotic drinks using aqueous leaf extracts of *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides* using different volumes.

4. To determine the microbial load of the probiotic drinks during preservation.

5. To determine the organoleptic properties and shelf-life extension properties of the preserved drinks.

6. To isolate and identify microorganisms associated with the preserved probiotic drinks.

1.4 Research Questions

1. What are the phytochemical constituents of the leaf extracts of *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides*?

2. What is the microbial load of probiotic drinks produced from calyces and tigernut after production?

3. Could aqueous leaf extracts of *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides* preserve probiotic drinks at different volumes?

4. What are the microbial loads of the probiotic drinks during preservation?

5. What are the organoleptic properties and shelf-life extension properties of the preserved drinks?

6. What are the microorganisms associated with the biopreserved probiotic drinks?

1.5 Research Hypothesis

Ho: There is no significant relationship between the preservative efficacy of the different medicinal plants andthe shelf-life extension of probiotic drinks.

Ha: There is significant relationship between the preservative efficacy of the different medicinal plants and the shelf-life extension of probiotic drinks.

1.6 Significance of the Study

This thesis is of great importance as it reveals the importance of medicinal plants in preserving some local beverages. It will benefit the producers of these local drinks, the beverage and food industries, the general public, students, and future researchers.

The results of this thesis will help local drink producers understand the importance of some of the medicinal plants available in society in extending the shelf life of these local drinks. By knowing this, they will effectively utilize these plants in preservation, thereby reducing the rate of spoilage and having greater opportunities to produce them in large quantities that could be resold after one day.

To the beverage and food industries, the results of this thesis will enlighten them in the exploitation of these medicinal plants and their incorporation into foods and drinks with the sole aim of extending the shelf-life as well as reducing the cost used in the acquisition of synthetic chemicals used in these industries. This will also help reduce the risks associated with the continual use of synthetic preservatives, thereby prolonging life and good health among the society.

The results of this thesis will enlighten the general public on the importance of nature's gift to man. They will be encouraged to utilize these plants in their individual preservation

of foods and drinks. This will help reduce the rate of food and drink spoilage in homes and maintain food security at all levels.

1.7 Scope of the Study

This thesis is delimited to the identification and collection of some medicinal plants, extraction of the active ingredients and phytochemical constituents of the medicinal plant extracts, production and determination of the microbial load of the produced probiotic drinks after production, preservation of the produced probiotic drinks, determination of the microbial load of the drinks during preservation for two (2) months at seven (7) days interval and determination of the organoleptic properties and shelf-life extension properties of the preserved drinks.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conceptual Framework

2.1.1 Plants and medicinal plants

Plants have provided a source of inspiration as plant-derived medicines, which have made large contributions to human health and well-being. A plant is a living thing that grows on the earth and has stems, leaves, and roots. Plants contain many biologically active compounds with potentials for development as medicinal agents. Plants have traditionally been used as a source of medicine all over the world of different ethnic groups inhabiting various terrains for the control of various ailments aflicting humans and their domestic animals (Uwimbabazi *et al.*, 2015; Harholt *et al.*, 2016).

Medicinal plants are those plants that are commonly used (parts, extract etc) in treating and preventing specific ailments and disease that are generally considered to be harmful to human. Medicinal plants have a long history of use that is beneficial to mankind. In 2000, World Health Organization reported that about 80% of the world's population relies mainly on traditional therapies which involve the use of plant extract or their active substances. They play an important role in traditional health care systems for curing many diseases (Kumar *et al.,* 2014). Medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anemia for a long time now, but the potential of higher plants as source for new drugs is still largely unexplored. Systematic screening of them may result in the discovery of novel effective compounds (Gajendrasinh *et al.,* 2014).

2.1.2 Probiotics

According to the National Health Service (2018), probiotics are live microorganisms to provide health benefits when consumed, generally by improving or restoring the gut flora. Probiotics are considered generally safe to consume but may cause bacteria-host interactions and unwanted side effects in rare cases.

Sources of probiotics

Probiotics are sourced from dairy products and fruits. Live probiotic cultures are part of fermented dairy products, other fermented foods, and probiotic-fortified foods. Some fermented products that contain lactic acid bacteria (LAB) include vegetables such as pickled vegetables, kimchi, pao cai, and sauerkraut; soy products such as tempeh, miso, and soy sauce; and dairy products such as yoghurt, kefir, and buttermilk (Hao *et al.,* 2015; World Gastroenterology Organization, 2016).

More precisely, sauerkraut contains the bacteria *Leuconostoc mesenteroides, Lactobacillus plantarum, Pediococcus pentosaceus, Lactobacillus brevis, Leuconostoc citreum, Leuconostoc argentinum, Lactobacillus paraplantarum, Lactobacillus coryniformis,* and *Weissella* species. Kimchi contains the bacteria *Leuconostoc* species, *Weissella* species, and *Lactobacillus* species. Pao cai contains *L. pentosus, L. plantarum, Leuconostoc mesenteroides, L. brevis, L. lactis,* and *L. fermentum* (Swain *et al.,* 2014).

These microorganisms, through various mechanisms, create inappropriate conditions for the growth of harmful microorganisms, playing a significant role in preventing gastrointestinal infections. The main criteria for selecting probiotic strains include human origin, resistance to the digestive system's acid and bile, and the ability to stick to the

intestinal wall and combat the pathogenic microbes of the environment (Sirin & Aziz, 2017).

2.1.3 Probiotic drinks

Zobo

Zobo is one of the well-known non-alcoholic beverages that are majorly patronized by students due to its cheapness and availability. Zobo is an aqueous extract of the dried reddish-brown petals (calyces) of *Hibiscus sabdarffa* which is sweetened to taste with sugar and sometimes flavoured with spices like ginger, garlic, hot pepper, etc., along with natural flavours such as pineapple juice or lime juice or artificial flavourings like strawberry vanilla, etc., depending on individual taste (Salami & Afolayan, 2020). Zobo is commonly found hawked around, packaged in transparent polythene sachets or plastic containers in most northern and some southern parts of Nigeria. Zobo drink has recently become a household name in almost every Nigerian home. The drink, if not preserved, is prone to deterioration by food-borne microorganisms. Although the popularity of Zobo juice is increasing, one of its greatest limitations for large-scale production is its short shelf-life of 24 hours if not refrigerated (Saraswati *et al.*, 2020).

Health benefits and nutritional values of *zobo*

The edible calyces are commonly usedto prepare hot or cold zobo beverages. The drink has been reported to have antioxidant, antihypertensive, antihypolipidaemic, anticancer, antibacterial, hepatoprotective and antistress, antidiuretic, antispasmodic, and antidiarrheal activities (Eaton & Groopman, 2013). Another study found that the drink affects metabolism, preventing obesity and fat build-up in the liver. Roselle has generally been

considered safe as a foodstuff; a dosage of 1.5 g is recommended for daily consumption (Salami & Afolayan, 2020).

The safety profile of roselle is excellent, with no proven adverse reactions. Noteworthy, Hudson (2013) affirmed that studies recommend 1,000 mg of dried herb three times daily, 1 cup of the drink two times daily, or 100 mg of standardized extract twice daily for reducing cholesterol levels. Also, for hypertension, 1 cup of the drink twice daily or dried powdered roselle extract providing 250 mg anthocyanin per day was recommended.

In the countries of origin, the flowers have been. Still, they are, widely used in the folk medicine as an antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, cooler, sedative, and tonic, among many other applications. Hibiscus and its derived extracts have an important inhibitory activity on the growth of fungus and yeasts. Additionally, they have a remarkable inhibitory activity on enzymes such as acid phosphatase, alkaline phosphatase, glutamate-oxalacetate transaminase and glutamate-pyruvate transaminase. Hibiscus flowers are currently included in different laxative infusion mixtures because of the osmotic effects of their organic acid contents, which make them hardly absorbable. It is also used to adjust the colour and flavour of different phytotherapeutic preparations (Saraswati *et al.*, 2020).

Further, anthocyanins from *Hibiscus* were extracted and tested against human promyelocytic leukemia cells (HL-60) to determine their effectiveness at causing apoptosis of these cells. The anthocyanins demonstrated to be inhibitory against the HL-60 cells and were determined to be possible chemopreventative agents. Additionally, consuming 1.5 g of *Hibiscus* tea twice a day for 15 days showed a uricosuric effect, which

helps with uric acid secretions and decreases the risk of renal stones (Bankole *et al.*, 2013).

The proximate composition of the calyces has been examined. Red calyces (based on 100 g dry weight) contain 6.4% protein, 79.25% carbohydrates, 5.13% fat, 2.7% crude fiber, and 6.52% ash. The calyces of *Hibiscus sabdariffa* are rich in bioactive compounds. Organic acids are prevalent in the calyces resulting in the low pH of approximately 2-2.5. The major organic acids in the calyces are citric and malic acids while other acids such as tartaric have also been identified. The calyces are also rich in phenolic compounds. Some of the phenolic acids identified from *Hibiscus* calyces include gallic, chlorogenic, and protocatechuic acid (Shruthi *et al.*, 2019).

Method of preparation of Zobo

Roselle drink is prepared in several ways using various ingredients, with dried calyces being the major ingredient. All the methods result in the drink but with a slightly different taste (based on the sweeteners used, such as sugar, honey, pineapple juice, sugar cane, and maple syrup) and flavour based on the spices used. The selection of preservatives and flavours for the drink depends on individuals. These preservatives include garlic, ginger, lime, clove, cinnamon, nutmeg, and pepper fruit (Ezeigbo *et al.*, 2015).

More recently, other plant materials are added as a blend in the preparation of the drink to enhance the flavor and keeping qualities. Te infusion of roselle calyces is usually sweetened with fruit juice, sugar cane, or granulated sugar or pineapple, depending on preference (Ezeigbo *et al.*, 2015; Saraswati *et al.*, 2020). Straw berry is another additive used in the preparation of the drink. Although the drink has different names all over the world, the flavour is very similar usually having a tangy flavour profile. There are two

main methods of preparing the drinks: the steeping and boiling using different procedures. Generally, the steeping method is carried out by pouring boiled water into clean calyces inside a bowl and set aside for two to four hours. The juice is then separated from the calyces using sieve; fruit juice and sweeteners are then added based on preference (Salami & Afolayan, 2020).

The drink can be taken hot or cold. The boiling method, on the other hand, is carried out by putting clean calyces with sliced fruits pineapple, oranges, and lime (with peel) (based on individuals) into a clean pot, covered with water, and boiled for five minutes, and then the desired spices were added such as cinnamon, garlic, and ginger. More water is added to the content and then left to boil for 30 minutes. The juice is then collected from the content using sieve and sweeteners added if desired. The drink is refrigerated afterwards and served chilled (Salami & Afolayan, 2020).

There are other reports on laboratory preparation of the drink. For examples, Umaru *et al.* (2014) prepared the drink by hurling the dried calyces into the water, leave for 1-2 hours and then sieve after cooling. Some researchers, Ezeigbo *et al*. (2015) have severally reported a stricter soaking period of 10–15 minutes. Then, preservatives (such as ginger, clove, and garlic) and sugar are added before cooling. The resultant filtrate is then consumed as hot tea or taken like a refreshing drink when chilled (Ezeigbo *et al.*, 2015).

Similarly, the International Organization for Standardization has specified procedures for producing the tea by means of infusing the calyces. “Shandy Sorrel” produced in Trinidad and Tobago is a combination of roselle drink and beer. The drink is one of the common beverages consumed in Mexico and Central America. In the Middle East and Sudan, “Karkade” calyces are steeped or boiled for about one hour. Mint leaves, menthol, fruit

juice, and sweeteners are added to make the drink especially in social events. In West Africa, the refreshing drink is prepared by leaving the calyces in the water overnight or boiling for about an hour, sieving, and then adding juice and sweeteners for good taste (Salami & Afolayan, 2020).

Prevalence of microbial contaminants in Zobo

The calyces used to prepare zobo drinks have been reported to be a major source of contamination as they harbour many microorganisms. Other sources of contamination include unhygienic states of other materials for preparation, such as water, sweeteners, preservatives, the equipment used, packaging materials, the place of preparation, the processors' unhygienic conditions, and poor storage (Salami & Afolayan, 2020). The calyces have been reported to get infested with microorganisms through the seed stock used, local growing conditions, and postharvest handling, especially during drying and storage and in the market.

Studies confirm that the bulk of contamination of the drink is through the calyces used as a major raw material. The high content of water in fresh calyces predisposes the calyces to infection; even if the calyces are later dried some of the microorganisms remain. At the local market, roselle calyces are displayed in big containers (uncovered) or polyethene sheets for sale; these expose calyces to microbial contamination (Izah *et al.,* 2015). The presence of contaminants and toxigenic fungi from roselle drink is a function of contamination of the calyces used.

Water is another raw material for the preparation of the drink. The quality of water also determines the quality of the drink. Clean and sterile water is good for a quality drink as poor water introduces some microbes that are dangerous to health. Other materials used

such as sweeteners (sugar, sugar cane juice, and pineapple juice), preservative materials such as garlic, lime, ginger, and benzoic acid as well as equipment such as bowls, pots, and stirrer used during preparation are possible means of contamination.

Risiquat (2013) reported that contamination may occur when the hot extract is left to cool, during the introduction of flavour and sweeteners and bottling. The place of preparation if not hygienic can be a source of contamination. In addition, the level of hygiene of the processor and the activities of the handlers such as cleaning children after defecating, cleaning mucus, sneezing, coughing, and excessive talking can be means of introducing toxigenic microbes into the drink. Akhigbemidu *et al*. (2015) affirmed that *Streptococci* species which are often found in the mouth enter the drink during preparations via talking.

Another means of zobo drink contamination is during packaging. In developing countries such as Nigeria, the drink is locally packaged by the use of polyethene sachet/films and plastic bottles. These materials if already polluted can contaminate the drink. Majority of the processors of the drink in the developing countries are small-scale women and even some children who may not attach more importance to hygiene. Due to ignorance and poverty level of some processors, used bottles are reused for packaging; if such bottles were not thoroughly washed, it can cause contamination of the drink (Salami & Afolayan, 2020).

Besides, some processors use mouth to blow air into polyethene sachets to open them; this is a means of introducing microorganisms into the drink. All other unhygienic activities like singing during packaging can cause contamination. *Staphylococcus* species and *Pseudomonas* species may occur in the drink due to handling and other utensils used after processing (Salami & Afolayan, 2020).

In addition to other sources of contaminants, conditions of a place of storage and duration of storage can also encourage contamination. If the storage environment is unhygienic or favourable for microbes' multiplication, contamination can occur. The values of microbial counts depend on the type of flavour, preservatives used, and storage duration (Izah *et al.,* 2015). Nevertheless, Risiquat (2013) found that the microbial population of the drink seldom exceeds acceptable/level and tolerant level for ready-to-eat food the day it is prepared.

Soyamilk

Soymilk is an aqueous, white, creamy extract produced from soybeans which is similar to cow milk in appearance and consistency. Soybean (*Glycine max*) is recognized as one of these legumes with a huge protein potential. Its edible products including; soymilk, soy bean flour and other soy based products. They are food items consumed on purchase from vendor, hawkers and consumed immediately without any further preparation. Some of them are snacks which are also vended along highways linking several geographical areas in the country (Oranusi & Braide, 2012). But most of them are found in public places including markets, motor parks, and streets, outside schools, hospitals and even express way (Izah *et al.,* 2015).

The increasing popularity of soymilk as a beverage worldwide is credited to health benefits e.g. low cholesterol and lactose, its ability to reduce bone loss and menopausal symptoms, prevention and reduction of heart diseases and certain cancers (Kohli *et al.,* 2017). As this drink is cholesterol free and low in energy, it could enhance health benefits in terms of reducing body weight and blood lipids (Kabiru *et al.,* 2012). With its unique nutty flavor and rich nutrition, soymilk can be used as supplementary way of dairy milk. It

is available as a plain, unflavored beverage or in a variety of flavored beverage including chocolate, vanilla and almond. Soy-based diets can reduce blood pressure in spontaneously hypertensive rats but apparently not in hypertensive humans (Khodke *et al.*, 2015).

Health benefits and nutritional values of soyamilk

Health benefits of soybeans and related products include low lactose and cholesterol, reduced bone loss, prevention and reduction of heart diseases. Soybean products also have protective properties against breast, prostate, colon and lung cancers because of the isoflavones content. Other than the whole seed, many processed soy products are available in the market. They include soya milk, soya flour, soya curd and tofu (soya paneer) (Kant & Broadway, 2015).

Soya protein is a complete protein, meaning it contains all of the indispensable amino acids required by the body in the correct proportions and amounts to meet human needs for growth, maintenance and repair of living tissues. Soya protein is the only complete plant based protein which is available to those maintaining a vegetarian lifestyle and is equal in protein quality to milk, meat and egg proteins. Muscles need protein to repair, rebuild and grow. In accordance with the guidelines given by WHO/FAO/UNU, used of soy protein as a whole source of protein in the daily diet will support normal muscle formation and maintain nitrogen balance in both children and adults (Kant & Broadway, 2015).

Soya milk is a highly nutritious as it contains protein, fat, carbohydrates vitamins and minerals. It is because of this nutritious value and comparative low cost, that soymilk plays an important role in the dietary pattern of people in most developing countries. The

nutrients content in eight ounces of plain soymilk are 140 gm calories, 10 gm protein, 4 gm fat, 14 gm carbohydrate, 120 mg sodium, 1.8 mg iron, 0.1 mg riboflavin and 80 mg calcium. It has about the same amount of protein as cow's milk, though the amino acid profile differs (Dauda & Adegoke, 2014). Soybeans contribute approximately 20% fat to the diet. Soymilk is rich in protein, carbohydrate and oil, made by soaking the soybeans in water before grinding and straining, the milk produced resembles cow milk in both appearance and constituency. They could be used as a potential substitute to cow milk in terms of quality and nutritive properties (Kant & Broadway, 2015).

Method of preparation of soymilk

Soybean is sorted and cleaned to remove stones and damaged, deformed seeds. Then the dry soybean is washed and soaked in water (500 g in 1 Liter) for 12 hours. It is then rinsed and blanched in 1.25% $NaHCO_3$ for 30 minutes. The rehydrated soybean is washed, manually dehulled and rinsed. The soybean seeds is ground in blender and expressed in the ratio of 3:1 (water to beans on a weight basis). The obtained milk is then formulated by adding anti-oxidants and preservatives. The milk was then pasteurized at the temperature 70 ^{0}C for 15 seconds and subsequently bottled and stored at ambient and refrigeration temperature (Kohli *et al.*, 2017).

Prevalence of microbial contaminants in soymilk

The sources of microbial contaminants could be from the soya bean seeds used to prepare soya milk, water, other raw materials, environment and packaging materials (Kohli *et al.*, 2017).

Kunun-zaki

Kunun-Zaki drink is a locally prepared indigenous non-alcoholic beverage (Maji *et al*., 2011), which is widely produced and consumed in large quantities in Nigeria, especially the northern part of the country. This local beverage is consumed in both wet and dry season because of its optimal thirst quenching abilities. *Kunun-zaki* drinks are usually produced using maize, guinea corn, millet or sorghum in varying proportions (Maji *et al*., 2011) to which sweet potato is sometimes added so as to increase the taste of the *Kunun-zaki* which is a major factor that attracts consumers to the product. The ingredients used are usually not quantified and are very unique because they are sourced locally.

Health benefits and nutritive values of kunun-zaki

Kunun-zaki drinks have been reported to have high nutritional value because of the raw materials such as; maize, guinea corn, millet or sorghum from which it is made. Maize has various health benefits. The B-complex vitamins in maize are good for skin, hair, heart, brain, and proper digestion. They also prevent the symptoms of rheumatism because they are believed to improve the joint motility. The presence of vitamins A, C, and K together with beta-carotene and selenium helps to improve the functioning of thyroid gland and immune system. Potassium is a major nutrient present in maize which has diuretic properties (Kumar & Jhariya, 2013).

Maize silk has many benefits associated with it. In many countries of the world such as India, China, Spain, France and Greece it is used to treat kidney stones, urinary tract infections, jaundice, and fluid retention. It also has a potential to improve blood pressure, support liver functioning, and produce bile. It acts as a good emollient for wounds, swelling, and ulcers. Decoction of silk, roots, and leaves are used for bladder problems,

nausea, and vomiting, while decoction of cob is used for stomach complaints (Kumar & Jhariya, 2013). Maize kernel is an edible and nutritive part of the plant. It also contains vitamin C, vitamin E, vitamin K, vitamin B_1 (thiamine), vitamin B_2 (niacin), vitamin B_3 (riboflavin), vitamin B_5 (pantothenic acid), vitamin B_6 (pyridoxine), folic acid, selenium, N-p-coumaryl tryptamine, and N-ferrulyl tryptamine. Potassium is a major nutrient present which has a good significance because an average human diet is deficient in it (Kumar & Jhariya, 2013).

The two main forms of vitamin E present in diet are alpha (α) and gamma (γ) tocopherols. Maize oil is amongst the rich sources of these tocopherols, especially γ-tocopherol and their reported concentration was 21.3 and 94.1 mg/100 g, respectively. Maize silk contains various constituents essential for our diet such as maizenic acid, fixed oils, resin, sugar, mucilage, salt, and fibers (Kumar & Jhariya, 2013). Millets include among other raw materials that can be used in the preparation of *Kunun-zaki*. In addition to their nutritive value, several potential health benefits such as preventing cancer and cardiovascular diseases, reducing tumor incidence, lowering blood pressure, risk of heart disease, cholesterol and rate of fat absorption, delaying gastric emptying, and supplying gastrointestinal bulk have been reported for millet (Gupta *et al.,* 2012). Millet grains, before consumption and for preparing of food, are usually processed by commonly used traditional processing techniques include decorticating, malting, fermentation, roasting, flaking, and grinding to improve their edible, nutritional, and sensory properties.

Method of preparation of kunun-zaki

The methods involved in the production of this local beverage include steeping the grains in some containers such as buckets and some other household utensils (Maji *et al*., 2011;

Oluwajoba *et al*., 2013) followed by grinding the steeped grains into a mash and then mixed up with some spices of choice such as pepper and ginger before it is then divided into two in equal proportions (Oluwalana & Adedeji, 2013). One of the proportions is then mixed up with hot or boiled water and the other with some ingredients such as malted rice and sweet potato paste. The two proportions are then mixed together at a temperature of about 75-80 °C as the mixture is left to undergo fermentation at room temperature within 20-24 h, after which it is sieved before being considered ready for consumption (Akoma *et al*., 2013).

Some individuals and communities prefer *Kunun-zaki* with much pepper and sugar (Adedokun *et al*., 2012). This common drink is usually packaged and sold in 50 ml to 1 litre plastic bottles and at times tied in some disposable polythene bags. The drink is mostly consumed within 20-35 h of its production due to its poor keeping quality (Akoma *et al*., 2012). This drink is not expensive because the grains and other ingredients used for production are locally sourced and are mostly grown within the savannah region and almost throughout the years, most especially the savannah belt of West Africa. The packaging materials are also available, cheap and easily affordable. Kunun-zaki has been reported to be rich in vitamins, minerals, carbohydrates and proteins (Folasade & Oyenike, 2012; Oluwajoba *et al*., 2013). No elaborate equipment and expertise are required for its production (Akoma *et al*., 2013; Oluwalana & Adedeji, 2013).

Prevalence of microbial contaminants of kunun-zaki

Spices are usually added in small quantities to improve taste and flavour as these are agricultural commodities which may contain a high level of microbial impurities and these can be sources of contaminants, spoilage and pathogenic microorganisms. The water

content coupled with the crude method of production and packaging that is under an improper sanitary conditions predisposes the drink to sudden microbial contamination (Akoma *et al*., 2012). The ubiquitous nature of microorganism guarantees them the opportunity to be found in this locally made beverage drink and possibly in the water used for its preparation, during storage and other processes involved. Some of the bacterial species that are associated with this locally produced beverage drink include spoilage species, pathogenic species, coliforms and lactic acid bacteria (Akoma *et al*., 2012).

2.1.4 Shelf-life and shelf-life extension

Shelf-life is defined as the length of time that a commodity such as; foods and drinks can be stored without spoilage or becoming unfit for use, consumption or sale. It has to do with the recommended maximum time for which products or fresh produce can be stored during which the defined quality of a specified proportion of the goods remains acceptable under expected conditions of distribution, storage and display (Yee, 2014). Shelf-life depends on the degradation mechanism of the specific product. Most products can be influenced by several factors such as; exposure to heat, moisture, light, transmission of gases, mechanical stresses and contamination by microorganisms (Magoulas, 2014).

Shelf-life extension is an effort to ensure that food products are safe for a long period while maintaining their original quality (Tucker, 2016). Different ingredients and processes can be employed to extend the shelf-life of food products.

2.1.5 Medicinal plants used in shelf-life extension of probiotic drinks

Medicinal plants and vegetables have been known to possess important phytochemicals, antioxidants and antimicrobial activities with nutritional and therapeutic values. Different extracts and essential oils have been identified from medicinal plants and tested for their

antimicrobial properties against various food-borne microorganisms (Boziaris *et al*., 2014). Extracts from medicinal plants are soluble fractions that can be removed from plant materials by solubilising the component(s) of interest in an aqueous, alcohol, lipid, solvent, or supercritical CO_2phase and then removing it (Brewer, 2014).

Extracts from medicinal plants contain essential oils (EOs). Essential oils (EOs) are volatile liquid, limpid and aromatic oily liquids derived from plant materials (Arvind & Vyas, 2014). EOs are usually isolated by steam distillation, extraction, or mechanical expression from the medicinal plant materials (Brewer, 2014). The phenolic components are chiefly responsible for the antibacterial and antioxidant properties of EOs and extracts (Carocho & Ferreira, 2014). Several researchers have revealed that medicinal plants contain rich sources of natural antioxidant compounds. Phenolic compounds are commonly found in medicinal plants, and have been reported to have a wide range of biological activities including antioxidant properties that directly or indirectly contribute to the inhibition or suppression of oxidation processes in microorganisms (Cox *et al*., 2015).

2.1.6 Important phytochemical compositions in medicinal plants

Alkaloids

Alkaloids are plant-derived compounds that are toxic or physiologically active. Some examples of alkaloids include; isopteropodine and pteropopine have anti-microbial activity. These components act by promoting white blood cells to dispose harmful microorganisms and cell debris (Ekeleme *et al.,* 2017). Highly aromatic planar quaternary alkaloids like berberine, piperine and harmane work by intercalating the DNA and cell wall. Others, by simulating neurotransmitters such as acetylcholine, dopamine and

serotonin, they affect central nervous system (CNS) at the synapses. They also act as narcotics, as antimalaria, as topical anesthetic for ophthalmology; in treating hypertension, neuralgia, rheumatism, motion sickness, and also in extending the life of hormones. They have analgesic activity and hence used to alleviate pain in cases of boils, septic wounds, and complain such as headaches, abdominal pains and eye conditions.

Similarly, alkaloids have antineoplatic activity. Indole alkaloids have been used in leukemia and Hodgkin's disease chemotherapy. They act by terminating and depolymerization of protein microtubules that form the mitotic spindle in cell division. This process helps in terminating the tumor cells from separating or dividing and henceforth resulting to reduction of cancer. Nevertheless, some types of alkaloids are hallucinative, addictive, and toxic and hence used as arrow poison for hunting wild game (Ngoci *et al.,* 2014).

Tannins

Tannins are astringent, bitter plant polyphenols that work by binding and precipitating or shrinking proteins. They have physiological role by acting as antioxidants through free radical scavenging activity, chelation of transition metals, inhibition of prooxidative enzymes and lipid peroxidation (Ngoci *et al.,* 2014), hence modulating oxidative stress and preventing degenerative diseases. They also inhibit tumor growth by inducing apoptosis and inhibiting mutagenecity of carcinogens. They exhibit anti-microbial activity by complexing nucleophilic proteins by hydrogen bonding, covalent bonding, and nonspecific interactions.

The main targets for complexing are cell wall and cell membrane adhesin proteins, hence inactivating microbial adhesion which is the first step in establishment of infections. They

also cause cell wall/membrane disruption. This also inactivates microbial enzymes and cell envelope transport proteins by processes that may involve reaction with sulfhydryl groups of proteins. They also accumulate /complexes metal ions (e.g. cobalt, manganese, iron, copper, etc.) necessary for microbial growth as co-factors and activators of enzymes. They also inhibit viral reverse transcriptase (Ngoci *et al*., 2014; Ogunwenmo *et al*., 2014).

Phenolic compounds

Toxicity to microorganisms in phenolic compounds depends on the site and the number of hydroxyl groups, with evidence that increased hydroxylation results to increased toxicity (Ngoci *et al*., 2014). They have endocrine role by interacting with estrogen receptors. They are also anti-inflammatory, molluscicidal and hence important in the control of schistosomiasis. They also have antidiarrhoeal, anti-septic anti-fungal properties, anti-parasitic, anti-irritant properties and also used in curbing hemorrhage, in wound healing, and improving vascular health by suppressing peptides that harden arteries (Ngoci *et al.,* 2014). Also, they have economic role of tanning leathers in leather industry. Nevertheless they affect intake and digestibility of feeds among livestock, and excess can be carcinogenic on normal tissues (Ngoci *et al.,* 2014).

Flavonoids

Flavonoids are structural derivatives of flavones that contain conjugated aromatic systems that bound to sugar(s) as glycosides, and they are phenolic and water soluble in nature. They exert their roles as anti-oxidants, and hence protecting against degenerative diseases. Flavonoids such as quercetin, act as chain breaking anti-oxidants, and by preventing oxidation of low-density lipoprotein by macrophages and metal ions like copper. This reduces the oxidative stress. They also act as; 'nature's biological modifiers' as anti-

allergens, anti-inflammatory, and induces phase two enzymes that eliminate mutagens and carcinogens (Ogunwenmo *et al.,* 2014). They also act as antimicrobial's by complexing extracellular and soluble proteins, and by complexing bacteria cell wall.

Probable targets on microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane bound enzymes. Still others like catechins found in oolong green tea inactivates bacterial toxins (e.g. cholera toxin) and inhibits bacterial glucosyltransferases. Flavonoids are also known to increase coronary flow, to reduce the myocardial oxygen consumption and to lower the arterial pressure. They are also known to reduce capillary fragility, to be antiallergic and also to be anti-spasmodic and hence applied to relief asthma and nose bleeding (Ngoci *et al.,* 2014). Flavonoids lacking hydroxyl groups (-OH) on their structure are more active against the micro-organism than those having -OH, and this supports the idea that their microbial target is the membrane (Ngoci *et al.,* 2014).

Saponins

Saponins are natural glycosides that act as hypo-glycemic, antifungal and serum cholesterol lowering agents in animals. They are essential elements in ensuring hormonal balance and synthesis of sex hormones.They have theability to precipitate and coagulate red blood cells and are characteristically able to form foams in aqueous solutions, brings about hemolytic activity, have bitterness properties as well as cause cholesterol binding (Okwuonu *et al.,* 2017). The modes of action for the antibacterial effects involve membranolytic properties of the saponin as well as lowering of the surface tension of the extracellular medium. Saponins have antineoplastic activity without killing normal cells. This is by reacting with cholesterol rich membranes of cancer cells, and inducing mitotic arrest that causes apoptosis of cell (Ngoci *et al.,* 2014).

They have a host of biological roles including boosting respiratory system as expectorant, and hence activity against cough. They also have anti-protozoa activity whereby they act by reacting with cholesterol in the protozoal cell membranes causing cell lysis, e.g. Yucca saponins are effective against protozoan *Giardia lamblia*. They serve as vaccine boosters by acting as adjuvant. They have anti- inflammatory, emetics, antiviral, antifungal, insecticidal, molluscicidal, piscidal and anti-bacterial activity (Ngoci *et al*., 2014). The mode of action for the anti-bacterial effects involve membranolytic properties of the saponin as well as lowering of the surface tension of the extracellular medium (Ngoci *et al.*, 2014).

Also, some saponins like Radix Notoginseng have been reported to increase the blood flow of the coronary arteries, prevent platelet aggregation and to decrease the consumption of oxygen by heart muscles. They also have anti-oedema, antitussive, purgative and immunoregulatory properties (Ngoci *et al*., 2014).

Phytosteroids

Phytosteroids are plant steroids that may or may not act as weak hormones in the body. They share common basic ring structures with animal steroids though they are not equivalent because of varying chemical groups attached to the main ring in different positions (Ngoci *et al*., 2014). Phytosteroidsare mainly used to treat reproductive complications such as treatment of venereal diseases, used during pregnancy to ensure an easy delivery, as well as to promote fertility in women and libido in men. They also act as sex hormones derivatives, (for example, they can be metabolized to either androgen or estrogen-like substances) and hence they are potential source of contraceptives (Ngoci *et al.*, 2014).

Phytosteroids are also have antimicrobial, analgesic, anti-inflammatory, and of use in treating stomach ailments and in decreasing serum cholesterol levels. They have also been indicated as potent inhibitors of macrophage activation, blocking the production of pro-inflammatory cytokines and LPS-induced lethality and therefore they have potential use as immunosuppressive agents especially the physalins (Soares *et al*., 2015).

Terpenoids

Terpenoids are derivatives of isoprene molecule having a carbon skeleton built from one or more of C15 units. They exert their roles as antibacterial, antifungal, antiviral, antiprotozoan, antiallergens, as immune boosters and as antineoplastic. The mechanism of action is speculated to involve membrane disruption by these lipophilic compounds (Ogunwenmo *et al*., 2014). Petalostemumal, an example of terpenoids, has demonstrated activity against *B. subtilis, S. aureus*, and *C. albicans* and to a lesser extent to Gram-negative bacteria (Ngoci *et al.,* 2014).

The mechanism of terpenoids could be due to a perturbation of the lipid fraction of bacterial plasma membranes, resulting in alterations of membrane permeability and in leakage of intracellular materials. This is related to physicochemical characteristics of the active principle (such as lipophilicity and water solubility), lipid composition and net surface charge of the bacterial membranes. These phytochemicals can cross the cell membranes, penetrating the interior of the cell and interacting with intracellular targets critical for antibacterial activity. They are also used to alleviate epilepsy, to relieve cold, influenza, cough and acute bronchial disease (Okwuonu *et al.,* 2017). From laboratory studies of terpenes from Ginseng, it has been suggested that the possible target of these

compounds involves hypothalamus-pituitaryadrenal axis due to the observed effects on the levels of adrenocorticotropic hormone and corticosterone.

Cardiac glycosides

Cardiac glycosides (also called cardenoloids) are phytochemicals that occur as a complex mixture together in the same plant. Most cardiac glycosides are toxic, however many have pharmacological activity especially to the heart. They are used in treatment of congestive heart failure, whereby they inhibit Na+ /K+ -ATPase pump that causes positive ionotropic effects and electrophysiological changes. This strengthens heart muscle and the power of systolic concentration against congestive heart failure (Ogunwenmo *et al.*, 2014). They are also used in treatment of atrial fibrillation, flatter, and they acts as emetics and as diuretics.

2.1.7 Plant-derived compounds used in shelf-life of foods

Essential oils (EOs)

Essential oils are complex mixtures of volatile organic compounds (VOCs) produced as secondary metabolites in plants and frequently responsible for the characteristic odor of plants. They are characterized by two or three major VOCs at fairly high concentrations (20–70%) compared to other VOCs. Some EOs have antimicrobial and antioxidant properties and an increasing demand for natural preservatives has led to EOs as potential alternatives for antimicrobials and antioxidants (Calo *et al.*, 2015).

EOs have been proved to be effective antimicrobials against some food-borne pathogens including *S. typhimurium, E. coli* O157:H7, *Campylobacter, L. monocytogenes, S. aureus,* and others. Studies show that the efficacy of EOs depends on chemical structure,

concentration, matching the antimicrobial activity spectrum with the target microorganism(s), interactions with the food matrix, and application method.

It has been observed that some EOs show inhibitory effect on membrane integrity against the tested food-borne pathogenic bacteria (Kang *et al.,* 2019; Wan *et al.,* 2019). Intracellular material leakage is a general phenomenon results in cell death. The hydrophobic nature of EOs could interfere with bacterial lipid membrane resulting in increased permeability of the cell constituents, which is in agreement with other phenolic compounds (Cui *et al.,* 2019; Guo *et al.,* 2019). So far, most studies concerning the antimicrobial action mode of EOs have been carried out on bacteria, while less is known about their effects on molds and yeast. Gram-positive bacteria are generally more susceptible than Gram-negative ones. The cell wall lipopolysaccharides (LPS) of Gram-negative bacteria can create a barrier toward macromolecules and hydrophobic compounds, preventing active compounds in EOs reaching to cytoplasmic membrane (Guo *et al.,* 2019). The combinations of EOs with other natural preservatives or even other chemical ones also show positive effects.

Plant extracts

Plant extracts have broad application prospects in fish preservation. The antimicrobial activities of plant extracts may be attributed to the combined effects of polyphenols adsorption to bacterial membrane with membrane disruption and subsequent cellular contents leakage, and the generation of hydroperoxides from polyphenols (Bouarab-Chibane *et al.,* 2019). Plant extracts also show antifungal activities, antioxidant, antimutagenic activities, and inhibit lipid oxidation in food (Ashrafi *et al.,* 2018; Kharchoufi *et al.,* 2018).

Numerous studies have been done *in-vitro* to evaluate the antimicrobial activities of plant extracts; however, only a few studies are available for fish preservation as the antimicrobial activity of plant extracts does not produce as marked inhibition as many of the chemical preservatives in fish. The plant crude extracts generally contain flavonoids in the form of glycosides, in which the sugar presenting in them decreases the effectiveness against some food-borne pathogens (Kumar & Pandey, 2014).

The antimicrobial and antioxidant effects of various plant-derived compounds on fish have been performed. The plant-derived compounds could extend fish shelf life by reducing the total aerobic plate count and retarding lipid oxidation and may also be used together with other natural preservatives or different packaging ways. Rainbow trout (*Oncorhynchus mykiss*) using turmeric extract, shallot extract, and their combination with vacuum packaging could reduce the growth of total viable count and extend the shelf-life. The plant-derived compounds are also combined with nisin to extend the shelf-life of fish and fish products (Gao *et al.*, 2014).

2.1.8 Some medicinal plants used in shelf-life drinks

Alstonia boonei

Alstonia boonei belongs to the Apocynaceae family.The plant is used by traditional health practitioners especially in rural areas. Medicinally, the stem bark has been found to possess anti-rheumatic, anti-inflammatory, analgesic/pain-killing, antimalaria/antipyretic, antidiabetic (mild hypoglycaemic), antihelminthic, antimicrobial and antibiotic properties. Decoction from the plant has antibacterial activity, reliefs aches and pains associated with malaria fever. Preparations from *Alstonia boonie* are taken in the form of preparations that

exhibits anti-pyrexia and anti-malaria effects, to combat rheumatic and arthritic pains (Nkono *et al.*, 2014).

The decoction of *Alstonia boonie* bark could be taken alone as an effective pain-killing agent. A cold infusion made from the fresh or dried bark of *Alstonia boonie* taken orally two to three times daily exerts a mild hypoglycemic effect on diabetic patients. In some African countries *Alstonia boonei* is considered a sacred tree and worshiped in the forest and hence human beings in those countries do not eat its parts (Osuntokun & Ajiga, 2020).

Pterocarpus santalinoides

Pterocarpus santalinoides is a plant species that belongs to the Fabaceae family. Its leaves and fruits are used in human food as a vegetable and as a snack. In traditional medicine, the leaves or the bark of the stem are used to treat gastroenteritis. *P. santalinoides* leaves are used in the treatment of diarrhea of bacterial and non-bacterial origin (Ogbonna *et al.*, 2018; Ihedioha *et al.*, 2019; Emencheta *et al.*, 2019).

Moringa oleifera

Moringa oleifera Lam. belongs to the family Moringaceae and is a valuable plant, found in many countries of the tropics and subtropics. Its leaves, fruit, flowers and immature pods are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa. Seed extract is observed to have a protective effect by decreasing liver lipid peroxides and is antihypertensive (Muste *et al.*, 2019). *M. oleifera* roots, leaves, seed, fruit, flowers, bark and immature pods are used as cardiac and circulatory stimulants, contain antipyretic, antiepileptic, antitumor, antiinflammatory, antiulcer, diuretic, antihypertensive, cholesterol lowering, antispasmodic, antidiabetic, hepatoprotective, antioxidant, antibacterial and antifungal

activities, and are being used for the treatment of various ailments in the indigenous system of medicine (Aondo *et al.,* 2018).

Turmeric extract (*Curcuma longa)*

Turmeric is a rhizome of *Curcuma longa* with a flavourful yellow-orange spice. The rhizomes of the plant are oblong, ovate, pyriform, often short branched, and a good source of turmeric (Muste *et al.,* 2019). Turmeric has been shown to inhibit the growth of numerous microorganisms including bacteria, viruses, and fungi. In few cases, turmeric has been shown to act as a preservative by retarding microbial growth. Turmeric extract has also shown activity against food-borne pathogens. The bactericidal activities of turmeric against *Escherichia coli* BL-21 strain were reported by another study. Turmeric exhibits antifungal activity against numerous strains of fungus. This spice can also inhibit the production of aflatoxin.

Turmeric acts as a free radical scavenger in a number of *in vitro* studies (Gupta *et al.*, 2015). The effects of turmeric extract (T), shallot extract (Sh), and their combination (T + Sh) on the quality of vacuum-packaged rainbow trout (*Oncorhynchus mykiss*) examined during refrigerated storage (4 ± 1 °C) over a period of 20 days, it was observed by Pezeshk *et al.* (2014). The study concluded that dipping of whole gutted rainbow trout in turmeric extract (1.5%), shallot extract (1.5%), and combined treatment of both turmeric and shallot extracts (1.5 + 1.5%) can retard the microbial growth, delay the chemical changes, maintain the sensory attributes, and extend the shelf life of the rainbow trout during refrigerated storage. Therefore, turmeric extract, shallot extract, and their combination can be utilized as safe methods for preservation of fish under refrigerated storage (Pezeshk *et al.*, 2014).

Black seed oil (*Nigella sativa*)

Nigella sativa is a small black seed that has been used for centuries in herbal medicine. The seed comes from a flowering plant (part of the Ranunculacea family) native to southwest Asia and the Mediterranean. *Nigella sativa* is sometimes used to treat certain health conditions including asthma, bronchitis, and inflammation, and has long been used as a spice and food preservative (Muste *et al.*, 2019).

Garlic extracts (*Alliium sativum*)

Alliium sativum commonly known as Garlic belongs to the family Liliacea. Garlic has a wide spectrum of activity; including antibacterial, antiviral, antifungal and antiprotozoal activities. It also has beneficial effects on the cardiovascular and immune systems (Njue *et al.*, 2014). Garlic can rightfully be called one of nature's wonderful plants with healing power. Garlic is widely used around the world for its pungent flavor as a seasoning or condiment. The garlic plant's bulb is the most commonly used part of the plant. They have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking (Wang *et al*., 2015).

Redshank extract (*Persicaria maculosa*)

This is an annual plant in the buckwheat family, Polygonaceae. The common names of the plant include; lady's thumb, spotted lady's thumb, Jesus plant and redshank. The young leaves are eaten as a leafy vegetable (Germplasm Resources Information Network, 2017).

Lemon grass (*Cymbopogon citratus*)

Cymbopogon citratus is popularly known as citronella grass or lemongrass. Lemon grass is a tufted perennial grass growing to a height of 1 meter with numerous stiff leafy stems

arising from short rhizomatous roots. *C. citratus* is also consumed as a tea, added to non-alcoholic beverages and baked food, and used as a flavoring and preservative in confections and cuisines. In cosmetics, it's essential oils are used as fragrance in the manufacture of perfumes, soaps, detergents, and creams (Promila & Madan, 2018).

Ginger (*Zingiber officinale*)

Ginger (*Zingiber officinale*) belongs to Zingiberacae family is one of the famous spices all over the world. It is a perennial creeping plant with long leaves, yellow green flowers and thick tuberous rhizome (Gupta & Sharma, 2014). From ancient times ginger has been exploited both as Ayurvedic and Chinese medicine for curing heart problems, menstruation disorder, food poisoning, osteoarthritis, epilepsy, nausea, inflammation, cough and cold, motion sickness, menstrual cramps, cancer and many more. Besides these it also exhibits antimicrobial and antioxidant properties. The medicinal properties of ginger are due to the presence of gingerol and paradol, shogaols, etc (Kumar *et al.*, 2014).

Drum stick extract and oil (*Moringa oleifera*)

Moringa oleifera is a tropical plant belonging to the family of Moringaceae. *Moringa* leaves have been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Abbas *et al.*, 2018).

Uziza (*Piper guineense* extract and oil)

Piper guineense (uziza) is a spice plant from the family Piperaceaeand from genus *Piper*. It is a West African spice plant commonly called ashanti pepper. It is known as Uziza in

Igbo and Iyere in Yoruba. Other common names are Benin pepper, guinea pepper, false cubeb and Kale. The plants that provide the pepper are vines that grow up to 20m tall climbing up bole of trees by means of adventitious roots (Besong *et al.*, 2016). *Piper guineense* have nutritional and non-nutritional factors which are responsible for its aroma, flavour and preservative properties. The vegetable plant (*Piper guineense*) contains crude protein, fat carbohydrates and vitamins (Nwankwo *et al.*, 2014).

Citrus plant extract and oil

Citrus belong to the family of Rutaceae, are one of the main fruit tree crops grown throughout the world. Although *Citrus sinensis* (sweet orange) is the major fruit in this group accounting for about 70% of citrus output, the group also encompasses small citrus fruits such as *Citrus paradisi* (grape fruit), *Citrus aurantium*, *Citrus reticulata* (tangerine tree), *Citrus aurantifolia* (lime tree) and *Citrus limonum* (lemon tree). Citrus plants are shrubs or trees usually spinous with alternate, foliolate leaves, and coriaceous petioles (Rafiq *et al.*, 2016).

Citrus fruits are notable for their fragrance, partly due to flavonoids and limonoids (which in turn are terpenes) contained in the rind, and most are juice laden. The juice exhibit higher antimicrobial activity and contains a high quantity of citric acid giving them their characteristic sharp flavour. They are also good sources of vitamin C, flavanones and flavones (Favela-Hernandez *et al.*, 2016).

Orange (*Citrus sinensis*) is a hybrid of pomelo (*Citrus maxima*) and mandarin (*Citrus reticulata*) (Khushwaha *et al*., 2014). Sweet orange oil (essential oil) is a by-product of the juice industry produced by pressing the peel. It is used in the flavouring of foods, drinks and as a fragrance in perfume and aromatherapy. *Citrus aurantifolia* (lime) fruit is

typically round, green to yellow in color and about 3-6 cm in diameter (Kumar *et al*., 2014). Lime (*Citrus aurantifolia*) juice has been shown to have both medicinal and cosmetic values. Lime oil is mainly used as antidepressant because it promotes refreshment to the tide mind. It can be helpful for rheumatism arthritis, obesity and cellulite and has an astringent and toning action to clear oily skin and acne, helps with herpes, cuts and insect bites (Kumar *et al*., 2014).

Citrus fruits are good sources of nutrition with an ample amount of vitamin C. Besides, the fruits are abundant in other macronutrients, including sugars, dietary fiber, potassium, folate, calcium, thiamin, niacin, vitamin B_6, phosphorus, magnesium, copper, riboflavin and pantothenic acid. These constituents, also known as phytochemicals, are small molecules that are not strictly necessarily for the survival of the plants but represent pharmacological activity (Favela-Hernandez *et al.,* 2016; Rafiq *et al.,* 2016).

2.2 Empirical Studies

2.2.1 Assessment of phytochemical and antimicrobial properties of *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides*

Okechukwu *et al.* (2021) reported on phytochemical constituents of methanol extract of *Moringa oleifera* Lam. whole leaf by Gas Chromatography-Mass Spectrometry and Fourier transform infrared spectroscopy analysis. The study showed that the methanol extract of *Moringa oleifera* whole leaves possesses phytochemicals, such as alkaloid, terpenoids, saponins, phenols, and glycosides that possess high therapeutic value. GC-MS analysis of the extract reveals the identification of twenty compounds, in which two compounds were identified in each peak. N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and 2-Myristynoyl pantetheine (100%), 2-Myristynoyl pantetheine and

Deoxyspergualin (92.05%), 5- Octadecenaland 9-Hexadecenoic acid (27.94%). N,N'-Pentamethylenebis[s-3-aminopropylthiosulfuric acid and Pentetic Acid are the major phytoconstituents. Most of the compounds in the list are bioactive and possess medicinal properties.

Unegbu *et al.* (2020) reported on phytochemical and antibacterial properties of *Moringa oleifera* leaf extracts on *Escherichia coli* and *Staphylococcus aureus*. Phytochemical analysis showed that terpenoids, phenols, flavonoids, glycosides, tannin, saponin, alkaloids, steroids and anthraquinolones were present in varying concentrations of the different extracts. The antibacterial activities of the ethanol and aqueous extracts of *M. oleifera* leaf at concentrations of 200, 100, 50, 25,12.5 mg/ml showed the zone diameter of inhibition for *S. aureus* in response to the different extracts ranged between 9mm to 20mm while that of *E. coli* was between 7mm to 19 mm. The MIC of aqueous and ethanol leaf extract on *S. aureus* is 25 mg/ml and 12.5 mg/ml respectively while the MIC of aqueous and ethanol leaf extract on *E. coli* is 12.5 mg/ml and 6.25 mg/ml respectively. The MBC of aqueous and ethanol leaf extract on *S. aureus* is 25 mg/ml and 12.5 mg/ml respectively while the MBC of aqueous and ethanol leaf extract on *E. coli* is 50 mg/ml and 25 mg/ml respectively. It was concluded that some secondary metabolites present in *Moringa oleifera* leaf may be responsible for the inhibition of the bacteria observed in this study; and the ethanol extract of *M. oleifera* leaf possesses more antimicrobial activity (10 – 20mm) in a concentration dependent manner than the aqueous extract (9 – 15 mm). This could justify its use as an antimicrobial agent. Therefore, *M. oleifera* leaf could be a promising natural antimicrobial agent with potential applications in pharmaceutical industries for controlling the pathogenic bacteria used in this work.

Elzein *et al.* (2018) reported on qualitative and quantitative phytochemical analysis of *Moringa oleifera* (Lam) pods. Five important phyto-constituents were evaluated using aqueous, ethanol, methanol and chloroform extracts. Phytochemical constituents such as alkaloids, flavonoids, saponins, sterols and tannins were detected in the pod extracts.

Etejere *et al*. (2015) reported on comparative studies of phytochemical constituents of leaf, bark and root of *Moringa oleifera* Lam. The results showed that aqueous extract of the plant parts tested positive for tannins, phlobatannins, saponins, flavonoids, terpenoids and alkaloids except cardiac glycosides which were present only in the root. Significant differences ($p \leq 0.05$) in phytochemical constituents were observed between the plant parts and they were found to be significantly higher in the leaf compared to the other parts. Among the phytochemical constituents investigated, saponin was found to be highest with mean value of 20.81 ± 2.38 mg/100g, followed in decreasing order by flavonoids, alkaloids, phenols and tannins with respective mean values of 4.00 ± 0.75 mg/100g, 2.00 ± 0.60 mg/100g, 1.73 ± 0.60 mg/100g and 1.03 ± 0.14 mg/100g. The present study therefore conclusively points out that *M. oleifera* is a good source of various pharmacologically active substances most especially saponin and cardiac glycoside, in which the latter is a drug of choice for the treatment of congestive heart failure.

Idowu and Oseni (2015) reported on compositional investigation of phytochemical and antioxidant properties of various parts of *Moringa oleifera* plant. The results of phytochemical screening revealed that saponin, terpenoid, steroid and flavonoid were present in all the parts of the plant. Phlobatamin was observed to be absent in all parts of the plant. Alkaloid and tannin were observed in the *Moringa* root as tannin was also observed in the leaf and cardiac glycosides were present in the seed and leaf of the plant. The antioxidant properties showed that total flavonoid, total phenol, iron reducing

antioxidant property (FRAP), vitamin C and the free radical scavenging ability against 1,1-diphenyl-2 picrylhydrazyl (DPPH) were evidenced in all the parts of the *Moringa* plant. The results however showed that the various parts of the plant contained varying amounts of phytochemicals and antioxidant properties of medicinal importance.

Babatunde (2017) reported on GC-MS analysis of leaf, stem-bark and root extracts of *Alstonia boonei.* Leaf, stem-bark and root extracts of *A. boonei* were prepared by maceration using 1:1 EtOAc/MeOH. The crude extract was successively macerated with hexane, dichloromethane (DCM) and methanol. Thin layer chromatography (TLC) by 2,2-diphenyl-1-picrylhydrazyl (DPPH) (TLC-DPPH) analysis was used to screening out DCM fraction for further analysis. Gas chromatography and mass spectroscopy studies were performed to profile phytochemical constituent of the plant. The GC-MS analysis of DCM extract of the leaf revealed ten chemical components with Eugenol as major component (54.58%); DCM extract of the stem-bark showed forty one components with alpha-amyrin (32.25%) while DCM extract of the root revealed twenty components with 1,2-benzenedicarboxylic acid (49.2%) as major component. This study shows that the *A. boonei* extracts of the leaf, stem-bark and root consist of different types of compounds with few components common to two of the parts. Quantitatively, common phytochemicals decrease from leave to root. The most prominent compounds identified by GC/MS were Eugenol, benzenedicarboxylic acid and alpha-amyrin.

Arogbodo (2019) reported on phytochemical screening and antimicrobial effect of ethanolic leaf extract of *Alstonia boonei* De Wild (Apocynaceae) on some selected pathogenic microorganisms. Preliminary phytochemical screening of the extract of Alstoniaboonei indicated the presence of tannins, cardiac glycosides, flavonoids, saponnins, steroids and terpenoids while phlobatannins and anthraquinones were absent.

The extract produced significant inhibitory effect on *S. aureus* and *P. aeruginosa* with inhibition zones of 19mm, 16mm, Minimum Inhibitory Concentration (MIC) of 6.25mg/ml and 12.50mg/ml respectively. *E. coli, S. typhi* and *P. mirabilis* were not sensitive to the extract. It was concluded that *Staphylococcus aureus* and *Pseudomonas* aeruginosa were highly sensitive to ethanolic leaf extract of *Alstonia boonei*.

Opoku and Akoto (2015) reported on antimicrobial and phytochemical properties of *Alstonia boonei* extracts. The results of the phytochemical studies revealed the presence alkaloids, cyanogenetic glycosides, flavonoids, terpenoids and steroids and saponins. Susceptibility testing by disc diffusion assay revealed significant antimicrobial activity of methanol and aqueous extracts of the roots against the pathogens tested. The minimum inhibitory concentrations (MIC) of the various extracts by agar dilution method ranged from 3.0 to 10.0 mg/ml. The ethanol extracts exhibited better antimicrobial activity than aqueous extract. The study findings provide supportive evidence for the use of *Alstoniaboonei* in traditional medicines.

Umeh *et al.* (2014) reported on preliminary study of the antibacterial and analgesic effect of the leaf extract of *Pterocarpus santalinoides* L'Hér. Ex DC. Phytochemical analysis of the plant extract revealed that the crude extract contains alkaloids, saponins, tannins, cardiac and cyanogenic glycosides, flavonoids, terpenoids, carbohydrates and protein. Trace elements tested using the Atomic absorption spectrophotometer showed the presence of iron, potassium, phosphorous, magnesium, manganese and calcium. Antibacterial screening of the crude extract showed inhibitory activity against some gram positive and gram negative bacteria (*Staphylococcus aureus*, *Escherichia coli, Salmonella* sp, *Enterobacter* sp and *Bacillus* sp). Analgesic activity of the crude ethanolic extract on

albino mice, using tail-flick method and hot-plate (20 – 40 ^{o}C) method showed that the plant leaves extract exhibit analgesic effects.

Ajah *et al.* (2015) reported on GC-MS analysis and anti-bacterial property of *Ptrocarpus santalinoides* leaf from Abakaliki, Ebonyi State, Nigeria.The result of GC-MS analysis revealed the presence of eight chemical constituents which includes: octa-1-ene (4.57%), nona-1,3-diene, hexadecanoic acid (22.87%), octadeca-9,11- enoic acid (3.66%), octadecanoic acid (14.02%), octadeca-10,12-enoic acid acid (18.90%), octadeca-11,13-enoic acid (6.40%) and octadeca-9-enoic acid (27.43%). The result of antibacterial activity of ethanol extract of *Pterocarpus santalinoides* leaf showed zone of inhibition on *Streptococcus pneumonia, klebsiella pneumonia, Escherichia coli* and *Staphylococcus aureus*. This result indicates the use of *Pterocarpus santalinoides* in ethno-medicine for the management of different ailment.

Njokuocha and Ewenike (2020) reported on antibacterial and phytochemical properties of crude leaf extracts of *Moringa oleifera* Lam., *Pterocarpus santalinoides*L'Herit DC and *Ceiba pentandra* L. on some clinical bacterial isolates in Nigeria. Alkaloids, steroidal aglycones, glycosides, proteins, carbohydrates, reducing sugars, tannins, saponins, vitamins A and E were present in all the plant samples. Flavonoids and cardiac glycosides were not detected in *Pterocarpus santalinoides* and *Ceiba pentandra,* respectively. Anthracene glycoside was absent in all samples. Aqueous and ethanolic extracts of *M. oleifera* showed antibacterial activities against all the bacterial isolates at minimum inhibitory concentration (MIC) of 3.125 mg/ml and 1.5625 mg/ml respectively. *Pterocarpus santalinoides* showed inhibitory activity only on *Salmonella typhi* at 3.125 mg/ml and *Escherichia coli* 1.5625 mg/ml MIC. *Ceiba pentandra* showed spectrum of antibacterial activity against all the bacterial isolates at 1.56 mg/ml MIC with exception of

Salmonella typhi. *E. coli* was the most susceptible to the leaf extracts. *Salmonella typhi* was not sensitive to the leaf extracts of *Ceiba pentandra,* while *Staphylococcus aureus* and *Pseudomonas aeruginosa* were not sensitive to the leaf extracts of *Pterocarpus santalinoides.* It can be concluded that both aqueous and ethanolic leaf extracts had antibacterial activity against the test organism, thus justifying their use in folklore medicine.

Nwokorie *et al.* (2015) reported on the phytochemical and antimicrobial analysis of *Pterocarpus santalinoides*plants. The results show that, the plant part is rich in bioactive substances such as alkaloid (2.64±0.01), flavonoid (2.0±0.00), tannin (1.52±0.01), saponnin (2.5±0.02), terpens (2.6±0.01), cardiac glycoside (2.5±0.00) and Steroid (1.82±0.01). The antimicrobial analysis shows that ethanol extract of the leaves had more inhibition against *S. aureus, E. coli, B. subtilis, K. pneumonia* and *P. aeruginosa*, than the methanol extract of the stem bark. The antifungal activities of the leaves and the stem bark showed a higher inhibition on *A. niger, A. flavus, T. rubrum* and *M. gypseum* on 40mg/ml concentration of the extract. The minimum inhibitory concentration (MIC) evaluated on the ethanol and methanol extract using a two-fold serial dilution showed little zone of inhibition ranging from (1-3 mm) in diameter. These results indicate that the plant leaves and the stem bark have potential medicinal uses, and could protect the body against some common microorganisms.

2.2.2 Assessment of the preservative properties of medicinal plants in the shelf-life extension of probiotic drinks and foods

Orishagbemi *et al*. (2020) reported on organic preservation and shelf–life evaluation of liquid kunu-zaki food drink, with extract of West African black pepper (*Piper guineense*).

The extract was applied at four (4) concentration levels (5.0, 10.0, 15.0 and 20.0g extract/L kunun) making four experimental samples and one control properly packaged in plastic bottle (all coded). Results showed that samples containing 5.0, 10.0 and 20.0g extract/L could maintain physical properties for less than 3 weeks, before deterioration of sensory properties set in.

Ekeke *et al.* (2015) reported on studies on optimal conditions for the preservation of zobo drink. Zobo drink was prepared from the calyx of *Hibiscus sabdariffa* using two types of spices - *Zingiber officinale* (ginger) and *Caryophillus oromatics* (cloves) were prepared and added in the following proportions 150g: 20g; 150g:10g; 100g:20g; and 100g:10g respectively, to 400 ml of zobo drink. Samples of the drink were pasteurized at two different temperatures 167 ^{0}C for 5min and 72 ^{0}C for 6 minutes. All the samples were stored at ambient temperature (30 ± 2 ^{0} C) for 9 days. Microbial analysis was performed on the samples every 2 days. The various combinations of the spices alone could not preserve the drink beyond 2 days, but, when coupled with pasteurization, the shelf life was extended. A combination of ginger (150g) and cloves (20g) was found to be more effective, and when in conjunction with pasteurization, kept the drink in sterile condition for 3 days, and extended the shelf-life to 5 days.

Ani (2021) reported on production, sensory and physicochemical evaluation of zobo and zobo-date wine from *Hibiscus sabdariffa* flower, pineapple, orange and lime juice using *Saccharomyces cerevisae.* Both zobo wine and zobo-date wine produced significantly different ($p < 0.05$) physicochemical properties with pH 2.747 and 3.333, titratable Acidity; 0.4143 and 0.1220%, brix content; 6.1667 and 2.11%, vitamin C; 12 mg/100ml and 20 mg/100ml, total dissolvable solids 6.52 Mg/L and 3.53 Mg/L, alcohol content; 9.18% and 6.56% respectively. The Zobo-Date wine however, tend to reduce microbial

growth when compared to the zobo wine as shown in the microbial count of both wines (9.703 $\times 10^6$ and 1.2967 $\times 10^7$ cfu/ml respectively). The sensory evaluation showed zobo-Date wine to compete favorably with zobo wine in terms of sensory attributes evaluated and overall acceptability showed no significant difference ($p > 0.05$).

Ajayi *et al*. (2015) reported on quality evaluation of zobo (*Hibiscus sabdariffa*) juice preserved with *Moringa* (*Moringa oleifera)* or ginger *(Zingiber officinale)* extracts at different storage conditions. Zobo juice infused with extracts of *Moringa* seeds or ginger at (0.5 and 1%), control (0% preservative) and, food vendor prepared (FVPZ) zobo were evaluated for 8 weeks. Samples were stored at ambient or refrigeration temperatures and physico-chemical, microbial load and sensory qualities of the juice were analyzed using standard methods. There were drops in pH values after pasteurization from (2.44- 2.75) to (2.31 - 2.58). Vitamin C increased with storage in preserved juice but reduced in control and FVPZ. There were significant ($p<0.05$) differences between samples in total titratable acidity. All of the samples had varying levels of microbial load. Microbial load of raw material ranged from (7.8×10^4 to TNTC), (3.0×10^3 to 3.0×10^4) and (5.6×10^4 to 8.0×10^4) CFU/g, while zobo juice on day 0 had counts ranging from (2.23×10^3 to TNTC); (3.0×10^2 to 8.0×10^4); (4.0×10^4 to 5.6×10^4), CFU/mL for total viable, staphylococcal and fungal count respectively. There was zero enterobacteriaceae count on day 0 but increased during storage. Moringa and ginger zobo juice overall had reduced microbial load during storage compared to zobo without preservative. On day 0 show that FVPZ was more liked: appearance (4.5), aroma (4.5), taste (4.4) and general acceptability (4.7) but scores degenerated during storage. At 8 weeks of storage, 0.5% GZ scored higher in all attributes. Refrigeration retarded microbial growth but did not influence sensory scores.

Ezekiel *et al.* (2016) reported on nutritional, sensory and bacteriological quality of two varieties of locally prepared zobo (*Hibiscus sabdariffa*) drink; dark red zobo (DRZ) and bright red zobo (BRZ). An average total heterotrophic bacterial counts of 1.87E+06 and 1.49E+05 cfu/g were obtained for dark red and bright red samples with total coliform counts of 1.63E+04 cfu/ml (DRZ) and 1.56E+03 cfu/ml (BRZ). Their results showed that both zobo drink varieties met the International Commission on the Microbiological Specifications for Foods (ICMSF) limit of $1x10^7$cfu/ml set for total aerobic plate counts and so, should be consumed to boostlocal production and a healthy lifestyle.

Ouattara *et al*. (2017) reported on the preservative effects of different treatments and their flavor acceptability in cashew apple and pineapple blend juices. The microbiological analysis showed lower microbial counts of the treated samples compared to the control. The sensory analysis revealed a general acceptability for all the samples formulated. This acceptability value was higher for the sample supplemented with 10% aqueous extract of ginger. They concluded that the 10% aqueous ginger extract could help extend the shelf life of fruit juice drinks.

Edward and Ohaegbu (2014) reported on the effect of ginger and garlic on the microbial load and shelf life of Kunun-zaki. Kunun-zaki was produced using ginger and garlic and stored under ambient conditions for 10 days. Their result showed the potential of the combination treatment of ginger and garlic as antimicrobials and in extending the shelf-life of Kunun-zaki.

Kahramanoglu and Usanmaz (2017) in their study have evaluated the effects of black seed oil on the shelf-life of freshly squeezed pomegranate juice. According to the results obtained, black seed oil has delaying effect on the development of yeast and mould up to

37 days. Moreover, it was also found that combination of treatments with freezing increases the efficiency of tested natural, as well as the shelf-life.

Ashwini and Desai (2018) conducted an investigation on effect of extracts/bulb extracts garlic (5 and 10%) on shelf-life of mango fruits. The results showed that the physiological loss of weight in mango fruits showed an increasing trend in all the treatments. Contrary to this, acidity decreased with storage and there was an increase in pH with extension of storage period. The total soluble solids of fruits first increased up to a certain period and there after decreased. Bulb extracts of garlic at 10 percent were most effective of all the treatments with minimum spoilage percentage followed by garlic extracts at 5 percent concentration. The investigation concluded that the shelf-life of mango fruits can be extended from 3 to 7 days by treating with 10 percent garlic extract (Ashwini & Desai, 2018).

Tyagi *et al.* (2014) reported on chemical composition and anti yeast activity and fruit juice preservation potential of lemon grass. The anti-yeast activity of lemon grass oil was evaluated against several food spoiling yeasts (*Saccharomyces cerevisiae, Zygosaccharomyces bailii, Aureobasidium pullulans, Candida diversa, Pichia fermentans, Pichia kluyveri, Pichia anomala* and *Hansenula polymorpha*) through disc diffusion and microbroth dilution method. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) varied from 0.28 to 1.3 mg/ml and 0.56 to 4.5 mg/ml, respectively, where highest MIC (2.25 mg/ml) was shown by *A. pullulans* and lowest MIC (0.28 mg/ml) was shown by *C. diversa* and *P. anomala*. Kill time assay was conducted for selected three yeast strains where *S. cerevisiae* showed highest reduction (3 log cfu) in viability within 24 h exposure of lemon grass oil at MFC level.

Tavakoli *et al.* (2017) revealed that the Redshank extract and Redshank essential oil combined with ice seem to be a promising solution for extending fish shelf-life. Fish preserved with Redshank extract and oil were examined for chemical properties such as; (peroxide value (PV), thiobarbituric acids (TBA), total volatile nitrogen base (TVB-N), and free fatty acids (FFA)), microbiological (total viable count (TVC) and psychrotrophic viable count (PVC)) and sensory evaluations (texture, colour, flavour, and general acceptance) every 4 days during a 20-day storage period. Results revealed that the effect of both icing systems led to considerably lower bacterial counts and chemical indices in comparison with the traditional ice coverage without such phytogenic. According to sensory analyses, fish stored in ice containing Redshank essential oil had a longer shelf-life (>16 days) and those stored in ice medium included with Redshank extract possessed a shelf-life of 16 days, whereas lot stored in traditional ice showed a shorter shelf-life of 12 days.

Hayam *et al.* (2014) in their study on effect of adding lemongrass leaf extracts on chicken patties quality reported that lemon grass inhibited the growth of *Bacillus cereus, S. typhimurium* and *S. aureus* antibacterial agents in refrigerated chicken patties.

Adesokan *et al.* (2014) reported on the influence of ginger on fermentation, acceptability and shelf-life of ogi (maize pap). During storage, there was a slight decrease in pH of the samples which ranged between 3.27 and 3.65 while TA ranged between 0.009 and 0.12%. The study revealed that incorporation of 5% ginger into ogi significantly improved its sensory attributes, led to a relatively reduced microbial load during storage for 8 days and hence an improvement in the shelf stability of the product.

Zemenu (2014) reported on effect of ginger (*Zingiber officinale*) powder addition on pH, titratable acidity and total viable bacterial counts of minced meat under refrigerated storage. The overall results of the current study revealed that ginger powder addition has positive impacts on the shelf-life and keeping quality of meat samples.

Adedeji and Ade-Omewaye (2014) reported on the effects of *Aframomumdanielli* and *Zingiber officinale* crude extract on the storability of fried bean cake snacks. The fried bean cakes were spiced with 0.2, 0.4, 0.6, 0.8 and 1% of both spices, the untreated sample was also prepared. Sensory evaluation showed that there was no significant difference ($p<0.5$) among the treated and untreated samples in terms of all the sensory attributes evaluated. The fried bean cake snacks treated with 0.2% and 0.4% of both spices were more acceptable generally and stable than the ones treated with 0.6 and 0.8% of both spices. The fried bean cake snacks treated with 1% of both spices were unacceptable in terms of all the sensory attributes evaluated.

Salem *et al*. (2015) reported on the chemical, microbiological and organoleptic properties of sour cream dairy storage 4 week. The results indicated that addition of MOLE and MOO at all levels has remarkable effect on TS, fat, total protein and lactose. The acidity content increased with increasing the level of MOLE while it decreased with increasing level of MOO. Acidity increased gradually in all treatments during storage period. Sour cream fortified with MOO exhibited lowest PV than sour cream fortified with MOLE. The results indicated that TC decreased with advanced storage period in all treatments of sour cream fortified with MOLE and MOO than control.

Rahman *et al*. (2020) reported on effect of *Moringa oleifera* leaf extract and synthetic antioxidant on quality and shelf-life of goat meat nuggets at frozen storage. Goat meat

nuggets were formulated having five treatment groups namely control, 0.1% BHA, 0.1%, 0.2% and 0.3% *M. oleifera* leaf extract, respectively. pH and cooking loss of ready to eat nuggets decreased significantly ($p<0.05$) in comparison to control and BHA with prolonged storage period. *M. oleifera* leaf extract treated nugget's quality remained stable with minor changed in sensory, physicochemical and microbiological quality during frozen storage (-18±1°C) for 45 days.

Mubarak *et al.* (2018) reported on preservative characteristics of dehydrated Murunga (*Moringa oleifera*) leaf powder. There were significant differences ($P<0.05$) in the total plate counts in all cooked food samples with *Moringa* leaf powder when compared to the control. After 16 hours, colour, flavour, odor and overall acceptability of *Moringa* leaf powder treated food samples were significantly different ($P<0.05$) compared to the control samples. The study revealed that the dehydrated *Moringa* leaf powder could be potentially used to extent the shelf life of cooked food such as rice and curry.

Ansari *et al*. (2020) reported on the shelf-life assessment of *Moringa oleifera* fortified (leaf powder) instant soup mixes. Different combinations like 0%, 18%, 20%, 22% and 24% of *Moringa* leaves powdered and were used for formulations respectively. Prepared *Moringa* leaves powder were mix with other spices to prepare instant soup powder. Proximate study of selected sample were found high in protein 13.67%, ash 9.79%, fibre 5.99% and low in fat 3.04% and energy value which make the developed soup as an appropriate choice to fulfill nutritional demand of consumers. Instant soup mixes packed in aluminum foil was found to be successfully stored for 90 days at room temperature without any major changes, in physicochemical, microbial and organoleptic parameters.

According to Anyanwu and Nwosu (2014), *P. guineense* by its nature is aromatic and carminative and that it is natural antioxidant, act as anti-inflammatory, anti-cancer and anti-pyretic agents. The seeds and leaves of *Piper guineense*is consumed in Southern Nigeria and some parts of West Africa because of their spicy aroma. It is known to contain oils and its uses in traditional medicinal practices in Africa and beyond are well documented.

Ugwuona (2014) reported on effect of piper extracts on pork stability. Fresh and cooked ground pork patties were evaluated at inclusion levels of 0.25 and 0.50% of meat weight. Patties were stored for 16 weeks at room temperature (27±1C). Antioxidant effects of extracts increased with increased use levels from 0.25 to 0.50% of meat weight. The essential oil had better antioxidant effect than the oleoresin at both use levels. The TBA and PV decreased as the extracts increased in concentration. However, both were more effective antioxidants in cooked pork than in fresh pork.

Klangpetch *et al*. (2016) reported that ethanol and ethyl acetate extraction of three type of citrus peel (kaffir lime, lime and pomelo) were studied in stored raw chicken drumettes at 4ºC as antimicrobial and antioxidants for extension their shelf-life. They were found to be more effective against pathogenic bacteria at level ranged from 0.4-50 mg/ml. Also, they found that the treated chicken wing samples with ethyl acetate extracted from kaffir lime peel were lower than those of control sample in their values of total viable counts, 2-Thiobarbituric Acid Reactive Substances (TBARS) and increased the sensory properties during the period of storage (14 days).

Ben-Hsouna *et al.* (2017) investigated the effect of *Citrus limon* essential oil (CLEO) in situ against *L. monocytogenes* in minced beef meat model. They found that the CLEO was

more effective against gram positive and negative bacteria at concentration varied from 0.039 to 1.25 mg/ml and 0.25 to 2.5 mg/ml respectively. Also, they assessed the CLEO as preservative substances in minced beef meet during storage at 4ºC. The results showed that added 0.06 and 0.312 mg/g from CLEO prohibited the contamination in pathogenic bacteria, particularly *L. monocytogenes*.

Alfonzo *et al*. (2017) studied the effect of essential oil that extracted from lemon on the quality of salted sardines. They found that the concentrations of all microbial group under study were decreased during the entire period of monitoring.

Kavas and Kavas (2016) revealed that adding the orange essential oil (EO) to egg white protein powder as based film reduced the growth of microorganism in kashar cheese. Thereby, extend the storage period than the film without EO. In another study conducted by Espina *et al*. (2014), to investigated effect the combined treatment of thermal treatment and citrus fruit essential oil in apple juice to inactivate of *Escherichia coli* O157:H7. They observed that the adding 200 µL of lemon EO combined with temperature of 54-60 ºC in the inactivation of *E. coli* O157:H7, might suppose a reduction in the treatment time and temperature (5.7 min and 4.5 ºC) respectively, stated that the using of citrus extract and sodium-benzoate reduced the presence of spores (*Fusarium oxysporum*) in pineapple juice and improved the effect of homogenization in the same beverage.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

The study adopted an experimental study design. Experimental study design is the process of conducting research in an objective and controlled environment so that precision is maximized and specific conclusions can be drawn regarding a hypothesis statement (Jack, 2014).

3.2 Area of Study

This study was conducted in Owerri, Imo State, Nigeria. Historically, the indigenes of Owerri trace their ancestry to a man called Ekwem Arugo. With British influence and colonization in the early 1900s Owerri town was the headquarters for Owerri Division and later old Owerri Province. Also, when Imo State was created on the 3rd of February 1976, Owerri city was chosen as its capital. On the 15th of December 1996 Owerri city attained municipal status.

3.3 Instruments for Data Collection

The instruments used to collect data for this study were samples, glasswares and laboratory equipment. The samples included; calyces leaves, tigernut, three (3) medicinal plants (*Moringa oleifera, Alstonia bonnie* and *Pterocarpus santalinoides*) leaves. The laboratory equipment included; autoclave, microscope, weighing balance, incubator, thermometer, pH meter and electric blender. Glasswares included; test tubes, measuring cylinders, beakers, etc.

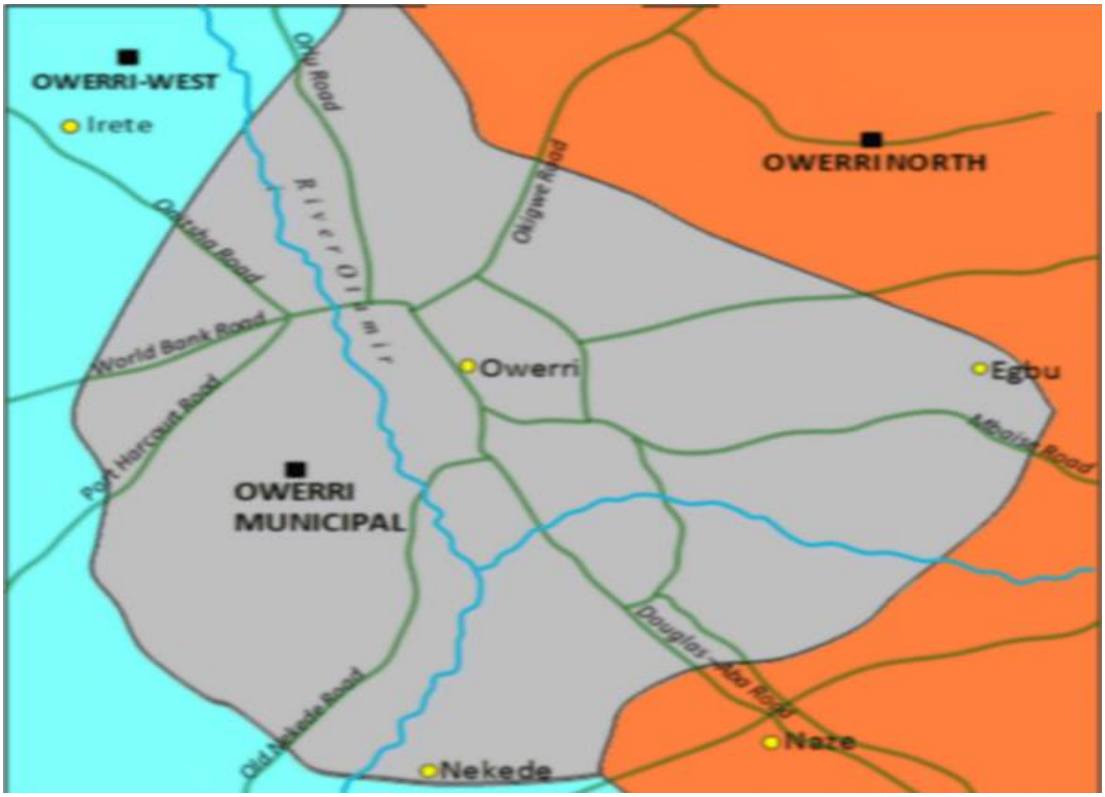


Figure 1: Map of Imo State showing local governments in Owerri

Source: Imo State Government (2010)

3.4 Validity of Instruments

The instruments used in the study were validated by adopting existing methods described by Ezearigo *et al.* (2014) for the production of probiotic drinks, Ushie *et al.* (2014) for the determination of phytochemical constituents of the medicinal plants and Salem *et al.* (2015) for the determination of the preservative efficacy of some medicinal plants. pH meter was calibrated using pH buffer solution of pH 4.00 (for acidity) and buffer solution of pH 6.86 (for alkaline). The different concentrations of the chemicals used were prepared following standardized procedures described by Ochei and Kolhatkar (2010).

3.5 Method of Data Collection

3.5.1 Source of samples and sample collection

Mature dried reddish-purple petals of *H. sabdariffa* and tigernut tubers used for this study were obtained from Relief Market, Egbu Road, Owerri, Imo State, Nigeria. The samples were transported to the laboratory for production and necessary analysis. Plants used as preservative such as; *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides* were identified and authenticated by a Plant Botanist and collected from the trees at Aboh Mbaise L.G.A, Imo State.

3.5.2 Sterilization of glassware and media

The media used in this study included nutrient agar, eosin methylene blue agar, sabouraud dextrose agar, peptone water, triple sugar iron agar, Simmon's citrate agar, Mannitol salt agar and Mueller-Hinton agar. Nutrients serve as general-purpose media for the isolation of different types of aerobic bacteria. Eosin methylene blue agar served to isolate coliform bacteria, while sabouraud dextrose agar was used to isolate fungi (yeasts and moulds). Triple sugar iron agar was used to identify sugar fermentation test, acid production, gas production and hydrogen sulphide production. Peptone water was used together with Kovac's reagent for the identification of indole-producing bacteria, while Simmon's citrate agar was in the identification of citrate-utilizing bacteria. Mannitol salt agar was used for the isolation of *Staphylococcus* species. All the glassware used in this study were sterilized using laboratory hot air oven at temperature of 160 ^{0}C for 1 hour and media used in this study was sterilized using the autoclave at a temperature of 121 ^{0}C at 15psi for 15 minutes. After the sterilization, the media were brought out together with the glasswares and kept on a clean laboratory bench. The media were poured into the petridishes when cooled to 45 ^{O}C and allowed to solidify (Cheesbrough, 2010).

3.5.3 Processing of the mature dried reddish-purple petals of *H. sabdariffa* into zobo

The method described by Ezearigo *et al.* (2014) was adopted to process the mature dried reddish-purple petals of *H. sabdariffa* into zobo. The mature dried reddish-purple petals of *H. sabdariffa* were poured in a sterile clean tray and were sorted. Thereafter, 400 g of the matured and dried petals of *H. sabdarifa* Linn was weighed into a clean sterile five (5) litres stainless pot with four (4) litres of water and boiled over the stove for 15 minutes. This was allowed to cool and thereafter filtered. The filtrate was collected in pre-sterilized wide-mouth glass bottles and dispensed into fifty (50 cl) centilitre reinforced plastic bottles (Figure 2).

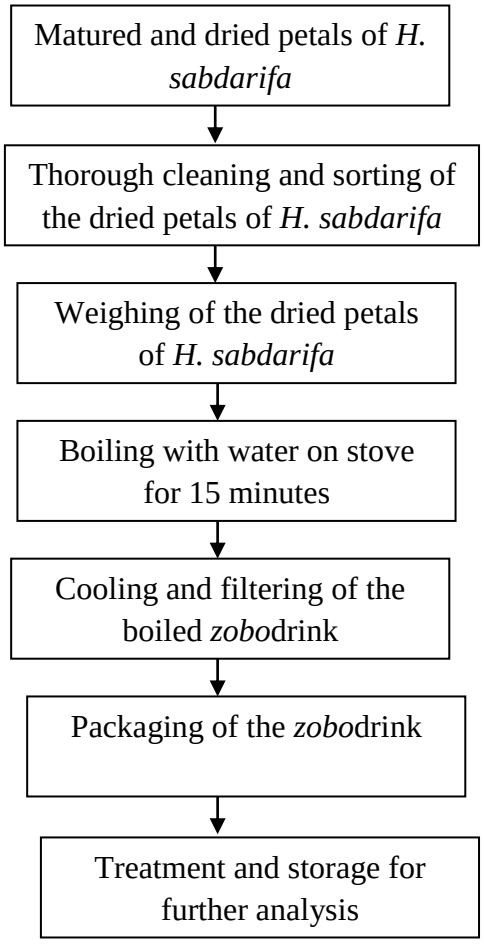


Figure 2: Flow chart for the production of zobo drinks
Source: Ezearigo *et al.* (2014)

3.5.4 Production of tigernut milk

The tigernut milk production method described by Udeozor and Awonorin (2014) was adopted. The tiger nut tubers were sorted, washed and dried. The electric blender was soaked in sterile hot water and allowed to stay for 15 minutes. Thereafter, the blender was washed and rinsed in sterile water to make it sterile. The tiger nut milk was produced by blending one kilogram (1kg) of fresh tiger nut seeds into a slurry with water in a sterile blender. The slurry was pressed using a muslin cloth to extract the milk and pasteurized by boiling in water bath at 70 ^{0}C for 15 minutes. The milk was homogenized, packaged and kept in the refrigerator at 4 ^{0}C for further studies. The flow chart for the tiger nut milk production is shown in Figure 3. The tigernut milk was pasteurized in a thermostatically maintained water bath at 75 °C for 15 minutes, 80 °C for 10 minutes and 85 °C for 5 minutes.

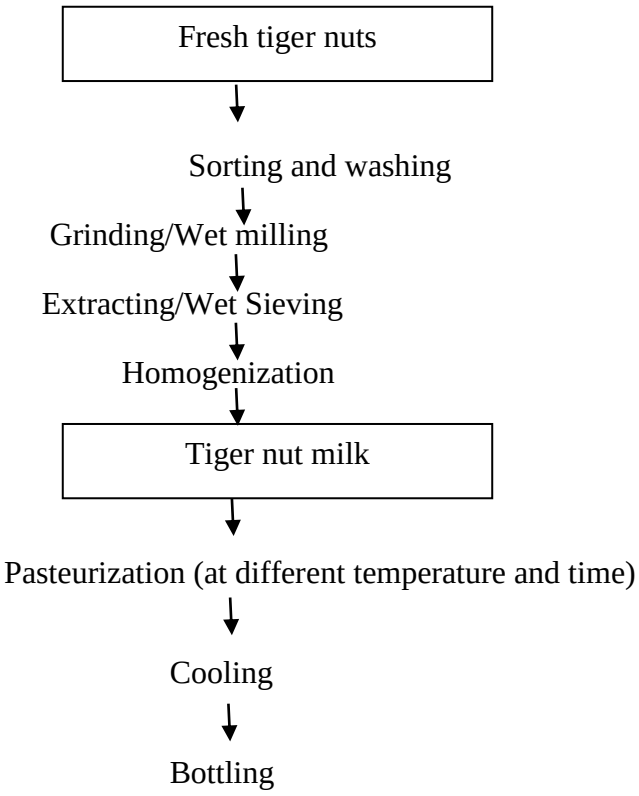


Figure 3: Flow chat for tiger nut milk production

Source: Udeozor and Awonorin (2014)

3.5.5 Extraction of aqueous extracts from the leaves of *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides*

Moringa oleifera, Alstonia boonei and *Pterocarpus santalinoides* leaves were washed and dried at room temperature. Each leaf was aseptically ground, and 100 grams each were extracted using 100 ml by boiling in sterile beakers and allowed for 2 hours with shaking at different intervals. Thereafter, the mixtures were filtered using sterile muslin clothes. The filtrates were cultured on nutrient agar and sabouraud dextrose agar to ascertain their sterility.

3.5.6 Qualitative phytochemical screening

Phytochemicals are active ingredients present in plants that make them have antimicrobial activities. The method described by Ushie *et al.* (2014) was adopted to determine the qualitative phytochemical screening of the aqueous extracts of the leaf extracts. The phytochemicals include; saponins, anthranoids, anthraquinones, phenols, alkaloids, tannins, phlobatannins, and cardiac glycosides.

Test for saponins

Ten milliliters (10ml) of distilled water was added to two milliliters (2ml) of each of the extracts in a test tube and shaken vigorously. Persistent frothing, even after heating, indicated the presence of saponins, while none indicated the absence of saponins.

Test for anthranoids

Two milliliters (2ml) of each of the extracts, five milliliters (5ml) of 0.5M potassium hydroxide was added and mixed properly. Then 6 drops of acetic acid was added followed by 2ml of toluene. 2ml of 0.5M potassium hydroxide was added to the upper layer formed.

A change in colour of the mixture was an indication of a positive test, while no colour change was an indication of a negative test.

Test for anthraquinones

Two (2ml) of each of the extracts were added to 5ml of 10% ammonia and shaken vigorously. Subsequently, 2ml of benzene was added. A colour change indicated a positive test, while none indicated a negative test.

Test for phenols

Five (5ml) of each extract was mixed with 8ml of distilled water in a test tube, and 6ml of ferric chloride was added to the mixture. A colour change to light brown indicated a positive test, while none indicated a negative test.

Test for alkaloids

Two (2ml) of each of the extracts and 5ml of 1% aqueous hydrochloric acid were added and placed in a water bath for 3 minutes. After that, 3 drops of Mayer's reagent were added. A white precipitate indicated a positive test, while none indicated a negative test.

Test for tannins

One (1ml) of each of the extracts and 2ml of 1% ferric chloride were added. A colour change indicated a positive test, while none indicated a negative test.

Test for phylobatannins

Two (2ml) of each of the extracts, 1% aqueous hydrochloric acid was added and boiled. The presence of white precipitate was an indication of a positive test while none was an indication of a negative test.

Test for cardiac glycoside

One (1ml) of the extracts, 2ml of chloroform, and then 2ml of concentrated tetra-oxo-sulphate (VI) acid was added to form a lower layer. A reddish brown colour at the interphase indicated a positive test, while none indicated a negative test.

3.5.7 Preservative efficacy of the leaf extracts at different concentrations

The prepared zobo and tigernut milk drinks were divided into different sterile bottles in 100 ml volumes and were preserved with the *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides* using the following concentrations; 10.0 ml, 30.0 ml, 50.0 ml and 100 ml. A sample of the zobo and tigernut milk drink with no additive served as a control for treated samples. The samples were kept on the laboratory shelf at a temperature of 28 ^{0}C ±2 ^{0}C. Samples were drawn from each preserved sample every seven (7) days and cultured until the 2nd month (8 weeks).

3.5.8 Organoleptic/Sensory Evaluation

The sensory evaluation was conducted using blind sensory evaluation method where the panelists were given the drinks without their knowledge of the preservation of the drinks. A 10-member panel consisting of students who are regular zobo and tigernut milk drinkers was used to evaluate the drinks. The panel tested the drinks by sipping the drinks and then rinsing their mouth with water after testing each drink. It was ranked by the colour (appearance), flavour, taste and overall general acceptability on a modified 4-point Likert scale of 1-4 as represented as; 1= Like, 2= Like very much, 3= Dislike, 4= Dislike very much. Significant difference was indicated by “a” superscript while non-significant difference was indicated by “b” superscript. General acceptability was calculated by adding colour (appearance), flavor and taste of each of the samples.

3.5.9 Microbiological analysis of the drinks

The method described by Salem *et al.* (2015) was adopted to determine the microbiological integrity of the preserved drinks. Bottles containing the different preserved and untreated zobo and tigernut milk samples were aseptically opened, and one milliliter (1 ml) aliquot of each sample for zero (0 h) and ambient stored samples was transferred into 9 ml of 0.1% (w/v). Sterile water was used as a diluent. Ten-fold (10-fold) serial dilution was carried out. Appropriate dilution (10^{-2}) was selected, and 0.1ml from the tube was aseptically plated using the pour plate technique for total viable aerobic bacteria count on nutrient agar (Biotech), eosin methylene blue agar and total fungi count on sabouraud dextrose agar (Biotech) supplemented with chloramphenicol (antibiotics). The procedure was repeated with the preserved and untreated zobo and tigernut milk samples every seven (7) days. The inoculated media were incubated at 37 ^{0}C for 24 hours for nutrient agar and eosin methylene blue agar and at 28 ^{0}C for 72 hours.

3.5.10 Microbial plate count

The method described by Salem *et al.* (2015) was adopted to determine the microbial load of the samples. After incubating the different plates, the different colonies formed on the media were counted using the digital colony counter. The total populations of the colonies were expressed as colony forming unit per milliliters (cfu/ml).

3.5.11 Colonial morphology identification

The method described by Cheesbrough (2010) was adopted in the colony morphology identification. Presumptive identification of the colonies was done by observing their shape, colour, elevation, edge, surface, consistency and appearance on the media used for isolation. Colonies with characteristic metallic sheen on EMB agar and lactose fermenters

on MacConkey agar were noted. The colonies were preserved in sterile agar slants in test tubes. Purified colonies were further characterized using Gram stain and biochemical tests.

3.5.12 Identification of the bacteria isolates

Gram staining techniques

Gram staining technique was used to characterize bacteria according to their Gram's reaction. The method described by Cheesbrough (2010) was adopted in the determination of the gram reactions of the bacterial isolates. A smear of each bacterial isolate was madeon clean, grease-free glass slides. After that, the smears were allowed to dry and heat-fixed to denature proteins. The smears werestained with crystal violet stain for 60 seconds and rapidly washed off with water after that. The smears were stained with Lugol's iodine for 60 seconds and were washed off with water. The smears were decolorized with acetone alcohol and were washed off after 10 seconds. The smears were finally flooded with safranin for 2minutes and were washed- off with clean water. The back of the slides were then wiped and placed in a draining rack for the smear to dry before they were viewed with x40 and x100 (oil immersion objective) lenses. Gram positive bacteria gave purple coloration while gram negative bacteria gave pinkish coloration.

Citrate utilization test

This test determines the ability of a bacterium isolate to utilize citrate as a sole source of carbon and ammonia as a sole of hydrogen and nitrogen for metabolism. It is therefore a useful test in the identification of organisms in the Enterobacteriaceae and other genera. Simon's citrate agar medium was used. Tube slants of the medium was prepared and lightly inoculated by streaking the isolates on the surface. Inoculated slants were incubated

at 37 ^{0}C for 24 hours and citrate utilization indicated by alkaline production, which change the color of the medium from green to blue (Ochei & Kolhatkar, 2010).

Motility test

This test is used to identify motile bacteria with the help of flagellar. The semi-solid nutrient agar was used for this study. The media was prepared in slants, and the organisms were inoculated by stabbing technique. Zig-zag growth along the line of stabs indicated a positive result, while none indicated a negative result (Ochei & Kolhatkar, 2010).

Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase-producing bacteria, such as streptococci. Five (5 ml) of hydrogen peroxide was poured into a tube and a sterile glass rod was used to collect a colony of the pure culture from the agar slant tube. It was dipped into the tube containing the hydrogen peroxide. Active bubbling indicated positive catalase test while none indicated catalase negative test (Ochei & Kolhatkar, 2010).

Indole test

Indole production test is used to identify those bacteria that are capable of fermenting the amino acid tryptophan present in peptone water to give indole on addition of Kova'c reagent. The test organisms were suspended in sterile peptone (about 3 ml) preparation in sterile test tubes and incubated at 37 ^{0}C for 48 hours after which 0.5ml of Kovac's reagent was added and shaken gently. A red coloration in the surface layer within 10 minutes was an indication of a positive test while none was an indication of a negative test (Ochei & Kolhatkar, 2010).

Oxidase test

This test is used to identify those bacteria that are capable of producing the enzyme oxidase. A piece of filter paper was placed in a clean petri-dish and three drops of fleshly prepared oxidase reagent was added in each case of the test organism. With a sterile piece of stick, each colony of the test organism was removed and smeared on each oxidase reagent drop on the filter paper. The development of a blue-purple coloration was an indication of a positive test while none was an indication of a negative test (Ochei & Kolhatkar, 2010).

Coagulase test

This test is used to identify those bacteria that are capable of producing the enzyme coagulase. A drop of distilled water was placed on each end of a slide for each of the test organisms. Thereafter a colony of each of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was then-added to one of the suspension and mixed gently for each of the test organism. Clumping within 10 seconds was an indication of positive test while none was an indication of a negative test (Ochei & Kolhatkar, 2010).

Sugar fermentation test

This test is used to identify those bacteria that are capable of fermenting the three sugars; mannose, sucrose and glucose. The fermentation of these sugars results in acid production, gas production and hydrogen sulphide. Each colony of the different test organisms was inoculated onto sterile agar slopes of triple sugar iron agar using stab inoculation. After this, the inoculated, agar slopes were incubated at 37 °C for 24 hours. The different colors of the slopes and butts in addition to the presence of gas production and hydrogen sulphide

(H_2S) blackening were indication of the type of bacteria present (Ochei & Kolhatkar, 2010).

3.5.13 Shelf-life properties of the probiotic drinks

The methods described by Association of Official Analytical Chemists (AOAC, 2015) and Babajide *et al.* (2013) were adopted in the determination of the shelf-life extension properties of the probiotic drinks.

Determination of total titratable acidity

The method described by Association of Official Analytical Chemists (AOAC, 2015) was adopted in the determination of total titratable acidity of the samples. Total titratable acidity is the total amount of acid content in the solution as determined by the titration using a standard solution of sodium hydroxide (titrant). To determine this, phenolphthalein was used as an indicator of the endpoint by titrating 100 ml (50 ml of the probiotic drinks plus 50 ml of distilled water) against 0.1 N NaOH.

pH determination

The method described by Association of Official Analytical Chemists (AOAC, 2015) was adopted in the determination of pH of the samples pH is a measure of the acidity/alkalinity of a substance. The pH of the probiotic drinks were determined using pH meter calibrated with standard buffer 7.0, 4.0 and 9.2. Fifty (50 ml) each of the probiotic drinks were dispensed into different sterile beakers. Thereafter, the probe of the pH meter was dipped into the beakers and the pH values were read and documented.

Determination of temperature

The method described by Association of Official Analytical Chemists (AOAC, 2015) was adopted in the determination of temperature of the samples. Five milliliters (5ml) each of samples were placed into different sterile beakers. The thermometer was dipped into the beaker containing the samples and was allowed for 5 minutes. Thereafter, the temperature of the samples was recorded.

3.6 Method of Data Analysis

All the collected data were analyzed using Statistical package for the social sciences version 2021. The raw scores were generated, organized and analyzed and the manual techniques were used to compute the data leading to the production of frequency count tables and cumulative percentages. Chi square test (χ^2) was used to test for significant association between the biopreservative efficacy of the medicinal plants and shelf-life extension of the probiotic drinks. Statistical tests were performed at 5% level of significant.

3.7 Ethical Considerations/Informed Consent

This research was approved by the FUTO School of Health Technology ethics committee. Written informed consent/verbal informed consent was obtained from the owners of the trees where the medicinal plant leaves were collected.

CHAPTER FOUR

RESULTS AND DISCUSSION

This chapter presents the results of the study on preservatives efficacy of some medicinal plants used in the shelf life extension of some probiotic drinks. The results include; phytochemical properties of the medicinal plants used, sensory/organoleptic properties of the biopreserved probiotic drinks, shelf-life extension properties of the probiotic drinks, microbial plate count, microorganisms associated with the biopreserved probiotic drinks.

4.1 RESULTS

4.1 Phytochemical constituents of medicinal plant leaf extracts

Table 1 shows the phytochemical constituents of medicinal plants. The presence of saponins, anthraquinones, phenols, alkaloids, tannins, phlobatannins and cardiac glycosides were detected in *Moringa oleifera*. Anthranoidswas not detected in the leaf extract of *Moringa oleifera.*

The presence of saponins, anthranoids, phenols, alkaloids and tannins were detected in *Moringa oleifera*. Phlobatannins, anthraquinones and cardiac glycosides were not detected in the leaf extract of *Alstonia boonei.*

The presence of saponins, phenols, alkaloids, tannins and cardiac glycosides were detected in *Pterocarpus santalinoides*. Anthranoids and anthraquinones were not detected in the leaf extract of *Pterocarpus santalinoides.*

Table 1: Phytochemical constituents of leaf extracts of the medicinal plants

Phytochemical parameters	**Medicinal plants/Results**		
	MO	**AB**	**PS**
Saponins	+	+	+
Anthranoids	-	-	-
Anthraquinones	+	+	+
Phenols	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Phlobatannins	+	+	+
Cardiac glycosides	+	+	+

Key: - = Absence of phytochemicals + = Presence of phytochemicals

MO = *Moringa oleifera* AB = *Alstonia boonei*

PS = *Pterocarpus santalinoides*

4.1.2 Organoleptic/sensory properties of the preserved probiotic drinks using *Moringa oleifera* leaf

Table 2 shows the organoleptic/sensory properties of the preserved probiotic drinks using *Moringa oleifera* leaf. There was no significant different in the organoleptic/sensory properties of the preserved probiotic zobo drinks from Week 1 to Week 6. However, there was a significant difference in the organoleptic/sensory properties of the preserved probiotic zobo drinks from Week 7 to Week 8.

There was no significant difference in the organoleptic/sensory properties of the tigernut drink preserved with *Moringa oleifera* leaf from week 1 to week 3, while there was a significant difference in the tigernut drink preserved with *Moringa oleifera* leaf from week 4 to week 8. There was also a significant difference in the concentrations of the preserved drinks.

Table 2: Organoleptic/sensory properties of the preserved probiotic drinks using *Moringa oleifera* leaf

Parameters	Probiotic drinks/Volume of medicinal plant extracts used							
	Zobo				Tigernut			
	10	30	50	100	10	30	50	100
Week 1								
Flavour	7.8[a]	8.0[a]	7.9[a]	8.1[a]	8.0[a]	7.6[a]	8.2[a]	8.3[a]
Colour	8.0[a]	8.1[a]	8.1[a]	8.2[a]	8.0[a]	8.0[a]	8.2[a]	8.2[a]
Taste	7.5[a]	7.5[a]	7.6[a]	7.8[a]	7.3[a]	7.6[a]	7.8[a]	7.8[a]
GA	7.8[a]	8.0[a]	8.0[a]	8.0[a]	8.2[a]	8.3[a]	8.3[a]	8.3[a]
Week 2								
Flavour	7.7[a]	7.8[a]	7.8[a]	8.0[a]	7.6[a]	7.7[a]	7.8[a]	7.8[a]
Colour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.7[a]	7.7[a]	7.8[a]	7.8[a]
Taste	7.8[a]	7.8[a]	7.8[a]	7.9[a]	7.5[a]	7.5[a]	7.6[a]	7.6[a]
GA	7.7[a]	7.8[a]	7.8[a]	7.8[a]	7.6[a]	7.6[a]	7.8[a]	7.8[a]
Week 3								
Flavour	7.8[a]	7.8[a]	7.8[a]	7.9[a]	7.7[a]	7.7[a]	7.8[a]	7.8[a]
Colour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.7[a]	7.7[a]	7.8[a]	7.8[a]
Taste	7.9[a]	7.9[a]	8.0[a]	8.1[a]	7.6[a]	7.6[a]	7.7[a]	7.7[a]
GA	7.7[a]	7.8[a]	7.8[a]	7.8[a]	7.6[a]	7.6[a]	7.8[a]	7.8[a]
Week 4								
Flavour	7.7[a]	7.8[a]	7.8[a]	8.0[a]	7.6[b]	7.7[b]	7.8[b]	7.8[b]
Colour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.7[b]	7.7[b]	7.8[b]	7.8[b]
Taste	7.8[a]	7.8[a]	7.8[a]	7.9[a]	7.5[b]	7.5[b]	7.6[b]	7.6[b]
GA	7.7[a]	7.8[a]	7.8[a]	7.8[a]	7.6[b]	7.6[b]	7.8[b]	7.8[b]
Week 5								
Flavour	7.8[a]	8.0[a]	7.9[a]	8.1[a]	8.0[b]	7.6[b]	8.2[b]	8.3[b]
Colour	8.0[a]	8.1[a]	8.1[a]	8.2[a]	8.0[b]	8.0[b]	8.2[b]	8.2[b]
Taste	7.5[a]	7.5[a]	7.6[a]	7.8[a]	7.3[b]	7.6[b]	7.8[b]	7.8[b]
GA	7.8[a]	8.0[a]	8.0[a]	8.0[a]	8.2[b]	8.3[b]	8.3[b]	8.3[b]
Week 6								
Flavour	7.8[a]	8.0[a]	7.9[a]	8.1[a]	8.0[b]	7.6[b]	8.2[b]	8.3[b]
Colour	8.0[a]	8.1[a]	8.1[a]	8.2[a]	8.0[b]	8.0[b]	8.2[b]	8.2[b]
Taste	7.5[a]	7.5[a]	7.6[a]	7.8[a]	7.3[b]	7.6[b]	7.8[b]	7.8[b]
GA	7.8[a]	8.0[a]	8.0[a]	8.0[a]	8.2[b]	8.3[b]	8.3[b]	8.3[b]
Week 7								
Flavour	7.2[b]	7.0[b]	7.2[b]	7.1[b]	7.3[b]	7.4[b]	7.2[b]	7.4[b]
Colour	7.6[b]	7.1[b]	7.4[b]	7.2[b]	7.5[b]	7.4[b]	7.2[b]	7.2[b]
Taste	7.2[b]	7.0[b]	7.2[b]	7.4[b]	7.3[b]	7.2[b]	7.4[b]	7.6[b]
GA	7.0[b]	7.0[b]	7.0[b]	7.0[b]	7.2[b]	7.3[b]	7.3[b]	7.3[b]
Week 8								
Flavour	7.0[b]	7.0[b]	7.3[b]	7.1[b]	7.0[b]	7.2[b]	7.2[b]	7.3[b]
Colour	7.0[b]	7.1[b]	7.4[b]	7.2[b]	7.0[b]	7.0[b]	7.2[b]	7.2[b]
Taste	7.2[b]	7.3[b]	7.2[b]	7.4[b]	7.3[b]	7.2[b]	7.4[b]	7.4[b]
GA	7.4[b]	7.0[b]	7.2[b]	7.0[b]	7.2[b]	7.3[b]	7.3[b]	7.3[b]

Key: GA = General Acceptability a = No significant difference b = Significant difference

4.1.3 Organoleptic/sensory properties of the preserved probiotic drinks using *Alstonia boonei* leaf

Table 3 shows the organoleptic/sensory properties of the preserved probiotic drinks using *Alstonia boonei* leaf. There was no significant different in the organoleptic/sensory properties of the preserved probiotic zobo drinks from Week 1 to Week 4. However, there was significant difference in the organoleptic/sensory properties of the preserved probiotic zobo drinks from Week 5 to Week 8.

There was no significant difference in the organoleptic/sensory properties of the tigernut drink preserved with *Alstonia boonei* leaf from week 1 to week 2, while there was a significant difference in the tigernut drink preserved with *Alstonia boonei* leaf from week 3 to week 8. There was also a significant difference in the concentrations of the preserved drinks.

Table 3: Organoleptic/sensory properties of the preserved probiotic drinks using *Alstonia boonei* leaf

Parameters	Probiotic drinks/Volume of medicinal plant extracts used							
	Zobo				Tigernut			
	10	30	50	100	10	30	50	100
Week 1								
Flavour	8.2[a]	8.2[a]	8.0[a]	8.0[a]	8.0[a]	8.0[a]	7.9[a]	7.9[a]
Colour	8.0[a]	8.1[a]	8.1[a]	8.2[a]	8.0[a]	8.0[a]	8.2[a]	8.2[a]
Taste	7.5[a]	7.5[a]	7.6[a]	7.8[a]	7.3[a]	7.6[a]	7.8[a]	7.8[a]
GA	7.6[a]	7.7[a]	7.8[a]	7.8[a]	8.0[a]	8.0[a]	8.1[a]	8.2[a]
Week 2								
Flavour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.6[a]	7.7[a]	7.8[a]	7.8[a]
Colour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.7[a]	7.7[a]	7.8[a]	7.8[a]
Taste	7.8[a]	7.8[a]	7.8[a]	7.9[a]	7.5[a]	7.5[a]	7.6[a]	7.6[a]
GA	7.7[a]	7.8[a]	7.8[a]	7.8[a]	7.6[a]	7.6[a]	7.8[a]	7.8[a]
Week 3								
Flavour	7.6[a]	7.6[a]	7.7[a]	7.7[a]	7.7[b]	7.7[b]	7.8[b]	7.8[b]
Colour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.7[b]	7.7[b]	7.8[b]	7.8[b]
Taste	7.9[a]	7.9[a]	8.0[a]	8.1[a]	7.6[b]	7.6[b]	7.7[b]	7.7[b]
GA	7.7[a]	7.8[a]	7.8[a]	7.8[a]	7.6[b]	7.6[b]	7.8[b]	7.8[b]
Week 4								
Flavour	7.7[a]	7.8[a]	7.8[a]	8.0[a]	7.6[b]	7.7[b]	7.8[b]	7.8[b]
Colour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.7[b]	7.7[b]	7.8[b]	7.8[b]
Taste	7.8[a]	7.8[a]	7.8[a]	7.9[a]	7.5[b]	7.5[b]	7.6[b]	7.6[b]
GA	7.7[a]	7.8[a]	7.8[a]	7.8[a]	7.6[b]	7.6[b]	7.8[b]	7.8[b]
Week 5								
Flavour	7.8[b]	8.0[b]	7.9[b]	8.1[b]	8.0[b]	7.6[b]	8.2[b]	8.3[b]
Colour	8.0[b]	8.1[b]	8.1[b]	8.2[b]	8.0[b]	8.0[b]	8.2[b]	8.2[b]
Taste	7.5[b]	7.5[b]	7.6[b]	7.8[b]	7.3[b]	7.6[b]	7.8[b]	7.8[b]
GA	7.8[b]	8.0[b]	8.0[b]	8.0[b]	8.2[b]	8.3[b]	8.3[b]	8.3[b]
Week 6								
Flavour	7.0[b]	7.0[b]	7.3[b]	7.1[b]	7.0[b]	7.2[b]	7.2[b]	7.3[b]
Colour	7.0[b]	7.1[b]	7.4[b]	7.2[b]	7.0[b]	7.0[b]	7.2[b]	7.2[b]
Taste	7.2[b]	7.3[b]	7.2[b]	7.4[b]	7.3[b]	7.2[b]	7.4[b]	7.4[b]
GA	7.4[b]	7.0[b]	7.2[b]	7.0[b]	7.2[b]	7.3[b]	7.3[b]	7.3[b]
Week 7								
Flavour	7.2[b]	7.0[b]	7.2[b]	7.1[b]	7.3[b]	7.4[b]	7.2[b]	7.4[b]
Colour	7.6[b]	7.1[b]	7.4[b]	7.2[b]	7.5[b]	7.4[b]	7.2[b]	7.2[b]
Taste	7.2[b]	7.0[b]	7.2[b]	7.4[b]	7.3[b]	7.2[b]	7.4[b]	7.6[b]
GA	7.0[b]	7.0[b]	7.0[b]	7.0[b]	7.2[b]	7.3[b]	7.3[b]	7.3[b]
Week 8								
Flavour	7.0[b]	7.0[b]	7.3[b]	7.1[b]	7.0[b]	7.2[b]	7.2[b]	7.3[b]
Colour	7.0[b]	7.1[b]	7.4[b]	7.2[b]	7.0[b]	7.0[b]	7.2[b]	7.2[b]
Taste	7.2[b]	7.3[b]	7.2[b]	7.4[b]	7.3[b]	7.2[b]	7.4[b]	7.4[b]
GA	7.4[b]	7.0[b]	7.2[b]	7.0[b]	7.2[b]	7.3[b]	7.3[b]	7.3[b]

Key: GA = General Acceptability a = No significant difference b = Significant difference

4.1.4 Organoleptic/sensory properties of the preserved probiotic drinks using *Pterocarpus santalinoides* leaf

Table 4 shows the organoleptic/sensory properties of the preserved probiotic drinks using *Pterocarpus santalinoides* leaf. There was no significant different in the organoleptic/sensory properties of the preserved probiotic zobo drinks from Week 1 to Week 7. However, there was significant difference in the organoleptic/sensory properties of the biopreserved probiotic zobo drinks from Week 8.

There was no significant difference in the organoleptic/sensory properties of the tigernut drink preserved with *Pterocarpus santalinoides* leaf from week 1 to week 5, while there was a significant difference in the tigernut drink preserved with *Pterocarpus santalinoides* leaf from week 6 to week 8. There was also a significant difference in the concentrations of the preserved drinks.

Table 4: Organoleptic/sensory properties of the preserved probiotic drinks using *Pterocarpus santalinoides* leaf

Parameters	Probiotic drinks/Volume of medicinal plant extracts used							
	Zobo				Tigernut			
	10	30	50	100	10	30	50	100
Week 1								
Flavour	7.8[a]	8.0[a]	7.9[a]	8.1[a]	8.0[a]	7.6[a]	8.2[a]	8.3[a]
Colour	8.0[a]	8.1[a]	8.1[a]	8.2[a]	8.0[a]	8.0[a]	8.2[a]	8.2[a]
Taste	7.5[a]	7.5[a]	7.6[a]	7.8[a]	7.3[a]	7.6[a]	7.8[a]	7.8[a]
GA	7.8[a]	8.0[a]	8.0[a]	8.0[a]	8.2[a]	8.3[a]	8.3[a]	8.3[a]
Week 2								
Flavour	7.7[a]	7.8[a]	7.8[a]	8.0[a]	7.6[a]	7.7[a]	7.8[a]	7.8[a]
Colour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.7[a]	7.7[a]	7.8[a]	7.8[a]
Taste	7.8[a]	7.8[a]	7.8[a]	7.9[a]	7.5[a]	7.5[a]	7.6[a]	7.6[a]
GA	7.7[a]	7.8[a]	7.8[a]	7.8[a]	7.6[a]	7.6[a]	7.8[a]	7.8[a]
Week 3								
Flavour	7.8[a]	7.8[a]	7.8[a]	7.9[a]	7.7[a]	7.7[a]	7.8[a]	7.8[a]
Colour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.7[a]	7.7[a]	7.8[a]	7.8[a]
Taste	7.9[a]	7.9[a]	8.0[a]	8.1[a]	7.6[a]	7.6[a]	7.7[a]	7.7[a]
GA	7.7[a]	7.8[a]	7.8[a]	7.8[a]	7.6[a]	7.6[a]	7.8[a]	7.8[a]
Week 4								
Flavour	7.7[a]	7.8[a]	7.8[a]	8.0[a]	7.6[a]	7.7[a]	7.8[a]	7.8[a]
Colour	7.8[a]	7.8[a]	7.9[a]	8.0[a]	7.7[a]	7.7[a]	7.8[a]	7.8[a]
Taste	7.8[a]	7.8[a]	7.8[a]	7.9[a]	7.5[a]	7.5[a]	7.6[a]	7.6[a]
GA	7.7[a]	7.8[a]	7.8[a]	7.8[a]	7.6[a]	7.6[a]	7.8[a]	7.8[a]
Week 5								
Flavour	7.8[a]	8.0[a]	7.9[a]	8.1[a]	8.0[a]	7.6[a]	8.2[a]	8.3[a]
Colour	8.0[a]	8.1[a]	8.1[a]	8.2[a]	8.0[a]	8.0[a]	8.2[a]	8.2[a]
Taste	7.5[a]	7.5[a]	7.6[a]	7.8[a]	7.3[a]	7.6[a]	7.8[a]	7.8[a]
GA	7.8[a]	8.0[a]	8.0[a]	8.0[a]	8.2[a]	8.3[a]	8.3[a]	8.3[a]
Week 6								
Flavour	7.8[a]	8.0[a]	7.9[a]	8.1[a]	8.0[b]	7.6[b]	8.2[b]	8.3[b]
Colour	8.0[a]	8.1[a]	8.1[a]	8.2[a]	8.0[b]	8.0[b]	8.2[b]	8.2[b]
Taste	7.5[a]	7.5[a]	7.6[a]	7.8[a]	7.3[b]	7.6[b]	7.8[b]	7.8[b]
GA	7.8[a]	8.0[a]	8.0[a]	8.0[a]	8.2[b]	8.3[b]	8.3[b]	8.3[b]
Week 7								
Flavour	7.2[a]	7.0[a]	7.2[a]	7.1[a]	7.3[b]	7.4[b]	7.2[b]	7.4[b]
Colour	7.6[a]	7.1[a]	7.4[a]	7.2[a]	7.5[b]	7.4[b]	7.2[b]	7.2[b]
Taste	7.2[a]	7.0[a]	7.2[a]	7.4[a]	7.3[b]	7.2[b]	7.4[b]	7.6[b]
GA	7.0[a]	7.0[a]	7.0[a]	7.0[a]	7.2[b]	7.3[b]	7.3[b]	7.3[b]
Week 8								
Flavour	7.0[b]	7.0[b]	7.3[b]	7.1[b]	7.0[b]	7.2[b]	7.2[b]	7.3[b]
Colour	7.0[b]	7.1[b]	7.4[b]	7.2[b]	7.0[b]	7.0[b]	7.2[b]	7.2[b]
Taste	7.2[b]	7.3[b]	7.2[b]	7.4[b]	7.3[b]	7.2[b]	7.4[b]	7.4[b]
GA	7.4[b]	7.0[b]	7.2[b]	7.0[b]	7.2[b]	7.3[b]	7.3[b]	7.3[b]

Key: GA = General Acceptability a = No significant difference b = Significant difference

4.1.5 Shelf-life properties of the probiotic drinks using *Moringa oleifera* leaf

Table 5 shows the shelf-life extension properties of the probiotic drinks using *Moringa oleifera* leaf. The temperature of the probiotic drinks remained stable between 26.5 ^{0}Cand 27.2 ^{0}C throughout the shelf-life properties determination. pH of the probiotic drinks became acidic from week 7 to week 8. The colour and odour were stable throughout the preservation period.

Table 5: Shelf-life properties of the probiotic drinks preserved using *Moringa oleifera* leaf

Parameters	Probiotic drinks/Volume of medicinal plant extracts used									
	Zobo					Tigernut				
	10	30	50	100	Ctrl	10	30	50	100	Ctrl
Temp. (0^C)										
Week 1	26.5	26.5	27.0	27.0	26.8	26.8	27.0	27.2	26.8	26.4
Week 2	26.6	26.6	26.8	26.6	26.8	26.8	26.7	26.8	26.8	26.4
Week 3	26.5	26.7	26.9	26.8	26.8	26.8	26.8	27.0	26.8	26.4
Week 4	26.6	26.5	27.0	26.9	26.8	26.8	27.0	27.0	26.8	26.4
Week 5	26.5	26.5	27.0	26.9	26.8	26.8	26.9	26.8	26.9	26.4
Week 6	26.5	26.5	27.0	27.2	26.8	26.8	27.0	27.2	26.8	26.4
Week 7	26.5	26.5	27.0	26.9	26.8	26.8	26.8	27.2	26.8	26.4
Week 8	26.5	26.5	27.0	26.9	26.8	26.8	27.0	26.9	26.9	26.4
pH										
Week 1	5.64	5.64	5.64	5.64	5.56	5.54	5.60	5.62	5.64	5.60
Week 2	5.64	5.64	5.64	5.64	5.46	5.54	5.60	5.62	5.64	5.56
Week 3	5.64	5.64	5.64	5.64	5.42	5.54	5.60	5.62	5.64	5.54
Week 4	5.64	5.64	5.64	5.64	4.90	5.54	5.60	5.62	5.64	5.52
Week 5	5.64	5.64	5.64	5.64	4.80	5.54	5.60	5.62	5.64	5.52
Week 6	5.64	5.64	5.64	5.64	4.86	5.54	5.60	5.62	5.64	5.50
Week 7	4.80	4.80	4.80	4.80	4.60	4.80	5.80	4.80	4.80	4.92
Week 8	4.70	4.70	4.70	4.70	4.56	4.70	4.70	4.70	4.70	4.90
Total titratable acidity										
Week 1	0.42	0.42	0.42	0.42	0.46	0.40	0.40	0.40	0.40	0.40
Week 2	0.42	0.42	0.42	0.42	0.46	0.40	0.40	0.40	0.40	0.42
Week 3	0.44	0.44	0.46	0.48	0.48	0.40	0.42	0.42	0.44	0.42
Week 4	0.46	0.46	0.48	0.48	0.48	0.41	0.41	0.42	0.44	0.44
Week 5	0.48	0.48	0.50	0.50	0.50	0.44	0.44	0.46	0.48	0.46
Week 6	0.48	0.48	0.50	0.50	0.52	0.44	0.44	0.46	0.48	0.46
Week 7	0.50	0.50	0.50	0.50	0.54	0.46	0.46	0.48	0.48	0.46
Week 8	0.50	0.50	0.52	0.52	0.58	0.48	0.48	0.48	0.50	0.48

Key: ^{0}C = Degrees centigrade Ctrl = Control (unpreserved sample)

4.1.6 Shelf-life properties of the probiotic drinks using *Alstoniaboonei* leaf

Table 6 shows the shelf-life extension properties of the probiotic drinks using *Alstonia boonei* leaf. The temperature of the probiotic drinks remained stable between 26.5 to 27.0 ^{0}C throughout the shelf-life extension properties determination. pH of the probiotic drinks became acidic from week 7 to week 8. The colour and odour were stable throughout the preservation period.

Table 6: Shelf-life properties of the probiotic drinks preserved using *Alstonia boonei* leaf

Parameters	Probiotic drinks/Volume of medicinal plant extracts used									
	Zobo					Tigernut				
	10	30	50	100	Ctrl	10	30	50	100	Ctrl
Temp. (0^C)										
Week 1	26.5	26.5	27.0	27.0	26.8	26.8	27.0	27.0	26.8	26.4
Week 2	26.6	26.6	26.8	26.6	26.8	26.8	26.7	26.8	26.8	26.4
Week 3	26.5	26.7	26.9	26.8	26.8	26.8	26.8	27.0	26.8	26.4
Week 4	26.6	26.5	27.0	26.9	26.8	26.8	27.0	27.0	26.8	26.4
Week 5	26.5	26.5	27.0	26.9	26.8	26.8	26.9	26.8	26.9	26.4
Week 6	26.5	26.5	27.0	27.0	26.8	26.8	27.0	27.0	26.8	26.4
Week 7	26.5	26.5	27.0	26.9	26.8	26.8	26.8	26.8	26.8	26.4
Week 8	26.5	26.5	27.0	26.9	26.8	26.8	27.0	26.9	26.9	26.4
pH										
Week 1	5.60	5.62	5.62	5.62	5.50	5.52	5.60	5.60	5.60	5.60
Week 2	5.60	5.62	5.64	5.64	5.46	5.54	5.60	5.62	5.64	5.56
Week 3	5.64	5.64	5.60	5.64	5.42	5.50	5.60	5.62	5.64	5.54
Week 4	5.64	5.64	5.64	5.64	5.00	5.54	5.62	5.62	5.64	5.52
Week 5	5.64	5.64	5.64	5.64	4.80	5.54	5.60	5.62	5.64	5.52
Week 6	5.62	5.64	5.64	5.64	4.82	5.54	5.60	5.62	5.64	5.50
Week 7	4.80	4.80	4.80	4.80	4.60	4.80	5.80	4.70	4.80	4.92
Week 8	4.80	4.80	4.80	4.70	4.60	4.60	4.60	4.70	4.70	4.90
Total titratable acidity										
Week 1	0.42	0.42	0.42	0.42	0.46	0.40	0.40	0.40	0.40	0.40
Week 2	0.42	0.42	0.42	0.42	0.46	0.40	0.40	0.40	0.40	0.42
Week 3	0.44	0.44	0.46	0.48	0.48	0.40	0.42	0.42	0.44	0.42
Week 4	0.46	0.46	0.48	0.48	0.48	0.41	0.41	0.42	0.44	0.44
Week 5	0.48	0.48	0.50	0.50	0.50	0.44	0.44	0.46	0.48	0.46
Week 6	0.48	0.48	0.50	0.50	0.52	0.44	0.44	0.46	0.48	0.46
Week 7	0.50	0.50	0.50	0.50	0.54	0.46	0.46	0.48	0.48	0.46
Week 8	0.50	0.50	0.52	0.52	0.58	0.48	0.48	0.48	0.50	0.48

Key: ^{0}C = Degrees centigrade Ctrl = Control (unpreserved sample)

4.1.7 Shelf-life properties of the probiotic drinks using *Pterocarpus santalinoides* leaf

Table 7 shows the shelf-life properties of the probiotic drinks using *Pterocarpus santalinoides* leaf. The temperature of the probiotic drinks remained stable between 26.2 ^{0}C and 27.0 ^{0}C throughout the shelf-life properties determination. pH of the probiotic drinks became acidic from week 7 to week 8. The colour and odour were stable throughout the preservation period.

Table 7: Shelf-life properties of the probiotic drinks preserved using *Pterocarpus santalinoides* leaf

Parameters	Probiotic drinks/Volume of medicinal plant extracts used									
	Zobo					Tigernut				
	10	30	50	100	Ctrl	10	30	50	100	Ctrl
Temp. (0^C)										
Week 1	26.2	26.3	26.8	26.6	26.8	26.8	26.6	27.0	26.4	26.6
Week 2	26.6	26.6	26.8	26.6	26.8	26.8	26.7	26.8	26.8	26.4
Week 3	26.5	26.7	26.9	26.8	26.8	26.8	26.8	27.0	26.8	26.4
Week 4	26.6	26.5	27.0	26.9	26.8	26.8	27.0	27.0	26.8	26.4
Week 5	26.2	26.4	26.8	26.9	26.8	26.8	26.9	26.8	26.9	26.4
Week 6	26.5	26.5	26.6	27.0	26.8	26.8	27.0	27.0	26.8	26.4
Week 7	26.5	26.2	27.0	26.9	26.8	26.8	26.8	26.8	26.8	26.4
Week 8	26.3	26.5	26.7	26.9	26.8	26.8	27.0	26.9	26.9	26.4
pH										
Week 1	5.60	5.62	5.62	5.62	5.50	5.52	5.60	5.60	5.60	5.60
Week 2	5.62	5.64	5.64	5.64	4.82	5.54	5.60	5.62	5.64	5.50
Week 3	5.64	5.64	5.60	5.64	5.42	5.50	5.60	5.62	5.64	5.54
Week 4	5.64	5.64	5.64	5.64	5.00	5.54	5.62	5.62	5.64	5.52
Week 5	5.64	5.64	5.64	5.64	4.80	5.54	5.60	5.62	5.64	5.52
Week 6	5.62	5.64	5.64	5.64	4.82	5.54	5.60	5.62	5.64	5.50
Week 7	4.80	4.80	4.80	4.80	4.60	4.80	5.80	4.70	4.80	4.92
Week 8	4.80	4.80	4.80	4.70	4.60	4.60	4.60	4.70	4.70	4.90
Total titratable acidity										
Week 1	0.42	0.42	0.42	0.42	0.46	0.40	0.40	0.40	0.40	0.40
Week 2	0.42	0.42	0.42	0.42	0.46	0.40	0.40	0.40	0.40	0.42
Week 3	0.44	0.44	0.46	0.48	0.48	0.40	0.42	0.42	0.44	0.42
Week 4	0.46	0.46	0.48	0.48	0.48	0.41	0.41	0.42	0.44	0.44
Week 5	0.48	0.48	0.50	0.50	0.50	0.44	0.44	0.46	0.48	0.46
Week 6	0.48	0.48	0.50	0.50	0.52	0.44	0.44	0.46	0.48	0.46
Week 7	0.50	0.50	0.50	0.50	0.54	0.46	0.46	0.48	0.48	0.46
Week 8	0.50	0.50	0.52	0.52	0.58	0.48	0.48	0.48	0.50	0.48

Key: ^{0}C = Degrees centigrade Ctrl = Control (unpreserved sample)

4.8 Microbial plate counts during storage of the preserved zobo drink using the *Moringa oleifera* leaf

Table 8 shows the microbial plate countsduring storage of the preserved zobo drink using the *Moringa oleifera* leaf. Microbial plate countsranged from 2.0×10^1 to 8.0×10^1 cfu/ml, with the control sample (unpreserved zobo drink) having the highest microbial load. The microbial plate counts increased from week 7 to week 8. There was a significant difference between the unpreserved sample and the sample preserved with 100 ml of the extract.

Table 8: Microbial plate counts during storage of the preserved zobo drink using the *Moringa oleifera* leaf

Weeks	Volume of medicinal plant extracts used				
	Ctrl	10	30	50	100
Week 1	3.0×10^1	3.0×10^1	2.0×10^1	2.0×10^1	2.0×10^1
Week 2	4.0×10^1	4.0×10^1	2.0×10^1	1.0×10^1	NG
Week 3	6.0×10^1	6.0×10^1	3.0×10^1	1.0×10^1	NG
Week 4	1.0×10^2	8.0×10^1	6.0×10^1	4.0×10^1	2.0×10^1
Week 5	3.0×10^2	1.0×10^2	8.0×10^1	4.0×10^1	2.0×10^1
Week 6	3.8×10^2	1.2×10^2	1.0×10^2	6.0×10^1	4.0×10^1
Week 7	4.4×10^2	1.6×10^2	1.0×10^2	6.0×10^1	6.0×10^1
Week 8	8.0×10^2	3.0×10^2	1.0×10^2	1.4×10^2	8.0×10^1
p-value	<0.001	0.006	0.009	0.005	<0.001

Key: NG = No growth Ctrl = Control

4.9 Microbial plate counts during storage of the preserved zobo drink using the *Alstonia boonei* leaf

Table 9 shows the microbial plate counts during storage of the preserved zobo drink using the *Alstonia boonei* leaf. Microbial plate counts ranged from 4.0×10^1 to 7.2×10^2 cfu/ml with control sample (unpreserved zobo drink) having highest microbial load. There was increase in the microbial plate counts from week 6 to week 8. There was significant difference in the unpreserved sample and the sample preserved with 100 ml of the extract.

Table 9: Microbial plate counts during storage of the preserved zobo drink using the *Alstonia boonei* leaf

Weeks	Volume of medicinal plant extracts used				
	Ctrl	10	30	50	100
Week 1	8.0×10^1	8.0×10^1	7.0×10^1	6.0×10^1	6.0×10^1
Week 2	4.0×10^1	6.0×10^1	4.0×10^1	2.0×10^1	1.0×10^1
Week 3	6.0×10^1	8.0×10^1	1.0×10^2	4.0×10^1	2.0×10^1
Week 4	1.0×10^2	1.0×10^2	6.0×10^1	4.0×10^1	3.0×10^1
Week 5	3.0×10^2	1.0×10^2	8.0×10^1	4.0×10^1	2.0×10^1
Week 6	3.8×10^2	1.2×10^2	1.0×10^2	6.0×10^1	4.0×10^1
Week 7	4.4×10^2	1.4×10^2	1.2×10^2	8.0×10^1	6.0×10^1
Week 8	7.2×10^2	4.0×10^2	3.0×10^2	2.0×10^2	1.0×10^2
p-value	<0.001	0.007	0.009	0.006	<0.001

Ctrl = Control

4.10 Microbial plate counts during storage of the preserved zobo drink using the *Pterocarpus santalinoides* leaf

Table 10 shows the microbial plate counts during storage of the preserved zobo drink using the *Pterocarpus santalinoides* leaf. Microbial plate counts ranged from 4.0×10^1 to 8.8×10^2 cfu/m, with the control sample (unpreserved zobo drink) having the highest microbial load. The microbial plate count increased from week 7 to week 8. There was a significant difference between the unpreserved sample and the sample preserved with 100 ml of the extract.

Table 10: Microbial plate counts during storage of the preserved zobo drink using the *Pterocarpus santalinoides* leaf

Weeks	Volume of medicinal plant extracts used				
	Ctrl	10	30	50	100
Week 1	8.0×10^1	6.0×10^1	6.0×10^1	4.0×10^1	4.0×10^1
Week 2	4.0×10^1	4.0×10^1	2.0×10^1	1.0×10^1	NG
Week 3	8.0×10^1	6.0×10^1	8.0×10^1	2.0×10^1	1.0×10^1
Week 4	1.2×10^2	8.0×10^1	1.0×10^2	8.0×10^1	2.0×10^1
Week 5	3.4×10^2	1.0×10^2	6.0×10^1	4.0×10^1	2.0×10^1
Week 6	4.4×10^2	1.2×10^2	1.0×10^2	6.0×10^1	4.0×10^1
Week 7	6.4×10^2	1.4×10^2	1.2×10^2	8.0×10^1	6.0×10^1
Week 8	8.8×10^2	3.4×10^2	2.0×10^2	1.4×10^2	1.0×10^2
p-value	<0.001	0.007	0.009	0.006	<0.001

Ctrl = Control

4.11 Microbial plate counts during storage of the preserved tigernut drink using the *Moringa oleifera* leaf

Table 11 shows the microbial plate counts during storage of the preserved tigernut drink using the *Moringa oleifera* leaf. Microbial plate counts ranged from 2.0×10^1 to 8.6×10^2 cfu/ml with control sample (unpreserved tigernut drink) having highest microbial load. There was increase in the microbial plate countsfrom week 5 to week 8. There was significant difference in the unpreserved sample and the sample preserved with 100ml of the extract.

Table 11: Microbial plate counts during storage of the preserved tigernut drink using the *Moringa oleifera* leaf

Weeks	Volume of medicinal plant extracts used				
	Ctrl	10	30	50	100
Week 1	2×10^1	6.0×10^1	4.0×10^1	4.0×10^1	2.0×10^1
Week 2	1.2×10^1	1.2×10^2	8.0×10^1	6.0×10^1	4.0×10^1
Week 3	1.6×10^2	1.4×10^2	1.0×10^2	8.0×10^1	6.0×10^1
Week 4	2.0×10^2	1.6×10^1	8.0×10^1	6.0×10^1	4.0×10^1
Week 5	3.0×10^2	2.0×10^2	1.2×10^2	8.0×10^1	6.0×10^1
Week 6	4.4×10^2	2.2×10^2	1.6×10^2	1.0×10^2	8.0×10^1
Week 7	6.8×10^2	3.6×10^2	2.8×10^2	1.8×10^2	1.0×10^2
Week 8	8.6×10^2	6.0×10^2	5.0×10^2	3.4×10^2	1.6×10^2
p-value	<0.001	0.004	0.003	0.008	<0.001

Ctrl = Control

4.12 Microbial plate counts during storage of the preserved tigernut drink using the *Alstonia boonei* leaf

Table 12 shows the microbial plate counts during storage of the preserved tigernut drink using the *Alstonia boonei* leaf. Microbial plate counts ranged from 4.0×10^1 to 9.2×10^2 cfu/ml with control sample (unpreserved tigernut drink) having highest microbial load. There was increase in the microbial plate counts from week 6 to week 8. There was significant difference in the unpreserved sample and the sample preserved with 100ml of the extract.

Table 12: Microbial plate counts during storage of the preserved tigernut drink using the *Alstonia boonei* leaf

Weeks	Volume of medicinal plant extracts used				
	Ctrl	10	30	50	100
Week 1	$1.0x10^1$	8.0×10^1	4.0×10^1	2.0×10^1	2.0×10^1
Week 2	6.0×10^1	1.6×10^2	8.0×10^1	6.0×10^1	6.0×10^1
Week 3	1.6×10^2	1.8×10^2	1.8×10^2	1.0×10^2	8.0×10^1
Week 4	2.4×10^2	2.4×10^1	2.0×10^2	1.2×10^2	1.0×10^2
Week 5	3.0×10^2	2.8×10^2	2.2×10^2	1.0×10^1	1.2×10^2
Week 6	7.4×10^2	3.2×10^2	2.6×10^2	1.4×10^2	1.4×10^2
Week 7	8.8×10^2	4.2×10^2	3.8×10^2	2.8×10^2	1.8×10^2
Week 8	9.2×10^2	6.0×10^2	5.4×10^2	4.4×10^2	2.2×10^2
p-value	<0.001	0.004	0.003	0.008	<0.001

Ctrl = Control

4.13 Microbial plate counts during storage of the preserved tigernut drink using the *Pterocarpus santalinoides* leaf

Table 13 shows the microbial plate counts during storage of the preserved tigernut drink using the *Pterocarpus santalinoides* leaf. Microbial plate counts ranged from 4.0×10^1 to 9.2×10^2 cfu/ml with control sample (unpreserved tigernut drink) having highest microbial load. There was increase in the microbial plate countsfrom week 6 to week 8. There was significant difference in the unpreserved sample and the sample preserved with 100ml of the extract.

Table 13: Microbial plate counts during storage of the preserved tigernut drink using the *Pterocarpus santalinoides* leaf

Weeks	Volume of medicinal plant extracts used				
	Ctrl	10	30	50	100
Week 1	$8.0x10^1$	7.0×10^1	6.0×10^1	4.0×10^1	4.0×10^1
Week 2	6.0×10^1	3.0×10^1	2.0×10^1	1.0×10^1	NG
Week 3	8.0×10^1	4.0×10^1	4.0×10^1	2.0×10^1	1.0×10^1
Week 4	1.2×10^2	8.0×10^1	1.0×10^2	6.0×10^1	2.0×10^1
Week 5	2.8×10^2	1.0×10^2	6.0×10^1	4.0×10^1	2.0×10^1
Week 6	4.4×10^2	1.2×10^2	1.0×10^2	6.0×10^1	4.0×10^1
Week 7	6.4×10^2	2.4×10^2	1.4×10^2	1.0×10^2	6.0×10^1
Week 8	9.2×10^2	3.4×10^2	2.0×10^2	1.4×10^2	1.0×10^2
p-value	<0.001	0.007	0.009	0.006	<0.001

Ctrl = Control

4.14: Cultural morphology and biochemical characteristics of the bacterial isolates from the preserved probiotic drinks

Table 14 shows the cultural morphology and biochemical characteristics of the bacterial isolates from the preserved drinks. A total of four (4) bacterial isolates comprising three (3) Gram positive and one (1) Gram negative bacteria were isolated from the probiotic drinks during preservation and storage. The bacterial isolates were; *Pseudomonas, Bacillus, Lactobacillus* and *Leuconostoc* species.

Table 14: Cultural morphology and biochemical characteristics of the bacterial isolates from the preserved probiotic drinks

Morphological Characteristics	Gram reaction	Oxidase	Indole	Spore	Catalase	Citrate	Coagulase	Motility	SFT				
									S	B	G	H_2S	Possible bacteria
Bluish-green, flat, non-mucoid colonies	Gram-negative rods in diploids	+	-	-	+	-	-	+	R	R	-	-	*Pseudomonas* species
Milkish, flat, rhizoid-like dry-surface colonies	Gram positive rods in short chains	-	-	+	+	-	-	-	Y	Y	+	-	*Bacillus* species
Milkish, raised, small, non-mucoid colonies	Gram positive rod	-	-	-	-	-	-	-	No Reaction		-	-	*Lactobacillus* species
Grayish, smooth, circular, colonies of 1mm	Gram positive cocci	-	-	-	-	-	-	-	No Reaction		-	-	*Leuconostoc*species

Key: -= Negative += Positive S = color of slope B = color of butt G= Gas production H_2S= Hydrogen sulphide production (blackening)
R= Reddish coloration (alkaline production) Y= Yellow coloration (Acidic production) SFT= Sugar fermentation test

4.15 Cultural morphology and microscopic characteristics of the fungal isolates from the preserved probiotic drinks

Table 15 shows the cultural morphology and microscopic characteristics of the fungal isolates from the preserved probiotic drinks. Four (4) fungal isolates were obtained from the samples. They were *Saccharomyces, Kluyveromyces*, *Aspergillus*, and *Rhizopus* species.

Table 15: Cultural characterization of the fungal isolates from the preserved probiotic drinks

Cultural morphology	Microscopy	Possible fungi
Whitish, raised, fluffy, cotton-like colonies that covered the plate after three days.	Non-septate hyphae	*Rhizopus* species
Whitish, circular, colonies with light yellow reverse.	Septate hyphae	*Aspergillus* species
Whitish, creamy, circular enlarge colonies	Budded yeast cells	*Kluyveromyces* species
White, creamy, oblong colonies	Budded yeast cells in diploid	*Saccharomyces* species

Hypotheses Testing

Hypothesis 1: There is no significance relationship between the preservative efficacy of the different medicinal plants and the shelf-life of probiotic drinks

The relationship between preservative efficacy of the different medicinal plants and the shelf-life properties of probiotic drinks using independent t-test. There was a significant association between the preservative efficacy of the medicinal plants and shelf-life properties of the preserved probiotic drinks. Probiotic drinks preserved using *Alstonia bonnie* leaf showed poor preservative efficacy compared to the those preserved using *Moringa oleifera* and *Pterocarpus santalinoides* leaf.

Table 18: Independent sample test showing the significant relationship between the preservative efficacy of the different medicinal plants in the shelf-life of probiotic drinks

Weeks	Preservative efficacy	Mean±SD	t- value	p-value
Moringa oleifera	Acceptable	$1.2\times10^2\pm1.3\times10^2$	-6.984	<0.001
Alstonia bonnei	Satisfactory	$4.2\times10^2\pm5.1\times10^2$	-15.154	<0.05
Pterocarpus santalinoides	Acceptable	$1.6\times10^2\pm3.7\times10^2$	-12.372	<0.001

4.2 DISCUSSION

4.2.1 Phytochemical constituents of *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides* leaf extract

Phytochemicals are chemical compounds produced by plants which help them to resist fungi, bacteria and virus infections. Table 1 shows the presence of saponins, anthraquinones, phenols, alkaloids, tannins, phlobatannins and cardiac glycosides were detected in *Moringa oleifera*. This is in line with the study of Okechukwu *et al.* (2021) who reported the presence of alkaloids, terpenoids, saponins, phenols, and glycosides in methanol extract of *Moringa oleifera* Lam. Similarly, Unegbu *et al.* (2020) reported the presence of terpenoids, phenols, flavonoids, glycosides, tannin, saponin, alkaloids, steroids and anthraquinolones in *Moringa oleifera* leaf extracts. Elzein *et al.* (2018) reported the presence of phytochemicals such as alkaloids, flavonoids, saponins, sterols and tannins in aqueous, ethanol, methanol and chloroform extracts of *Moringa oleifera*. Etejere *et al*. (2015) in their study on comparative studies of phytochemical constituents of leaf, bark and root of *Moringa oleifera* Lam reported that aqueous extract of the plant parts tested positive for tannins, phlobatannins, saponins, flavonoids, terpenoids and alkaloids except cardiac glycosides which were present only in the root.

The presence of phlobatannins, anthraquinones and cardiac glycosides were not detected in the leaf extract of *Alstonia boonei.* The results of this study is similar with the report of Arogbodo (2019) who reported the presence of tannins, cardiac glycosides, flavonoids, saponnins, steroids and terpenoids in ethanolic leaf extract of *Alstonia boonei.* Also, Opoku and Akoto (2015) reported that *Alstonia boonei*

extracts revealed the presence alkaloids, cyanogenetic glycosides, flavonoids, terpenoids and steroids and saponins.

The presence of saponins, phenols, alkaloids, tannins and cardiac glycosides were detected in *Pterocarpus santalinoides*. This is in line with the reports of Umeh *et al.* (2014) on leaf extract of *Pterocarpus santalinoides* showed that the crude extract contains alkaloids, saponins, tannins, cardiac and cyanogenic glycosides, flavonoids, terpenoids, carbohydrates and protein. Similarly, Njokuocha and Ewenike (2020) reported on the presence of alkaloids, steroidal aglycones, glycosides, proteins, carbohydrates, reducing sugars, tannins and saponins in crude leaf extracts of *Pterocarpus santalinoides.*

4.1.2 Organoleptic/sensory properties of the preserved probiotic drinks using *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides* leaf extracts

The organoleptic/sensory properties of the preserved probiotic drinks showed that there was no significant different in the organoleptic/sensory properties of the biopreserved probiotic zobo drinks from Week 1 to Week 6 using *Moringa oleifera* leaf, from Week 1 to Week 4 with zobo preserved with *Alstonia boonei* leaf and Week 1 to Week 7 with zobo preserved with *Pterocarpus santalinoides* leaf. The reports of Obasi and Mani (2023) recorded general acceptability ranging from 6.60 to 7.53 with tigernut milk sweetened with date palm fruit. Similarly, Ajayi *et al.* (2015) showed that zobo juice infused with extracts of *Moringa* seeds or ginger at (0.5 and 1%), control (0% preservative) showed that on day 0, food vendor produced zobo was more liked: appearance (4.5), aroma (4.5), taste (4.4) and general acceptability (4.7) but scores degenerated during storage. At 8 weeks of storage,

0.5% ginger zobo scored higher in all attributes. Preservation of zobo with good sensory properties during storage has been reported by Ezekiel *et al.* (2016). Their results showed that locally prepared zobo (*Hibiscus sabdariffa*) drink had 73.3% colour and 80% taste acceptability while the bright red zobo drink gave 26.7% colour with a 20% taste acceptance level.

4.1.3 Shelf-life properties of the probiotic drinks using *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides* leaf

The results of the pH recorded in this study were slightly acidic as it ranged from 4.56 to 5.64 while the temperature ranged from 26.5 ^{0}C to 27.2 ^{0}C. Total titratable acidity ranged from 0.40 to 0.54. pH of the probiotic drinks became acidic from week 7 to week 8. The colour and odour were stable throughout the preservation period. Ashaver *et al.* (2023) reported that tigernut milk drink preserved with *Moringa oleifera* seeds had pH values ranging from 6.30 to 6.80, total titratable acidity 0.62 to 0.66. The pH values recorded is slightly acidic and comparable to the reports of Obinna-Echem and Cookey (2022) who reported that zobo preserved at different storage conditions had pH values ranging from 4.57 to 4.60 from week 1 to week 3. The results of the pH, temperature and total titratable acidity are capable of extending its shelf-life because most bacteria do not thrive in acidic conditions. It also makes growth unfavourable for most food poisoning mesophilic microbes (Akama *et al.*, 2022). According to Ashaver *et al.* (2023), acidity inhibits the growth of bacteria and this attribute contributes to shelf-life of drinks and foods that could be deteriorated by spoilage microorganisms.

4.1.4 Total viable bacterial counts during storage of the biopreserved zobo drink using the *Moringa oleifera, Alstonia boonei* and *Pterocarpus santalinoides* leaf

The results of the microbial load of the preserved drinks showed that there was increase in the microbial plate counts from week 7 to week 8. There was significant difference in the unpreserved sample and the sample preserved with 100 ml of the extract. Fungal colonies were recorded within week 5 to week 8 during the storage of the probiotic drinks. Microbial load recorded ranged from 0 to 9.2 x 10^2 cfu/ml with drinks preserved with *Alstonia bonnie* having the highest count. Ajayi *et al*. (2015) revealed microbial load of raw material ranged from (7.8×10^4 to TNTC), (3.0×10^3 to 3.0×10^4) and (5.6×10^4 to 8.0×10^4) CFU/g, while zobo juice on day 0 had counts ranging from (2.23×10^3 to TNTC); (3.0×10^2 to 8.0×10^4); (4.0×10^4 to 5.6×10^4), CFU/mL for total viable, staphylococcal and fungal count respectively. There was zero enterobacteriaceae count on day 0 but increased during storage. *Moringa* and ginger zobo juice overall had reduced microbial load during storage compared to zobo without preservative.

Ezekiel *et al.* (2016) in their report showed an average total heterotrophic bacterial counts of 1.87E+06 and 1.49E+05 cfu/g were obtained for dark red zobo (DRZ) and bright red zobo (BRZ) samples with total coliform counts of 1.63E+04 cfu/ml (DRZ) and 1.56E+03 cfu/ml (BRZ). Their results showed that both zobo drink varieties met the International Commission on the Microbiological Specifications for Foods (ICMSF) limit of $1x10^7$cfu/ml set for total aerobic plate counts and so, should be consumed to boost local production and a healthy lifestyle. In a report by Edward

and Ohaegbu (2014), ginger and garlic were able to extend the shelf-life of Kunun-zaki stored under ambient conditions for 10 days. Their result showed the potential of the combination treatment of ginger and garlic as antimicrobials and in extending the shelf-life of Kunun-zaki. The efficacy of the medicinal plants in the preservation of the probiotic drinks is dependent on the phytochemical constituents present in the plants as shown in Tables 1. The presence of phytochemicals in medicinal plants has been reported to be associated with inhibition of microorganisms thereby effecting antimicrobial activities.

Saponins which were present in the medicinal plants have been reported to have anti- inflammatory, emetics, antiviral, antifungal, insecticidal, molluscicidal, piscidal and anti-bacterial activity. The mode of action for the anti-bacterial effects involves membranolytic properties of the saponin as well as lowering of the surface tension of the extracellular medium. They also bind to primary bile acids, which are metabolized by colon bacteria into secondary bile acids (Idowu *et al.*, 2015).

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. They exhibit antimicrobial activity by complexing nucleophilic proteins by hydrogen bonding, covalent bonding, and nonspecific interactions. Tannins form complexes with proline-rich proteins that inhibit cell protein synthesis. Synergistic action of tannins, flavonoids, alkaloids and saponins are known to inhibit the growth of pathogens. Tannins exert antimicrobial activities by iron deprivation, hydrogen bounding or specific interactions with vital proteins such as enzymes in microbial cells. Medicinal plants that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and

dysentery (Unegbu *et al.*, 2020). Similarly, the main targets of tannins are cell wall and cell membrane adhesin proteins, hence inactivating microbial adhesion which is the first step in establishment of infections. They also cause cell wall/membrane disruption. This also inactivates microbial enzymes and cell envelope transport proteins by processes that may involve reaction with sulfhydryl groups of proteins. They also accumulate /complexes metal ions (e.g. cobalt, manganese, iron, copper, etc.) necessary for microbial growth as co-factors and activators of enzymes. They also inhibit viral reverse transcriptase (Unegbu *et al.*, 2020).

Alkaloids are plant-derived compounds that are toxic or physiologically active. Some alkaloids such as isopteropodine, pteropopine have anti-microbial activity whereby they act by promoting white blood cells to dispose harmful micro-organisms and cell debris. Alkaloids can alter DNA, selectively deform cells, and cause locoism. Some alkaloid molecules, both natural and synthetic, can act as narcotics (Cushnie *et al*., 2014). Phenols are class of organic compounds containing a hydroxyl group and a benzene ring. Phenols protect against pathogens, prevent heart ailments and acts as anti-inflammatory agents. Cardiac glycosides phytochemical occurs as a complex mixture together in the same plant and most of them are toxic, however many have pharmacological activity especially to the heart.

4.1.5 Isolation and identification of microorganisms associated with the preserved probiotic drinks

The bacterial isolates were; *Pseudomonas, Bacillus, Lactobacillus* and *Leuconostoc* species. Four (4) fungal isolates were; *Saccharomyces, Kluyveromyces, Aspergillus* and *Rhizopus* species. *Lactobacillus, Bacillus, Saccharomyces* and *Leuconostoc*

species are known to be probiotic microorganisms (Fijan, 2014). Their presence in the probiotic drinks is an indication of health benefits which they could attribute to human microbiota. The results of this study had fewer bacteria and were more of probiotic bacteria compared to the reports of Izah *et al.* (2015) who reported the isolation of *Leuconostoc, Lactobacillus* species and other bacterial species from zobo drinks.

Nworie *et al.* (2016) isolated *Staphylococcus aureus and Escherichia coli* from locally made zobo drinks sold in Abakaliki Metropolis. Obasi et al. (2018) isolated *Staphylococcus epidermidis, Corynebacterium* species, *Klebsiella* species, *Pseudomonas* species, *Escherichia coli* and *Proteus* species from zobo drinks sold in Awka Metropolis, Anambra State. Ire *et al.* (2020) reported the isolation of *Staphylococcus, Corynebacterium, Pseudomonas, Enterobacter, Rhizopus, Aspergillus, Candida, Rhizopus, Fusarium* and *Saccharomyces* species from ready-to-drink tigernut drinks sold within Port Harcourt metropolis, Rivers State, Nigeria.

4.1.6 Significant relationship in the biopreservative efficacy of the different medicinal plants in the shelf-life of probiotic drinks

The association between biopreservative efficacy of the different medicinal plants and the shelf-life properties of probiotic drinks using independent t-test. There was a significant association between the biopreservative efficacy of the medicinal plants and shelf-life extension properties of the biopreserved probiotic drinks. Probiotic drinks preserved using *Alstonia bonnie* leaf showed poor preservative efficacy compared to the ones preserved using *Moringa oleifera* and *Pterocarpus santalinoides* leaf.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The results of this study revealed the presence of important phytochemicals in the three medicinal plants used. Probiotic drinks preserved with higher proportion of the medicinal plant extracts showed higher shelf-life properties compared to the ones preserved with lower proportion of the medicinal plant extracts.

The pH of the probiotic drinks became acidic from week 7 to week 8. The colour and odour were stable throughout the preservation period. There was increase in the total viable bacterial count from week 6 to week 8 with control sample (unpreserved zobo drink) having highest microbial load. There was a significant association between the biopreservative efficacy of the medicinal plants and shelf-life properties of the biopreserved probiotic drinks.

Probiotic drinks preserved using *Alstonia bonnie* leaf showed poor preservative efficacy compared to the ones preserved using *Moringa oleifera* and *Pterocarpus santalinoides* leaf.The study concluded that medicinal plants could be used in the preservation of probiotic drinks; hence, their uses should be adequately exploited in the society.

5.2 RECOMMENDATIONS

1. Combination of these medicinal plants in order to determine their synergistic effect on spoilage microorganisms of foods and drinks should be investigated.

2. Government should support researches on the exploitation of medicinal plants as preservatives in the society in order to ameliorate the rate of synthetic preservative usage in the society.

3. Also, there is need to adopt the use of natural plant materials in the preservation of foods and drinks in the society.

4. There should be further studies on the biopreservative efficacy of these medicinal plants in the shelf-life extension of some perishable food products in the society.

5.3 SUGGESTIONS FOR FURTHER STUDIES

Considering the findings from this study, it is suggested that further studies on Gas Chromatography – Mass Spectrometry of the extracts be conducted in order to evaluate the different phytoconstituents present in the extracts as well as their structures for high accuracy and precision of the chemicals.

Similarly, further studies on the use of these medicinal plant extracts in preservation of other food products such as meat and other low shelf-life food products should be considered.

5.4 CONTRIBUTION TO KNOWLEDGE

The findings from this study have helped in adding to existing knowledge on the importance of medicinal plants in the society. Apart from their medicinal uses, these plants could be used in the preservation of drinks and foods as they have the potential of inhibiting spoilage microorganisms thereby increasing shelf-life of the preserved drinks.

REFERENCES

Abbas, R.K., Elsharbasy, F.S., & Fadlelmula, A.A. (2018). Nutritional values of *Moringa oleifera*, total protein, amino acid, vitamins, minerals, carbohydrates, total fat and crude fiber, under the semi-arid conditions of Sudan. *Journal of Microbial and Biochemical Technology*, **10**, 56-58.

Adedeji, K., & Ade-Omewaye, O. (2014). Effects of *Aframomum danielli* and *Zingiber officinale* crude extract on the storability of fried bean cake snacks. *International Journal of Food Microbiology*, **111**(2), 164-169.

Adedokun, I.I., Okorie, S.U., Nwokeke, B.C., & Onyeneke, E.N. (2012). Effect of *Aframomum danelli* and black pepper crude extracts on physio-chemical and sensory properties of *Kunun-zaki* during storage. *Journal of Food Technology*, **10**, 97-102.

Adesokan, I.A., Abiola, O.P., Adigun, M.O., & Anifowose, O.A. (2014). Analysis of quality attributes of *Hibiscus sabdariffa* (Zobo) drinks blended with aqueous extract of ginger and garlic. *African Journal of Food Science*, **7**(7), 174 – 177.

Ajah, A., Umerie, S.C., Emelugo, B.N., & Nwobi, S.C. (2015). GC-MS analysis and anti-bacterial property of *Pterocarpus santalinoides* leaf from Abakaliki, Ebonyi State, Nigeria. *International Journal of Pharmaceutical Science Invention*, **3**(4), 17 – 22.

Ajayi, O.A., Lessor, A.G., & Akinwunmi, O.O. (2015). Quality evaluation of zobo (*Hibiscus sabdariffa*) juice preserved with *Moringa* (*Moringa oleifera)* or ginger *(Zingiber officinale)* extracts at different storage conditions. *Journal of Microbiology, Biotechnology and Food Sciences*, **6**(1), 730 – 736.

Akama, F.O., Amove, J., & Owoicho, M.E. (2022). Quality property of beverage drink from Tiger nut milk, malted yellow maize blend. *Therapeutic Health Care*, **4**(2), 11 – 15.

Akhigbemidu, W., Musa, A., & Kuforji, O. (2015). Assessment of the microbial qualities of noodles and the accompanying seasonings. *Nigerian Food Journal*, **33**, 48 – 53.

Akoma, O., Agarry, O.O., & Nkama, I. (2012). The microbiological quality of freeze-dried *Kunun-zaki* during production and storage. *International Journal of Biology and Pharmacy*, **1**, 1397-1410.

Akoma, O., Agarry, O.O., & Nkama, I. (2013). A study on the production and consumption pattern of *Kunun-zaki*: A cereal based ethnic fermented beverage of Northern Nigeria. *British Journal Applied Science Technology*, **3**, 1220-1227.

Al-Dalali, S., Zheng, F., & Al-Farga, A. (2019). Prolonged the shelf life of different foods using the *Citrus* by- products as antimicrobials: A review article. *Annals of Agriculture and Crop Science*, **4**(1), 1039-1045.

Alfonzo, A., Martorana, A., Guarrasi, V., Barbera, M., Gaglio, R., & Santulli, A. (2017). Effect of the lemon essential oils on the safety and sensory quality of salted sardines (*Sardina pilchardus Walbaum* 1792). *Food Control*, **73**, 1265-1274.

Ani, C.P. (2021). Production, sensory and physicochemical evaluation of zobo and zobo-date wine from *Hibiscus sabdariffa* flower, pineapple, orange and lime juice using *Saccharomyces cerevisae*. *International Journal of Applied Chemical and Biological Sciences*, **2**(4), 100-110.

Ansari, F., Singh, A., & Rana, K.G. (2020). Shelf-life assessment of *Moringa oleifera* fortified (leaf powder) instant soup mixes. *Asian Journal of Diary and Food Research*, **39**(3), 251-255.

Anyanwu, C.U., & Nwosu, G.C. (2014). Assessment of antimicrobial activity of aqueous and ethanolic extracts of *Piper guineese* leaves. *Journal of Medical Research*, **8(**10), 337 – 439.

Aondo, T.O., Odiaka, N.I., Akesa, T.M., & Olaleye, O.O. (2018). Phytochemical and antifungal efficacy of different parts of *Moringa oleifera* plant extracts. *Asian Journal of Biotechnology and Bioresource Technology*, **3**(2), 1-8.

Argyri, A., Panagou, E., & Nychas, G. (2014). Monitoring microbial spoilage of foods by vibrational spectroscopy (FT-IR and Raman). *Novel Food Preservation and Microbial Assessment Techniques*, **6**, 386-434.

Arogbodo, J.O. (2019). Phytochemical screening and antimicrobial effect of ethanolic leaf extract of *Alstonia boonei* De Wild (Apocynaceae) on some selected pathogenic microorganisms. *International Journal of Current Microbiology and Applied Sciences*, **8**(7), 1373 – 1379.

Arvind, S.R., & Vyas, P. (2014). Essential oils from plants: a review. *Indian Journal of Chemical Technology*, **2**, 129–134.

Ashaver, V.A., Ariahu, C.C., Yusufu, M.I., Ariahu, E.C., & Gbuusu, B. (2023). Formulation of aqueous drinks using tigernut and moringa seeds: effect of adding chemical hurdles and fortificants on physico-chemical and rheological properties. *Asian Journal of Food Research and Nutrition*, **2**(4), 847 – 862.

Ashrafi, A., Jokar, M., & Mohammadi-Nafchi, A. (2018). Preparation and characterization of biocomposite film based on chitosan and kombucha tea as active food packaging. *International Journal of Biology and Macromolecules*, **108**, 444–454.

Ashwini M., & Desai, N. (2018). Biopreservative effect of plant extracts on the shelf life of mango cv. Raspuri. *Journal of Pharmacognosy and Phytochemistry*, **7**(6), 2245-2248.

Association of Official Analytical Chemists (2015). Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC., USA, 9-12.

Babajide, J.M., Olaluwoye, A.A., Shittu, T.A., & Adebisi, M.A. (2013). Physicochemical properties and pytochemical components of spiced cucumber-pineapple fruit drink. *Nigerian Food Journal*, **32**, 40-50.

Babatunde, O. (2017). GC-MS analysis of leaf, stem-bark and root extracts of *Alstoniaboonei. African Journal of Pharmacy and Pharmacology,* **12**(46), 577 – 581.

Bankole, Y.O., Tanimola, A.O., Odunukan, R.O., & Samuel, D.O. (2013). Preservation of zobo drink (calyces of *Hibiscus sabdariffa*) using kola nut. *Nigerian Food Journal*, **33**, 48 – 53.

Ben-Hsouna, A., Ben-Halima, N., Smaoui, S., & Hamdi, N. (2017). *Citrus lemon* essential oil: Chemical composition, antioxidant and antimicrobial activities with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. *Lipids in Health and Disease*, **16**, 146-156.

Benner, R.A. (2014). Organisms of concern but not food-borne or confirmed food-borne: Spoilage microorganisms. *International Journal of Food Microbiology*, **109**, 146-150.

Besong, E.E., Balogun, M.E., Djobissie, F.D., Mbamelu, S.O., & Obinna, J.N. (2016). A Review of *Piper guineense* (African Black Pepper). *International Journal of Pharmacy and Pharmaceutical Research,* **6**(1), 368 – 384.

Bhat, R.M., Juyal, V., & Singh, A. (2014). *Amomum subulatum Roxb*: a critical review of pharmacological and phytochemical data. *International Journal of Pharmaceuticaland Phytopharmacology*, **3**, 362–364.

Bouarab-Chibane, L., Degraeve, P., Ferhout, H., Bouajila, J., & Oulahal, N. (2019). Plant antimicrobial polyphenols as potential natural food preservatives. *Journal of Science and Food Agriculture*, **99**, 1457–1474.

Boziaris, I.S., Proestos, C., Kapsokefalou, M., & Komaitis, M. (2014). Antimicrobial effect of *Filipendula ulmaria* plant extract against selected food-borne pathogenic and spoilage bacteria in laboratory media, fish flesh and fish roe product. *Food Technology and Biotechnology,* **49**, 263–270.

Brewer, M.S. (2014). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Food Science and Food Safety*, **10**, 221–247.

Calo, J.R., Crandall, P.G., O'Bryan, C.A., & Ricke, S.C. (2015). Essential oils as antimicrobials in food systems—A review. *Food Control*, **54**, 111–119.

Carocho, M., & Ferreira, I.C. (2014). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, **51**, 15–25.

Cheesbrough, M. (2010). *District Laboratory Practice in Tropical Countries*, 4th Edition. New York, USA: Cambridge University Press.

Clarke, D., Tyuftin, A.A., Cruz-Romero, M.C., Bolton, D., Fanning, S., & Pankaj, S.K. (2017). Surface attachment of active antimicrobial coatings onto conventional plastic-based laminates and performance assessment of these materials on the storage life of vacuum-packaged beef sub-primals. *Food Microbiology*, **62**, 196–201

Cox, S., Abu-Ghannam, N., & Gupta, S. (2015). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal*, **17**, 205–220.

Cui, S., Ma, X., Wang, X., Zhang, T.A., Hu, J., Tsang, Y.F., & Gao, M.T. (2019). Phenolic acids derived from rice straw generate peroxides which reduce the viability of *Staphylococcus aureus* cells in biofilm. *Industrial Crop Production*, **140**, 111-161.

Cushnie, T.P.T., Airaodion, A.I., & Ogbuagu, E.O. (2014). Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities". *International Journal of Antimicrobial*, **44**, 377-386.

Dauda, A.O., & Adegoke, G.O. (2014). Preservation of some physico-chemical properties of soymilk-based juice with *Aframomum danielli* spice powder. *American Journal of Food Science and Technology*, **2**(4), 116-121.

Djenane, D. (2015). Chemical profile, antibacterial and antioxidant activity of Algerian *Citrus* essential oils and their application in *Sardina pilchardus*. *Food Control*, **5**, 208- 228.

Doddanna, S.J., Patel, S., Sundarrao, M.A., & Veerabhadrappa, R.S. (2014). Antimicrobial activity of plant extracts on *Candida albicans*: An *in vitro* study. *Indian Journal of Dental Research*, **24**, 401–405.

Eaton, D.L., & Groopman, J.D. (2013). *The Toxicology of Aflatoxins*. Academic Press, New York, U.S.A, 383-426.

Edward, K.C., & Ohaegbu, C.G. (2014). The effect of ginger and garlic on the microbial load and shelf-life of kunun-zaki. *Journal of Applied Pharmaceutical Science*, **5**, 150-153.

Ekeke, I. C., Nkwocha, A.C., Kamen, F.L., Kamalu, C.I.O., & Uzondu, F.N. (2015). Studies on optimal conditions for the preservation of 'zobo' drink. *International Journal of Engineering and Management Research,* **5**(6), 121 – 125.

Ekeleme, K., Tsaku, P., Nkene, I., Ufomadu, U., Abimiku, R., Oti, V., & Sidi, M. (2017). Phytochemical analysis and antibacterial activity of *Psidium guajava* L. leaf extracts. *GSC Biological and Pharmaceutical Sciences*, **1**(2), 13-19.

Elzein, M.,Fahal, A.M., Babitha, R., Aklakur, T.I., & Neelam, S. (2018). Qualitative and quantitative phytochemical analysis of *Moringa oleifera* (Lam) pods. *International Journal of Current Microbiology and Applied Science,* **7**(05), 657-665.

Emencheta, S.C., Enweani, I.B., Oli, A.N., Okezie, U.M., & Attama, A.A. (2019). Evaluation of antimicrobial activities of fractions of plant parts of *Pterocarpussantalinoides*. *Biotechnology Journal International*, **23**(1), 1–11.

Espina, L., Somolinos, M., Loreen, S., Conchello, P., Garcia, D., & Pageen, R. (2014). Chemical composition of commercial *Citrus* fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Contamination,* **22**, 896-902.

Etejere, E.O., Olayinka, B.U., & Lawal, L.O. (2015). Comparative studies of phytochemical constituents of leaf, bark and root of *Moringa oleifera* Lam. *Jewel Journal of Scientific Research,* **3**(1), 173 -176.

Ezearigo, O.E., Adeniji, P.O., & Ayoade, F. (2014). Screening of natural spices for improving microbiological, nutritional and organoleptic qualities of the zobo drink. *Journal of Applied Biosciences,* **76**, 6397-6410.

Ezeigbo, O., Ekaiko, M., Agomo, K., Ojukwu, K., & Nnadozie, A. (2015). Antimicrobial effect of lime juice treatment on the shelf-life of zobo drink. *British Microbiology Research Journal,* **6**(3), 147-153.

Ezekiel, T., Solomon, L., Oforibika, A.G., &Daminabo, V. (2016). Nutritional, sensory and bacteriological quality of two varieties of locally prepared zobo (*Hibiscus sabdariffa*) drink. *World Rural Observations,* **8**(3), 99 – 104.

Favela-Hernandez, J.M., Gonzalez-Santiago, O., Ramirez, M., & Esquivel-Ferrino, P. (2016). Chemistry and pharmacology of *Citrus sinensis*. *Molecules,* **21**(2), 247-254.

Fijan, S. (2014). Microorganisms with claimed probiotic properties: An overview of recent literature. *International Journal of Research in Public Health,* **11**(5), 4745 – 4767.

Folasade, M., &Oyenike, O. (2012). Effect of sesame seed addition on the chemical and sensory qualities of sorghum based *Kunun-zaki* drink. *African Journal of Food Science and Technology*, **3**, 204-212.

Gajendrasinh, P.R., Bhavika, M.K., Rohit, S., Hetal, A., & Prajapati, P.K. (2014). *In vitro* Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (*Azadirachta indica L.*) and Tulsi (*Ocimum sanctum L.*). *International Journal of Pharmaceutical and Biological Association*, **3**(3), 582-586.

Gao, M., Feng, L., Jiang, T., Zhu, J., Fu, L., Yuan, D. & Li, J. (2014). The use of rosemary extract in combination with nisin to extend the shelf life of pompano (*Trachinotus ovatus*) fillet during chilled storage. *Food Control*, **37**, 1–8.

Germplasm Resources Information Network (2017). *Persicaria maculosa.* Agricultural Research Service, United States Department of Agriculture.

Guo, L., Sun, Q., Gong, S., Bi, X., Jiang, W., Xue, W., & Fei, P. (2019). Antimicrobial activity and action approach of the olive oil polyphenol extract against *Listeriamonocytogenes*. *Frontier Microbiology*, **10**, 1586 – 1599.

Gupta, K.V., & Sharma, S.K. (2014). Plants as natural antioxidants. *Indian Journal of Natural Products and Resources*, **5**, 326–334.

Gupta, N., Srivasta, A. & Pandey, V. (2012). Biodiversity and nutraceutical quality of some Indian millets. *Biological Sciences,* **82**(2), 110-118.

Hao, Q., Dong, B.R., & Wu, T. (2015). Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database System Review,* **2**, 6895-6901.

Harholt, J., Moestrup, O., &Ulvskov, P. (2016). Why plants were terrestrial from the beginning. *Trends in Plant Science*, **21**(2), 96-101.

Hasija, S., Ibrahim, G., & Wadia, A. (2015). Antimicrobial activity of *Citrus sinensis* (orange), *Citrus limetta* (sweet lime) and *Citrus limon* (lemon) peel oil on selected food-borne pathogens. *International Journal of Life SciencesResearch*, **3**, 35-39.

Hayam, M. I., Ferial, M., & Abu-Salem, S. (2013). Effect of adding lemongrass and lime peel extracts on chicken patties quality. *Journal of Applied Science Research,* **9**(8), 5035-5047.

Hudson, N.D. (2013). *Hibiscus sabdariffa*: A research review of its uses and safety. *International Journal of Biology and Pharmacy*, **1**, 1397-1410.

Idowu, L., & Oseni, O. (2015). Compositional investigation of phytochemical and antioxidant properties of various parts of *Moringa oleifera* plant. *African Journal of Pharmacy and Pharmacology,* **12**(46), 577 – 581.

Ihedioha, T.E., Asuzu, I.U., Anaga, A.O., & Ihedioha, J.I. (2019). Hepatoprotective and antioxidant activities of *Pterocarpus santalinoides* methanol leaf extract. *African Journal of Pharmacy and Pharmacology*, **13**(18), 359–373.

Imo State Government (2018). Map of Imo State and History. Imo State Government Report.

Ire, F.S., Goziem, K., Benneth, B., & Maduka, N. (2020). Microbiological evaluation of ready-to-drink tigernut drinks sold within Port Harcourt Metropolis, Rivers State, Nigeria. *Asian Food Science Journal*, **16**, 45 – 58.

Irkin, R., & Esmer, O.K. (2015). Novel food packaging systems with natural antimicrobial agents. *Journal of Food Science Technology,* **52**, 6095-6111.

Izah, S.C., Kigigha, L.T., & Aseibai, E.R. (2015). A review of the quality assessment of *zobo* drink consumed in Nigeria. *Journal of Microbiology, Food Science and Biotechnology Innovations*, **11**, 34-44.

Kabiru, Y.A., Makun, H.A., Saidu, A.N., Muhammad, L.H., Nuntah, L.C., & Amoo, S.A. (2012). Soymilk preservation using extracts of cloves *Syzygium aromaticum*Myrtaceae and guinea-pepper *Xylopia aethiopica. Annonaceae*, **3**(5), 44-50.

Kahramanoglu, L., & Usanmaz, O. (2017). Effects of black seed oil on the shelf-life of freshly squeezed pomegranate juice. *Food Science and Technology,* **7**, 54–62.

Kang, J., Jin, W., Wang, J., Sun, Y., Wu, X., & Liu, L. (2019). Antibacterial and anti-biofilm activities of peppermint essential oil against *Staphylococcus aureus*. *Food Science Technology*, **101**, 639–645.

Kant, R., & Broadway, A. (2015). The benefits of consuming soya milk – A review. *Trends in Biosciences,* **8**(5), 1159-1162.

Kavas, N., & Kavas, G. (2016). Physical-chemical and antimicrobial properties of Egg White Protein Powder films incorporated with orange essential oil on Kashar Cheese. *Food Science and Technology,* **36**, 672-678.

Kharchoufi, S., Licciardello, F., Siracusa, L., Muratore, G., Hamdi, M., & Restuccia, C. (2018). Antimicrobial and antioxidant features of 'Gabsi' pomegranate peel extracts. *Industrial Crop Production,* **111**, 345–352.

Khodke, S.U., Shinde, K.S., & Yenge, G.B. (2015). A study on the storage of sterilized soymilk. *International Journal of Farm Sciences*, **4**(4), 166-179.

Khushwaha, K., O'Bryan, C. A., Babu, D., Crandall, P. G., Chen, O., & Lee, S. O. (2014). Human health effects of isoflavones from soybeans. *Agriculture, Food and Analytical Bacteriology*, **4**, 122-142.

Klangpetch, W., Phromsurin, K., Hannarong, K., Wichaphon, J., & Rungchang, S. (2016). Antibacterial and antioxidant effects of tropical citrus peel extracts to improve the shelf-life of raw chicken drumettes. *International Food Research Journal*, **23**, 700-707.

Kohli, D., Kumar, S., Upadhyay, S., & Mishra, R. (2017). Preservation and processing of soymilk: A review. *International Journal of Food Science and Nutrition*, **2**(6), 66-70.

Kumar, D., & Jhariya, N.A. (2013). Nutritional, medicinal and economic importance of corn: A mini review. *Research Journal of Pharmaceutical Sciences*, **2**, 7–8.

Kumar, H., Shanmugavadivu, M., Rajamania, R., & Kuppsamy, S. (2014). Antibacterial activity of different solvent extracts of medicinal plant: *Ruta graveolens* L. *International Journal of Bioscience and Nanoscience*, **1**, 9–11.

Kumar, S., & Pandey, A.K. (2014). Chemistry and biological activities of flavonoids: An overview. *Science World Journal*, **201**, 162-170.

Liu, X., Jia, Y., Hu, Y., Xia, X., Li, Y., & Zhou, J. (2016). Effect of *Citrus wilsonii* Tanaka extract combined with alginate-calcium coating on quality maintenance of white shrimps (*Litopenaeus vannameiboone*). *Food Control*, **68**, 83-91.

Lukas, S., Carolina, S., Hoffman, G., Sven, K., Laure, A., Iqbal, K., Khalid, B., & Heiner, A. (2018). Food groups and risks of all-cause mortality: A systematic review and meta-analysis of prospective studies. *The Journal of Clinical Nutrition*, **105**(6), 34-46.

Magoulas, A.K. (2014). *Save Money by Knowing When Food is Safe.* U.S. Department of Health and Human Services.

Maji, A.A., Omale, J., & Chigozie, O.E. (2011). Effect of chemical treatment and pasteurization on the shelf-life of *Kunun-zaki* (sorghum and maize gruel). *European Journal of Food Research and Review*, **1**, 61-70.

Min, K.Y., Kim, H.J., Lee, K.A., Kim, K.T., & Paik, H.D. (2014). Antimicrobial activity of acid-hydrolyzed *Citrus unshiu*peel extract in milk. *Journal of Dairy Science*, **97**, 1955-1960.

Mubarak, A.J., Rifky, A.L.M., Shabry, C., & Ramalkeera, C.S. (2018). Food preservative characteristics of dehydrated murunga (*Moringa oleifera*) leaf powder. *International Journal of Academic and Applied Research*, **2**(8), 18-26.

Muste, S., Anamaria, P.O.P., Paucean, A., Chis, S., Man, S., Salanta, L., Marc, A., Muresan, A. & Martis, G. (2019). Herbs and spices in terms of food preservation and shelf-life. *Hop and Medicinal Plants*, **5**, 1-8.

National Health Service (2018). *Probiotics*. National Health Service Report.

Ngoci, S.N., Mwendia, C.M., & Mwaniki, C.G. (2014). Phytochemical and cytotoxicity testing of *Indigofera lupatana* Baker F. *Journal of Animal & PlantSciences*, **11**(1), 1364-1373.

Njokuocha, R.C., & Ewenike, A.E. (2020). Antibacterial and phytochemical properties of crude leaf extracts of *Moringa oleifera* Lam., *Pterocarpus santalinoides* L'Herit DC and *Ceiba pentandra* L. on some clinical bacterial isolates in Nigeria. *Journal of Complementary and Alternative Medical Research,* **10**(4), 1 – 15.

Njue, L., Kanja, L.W., Ombui, J.N., Nduhiu, J.G., & Obiero, D. (2014). Efficacy of antimicrobial activity of garlic extracts on bacterial pathogens commonly found to contaminate meat. *Eastern African Medical Journal*, **3**, 78-85.

Nkono, N.Y., Sokeng, S.D., Désiré, D.P. &Kamtchouing, P. (2014). Antihyperglycemic and antioxydant properties of *Alstonia boonei* De Wild. (Apocynaceae) stem bark aqueous extract in dexamethasone-induced hyperglycemic rats. *International Journal of Diabetic Research,* **3**(3), 27-35.

Nwankwo, C.S., Ebenezer, I.A., Ikpeama, A.I., &Asuzu, F.O. (2014). The nutritional and anti-nutritional values of culinary herbs – uziza leaf (*Piper guineense*) and scent leaf (*Ocimum grastissium)* popularly used in Nigeria. *International Journal of Science and Engineering Research,* **5**(12), 1160 – 1163.

Nwokorie, C.C., Nwachukwu, N.C., Ezeanokete, C.C., & Ike, C.C. (2015). The phytochemical and antimicrobial analysis of *Pterocarpus santalinoides* plants. *Asian Journal of Science and Technology,* **6**, 1411 – 1418.

Nzeako, B.C., Al-Kharousi, Z.S.N., & Al-Mahrooqui, Z. (2016). Antimicrobial activities of clove and thyme extracts. *Sultan Qaboos University Medical Journal*, **6**, 33–39.

Obasi, B.C., & Mani, V.N. (2023). Evaluation of sensory and microbiological quality of tigernut milk drink sweentened with date palm fruit. *International Journal of Science and Research Archive*, **10**(01), 281–288.

Obasi, C., Okafor, U.C., Ogonna, U.S., & Oluebube, O. (2018). Bacteriological examination of sorrel beverage (zobo) drinks sold in Awka metropolis. *Journal of Bioscience and Bioengineering,* **4**(2), 14 – 17.

Obinna-Echem, P.C., & Cookey, F.I. (2022). Physicochemical properties of *Hibiscus sabdarifa* drinks in different packaging materials during ambient and refrigeration storage. International Journal of Food Science and Nutrition, **7**(3), 170 – 178.

Ochei, J., & Kolhatkar, A. (2010). *Medical Laboratory Science: Theory and Practice.* New Delhi, India: Tata McGraw-Hill Publishers.

Ogbonna, P.C., Idumah, M.C., & Idumah, M.C. (2018). Phytochemical and mineral content in leaves, stem and bark of *Pterocarpus santalinoides* (nturukpa) from Afikpo, Ebonyi State, Nigeria. *Journal of Applied Sciences and Environmental Management,* **22**(8), 1147–1150.

Ogunwenmo, K.O., Idowu, O.A., Innocent, C., Esan, E.B., & Oyelana, O.A. (2014). Cultivars of *Codiaeum variegatum* (L.) Blume (Euphorbiaceae) show variability in phytochemical and cytological characteristics. *Journal of Biotechnology*, **20**, 2400- 2405.

Okechukwu, V.U., Eze, S.O., Omokpariola, D.O., & Okereke, J.F. (2021). Phytochemical constituents of methanol extract of *Moringa oleifera* Lam. whole leaf by Gas Chromatography-Mass Spectrometry and Fourier transform infrared spectroscopy analysis. *World News of Natural Sciences,* **37**, 18 – 30.

Okwuonu, U.C., Dorothea, B., Njoya, H., & Iyemene, P.T. (2017). Phytochemical, proximate and elemental constituents of *Aspilia africana* (Wild sunflower) flowers. *Journal of Medicinal Plants Studies*, **5**(4), 22-27.

Oluwajoba, S.O., Akinyosoye, F.A., & Oyetayo, O.V. (2013). Comparative sensory and proximate evaluation of spontaneously fermenting *kunu-zaki* made from germinated and ungerminated composite cereal grains. *Food Science Nutrition,* **1**, 336-349.

Oluwalana, I.B., & Adedeji, T.O. (2013). Nutritional composition of a non-alcoholic beverage spiced with *Zingiber officinale* extract produced from Sorghum bicolor stem sheath. *International Journal Food Science and Nutrition England*, **3**, 21-27.

Opoku, F., & Akoto, O. (2014). Antimicrobial and phytochemical properties of *Alstoniaboonei*Extracts. *Organic Chemistry and Current Research*, **1**, 137 – 142.

Oranusi, U.S., & Braide, W. (2012). A study of microbial safety of ready-to-eat foods vended on highways: Onitsha-Owerri, south east Nigeria. *International Research Journal of Microbiology,* **3**(2), 066-071.

Orishagbemi, C.O, Bushiratu, A., Isah, R.L., Igbatigbi, J.M., & Achimugu, S. (2020). Organic preservation and shelf-life evaluation of liquid kunu-zaki food drink, with extract of West African pepper (*Piper guineense*). *International Journal of Agricultural Research and Food Production,* **3**, 78-86.

Osuntokun, O.T., & Ajiga, P.J. (2020). Toxicological assessment of synergistic efficacy of *Alstonia boonei* and *Capacium frutescens* extract on *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) infected Swiss albino mice. *Annals of Pharmacology and Pharmacy*, **5**(4), 1187-1194.

Ouattara, L., Kudi, T.M.I., Banta, A.L., Akpoko, J.G., & Waynet, D. (2017). Preservative effects of different treatments and their flavor acceptability in cashew apple and pineapple blend juices. *Journal of Food Science and Technology,* **6**, 67-84.

Parlapani, F.F., Mallouchos, A., Haroutounian, S.A., & Boziaris, I.S. (2017). Volatile organic compounds of microbial and non-microbial origin produced on model fish substrate un-inoculated and inoculated with gilt-head sea bream spoilage bacteria. *Food Science and Technology*, **78**, 54–62.

Pezeshk, S., Rezaei, M., & Hosseini, H. (2014). Effects of turmeric, shallot extracts and their combination on quality characteristics of vacuum-packaged rainbow trout stored at 4 ± 1°C. *Food Science*, **76**, 387–391.

Promila, P., & Madan, V.K. (2018). A review on the phytochemistry and pharmacology of *Cymbopogon citratus* Stapf. *Food Science Technology*, **40**(4), 669-675.

Rafiq, S., Kaul, R., Sofi, S.A. & Bashir, N. (2016). *Citrus* peel as a source of functional ingredient: A review. *Journal of the Saudi Society of Agricultural Sciences*, **17**(4), 23-34.

Rahman, M.H., Alam, M., & Monir, M.M. (2020). Effect of *Moringa oleifera* leaf extract and synthetic antioxidant on quality and shelf-life of goat meat nuggets at frozen storage. *International Journal of Agricultural Research and Food Production*, **3**, 78-86.

Ramadan, H., Min, B., Tiwari, A.K., Reddy, G., Adesiyun, A., & Hinton, A. (2015). Antibacterial activity of pomegranate, orange and lemon peel extracts against food-borne pathogens and spoilage bacteria *in vitro* and on poultry skin. *International Journal of Poultry Science*, **14**, 229-239.

Risiquat, R.O. (2013). Bacteriology quality of zobo drinks consumed in some parts of Osun State, Nigeria. *Journal of Applied Sciences and Environmental Management*, **17**(1), 113-117.

Salami, S.O., &Afolayan, A.J. (2020). Assessment of Antimicrobial activities and toxicological effects of green and red cultivars of roselle – *Hibiscus sabdariffa* L. *European Journal of Medicinal Plants*, **31**(15), 11–22.

Salem, A.S., Salama, W., & Ragab, W.A. (2015). Prolonged shelf-life of sour cream by adding *Moringa oleifera* leaves extract (MOLE) or *Moringa oleifera* oil (MOO). *American Journal of Food Technology*, **10**(2), 58-67.

Sanchez-Ortega, I., Garcia-Almendarez, B.E., Santos, E.M., Amaro-Reyes, A., Barboza-Corona, J.E., & Regalado, C. (2014). Antimicrobial edible films and coatings for meat and meat products. *Preservation*, **5**, 89-95.

Saraswati, A.R., Mardiah, D., & Taniwiryono, D. (2020). Formulation of ready to drink (rtd) made from roselle (*Hibiscus sabdariffa. L*) tea and stevia (*Stevia rebaudiana*) leaf safe for diabetics. *Indonesian Journal of Applied Research*, **1**(1), 1–9.

Shruthi, V.H., Ramachandra, C.T., Udaykumar, N., Sharanagouda-Hiregoudar, N., & Naik, A.R. (2019). Physico-chemical, nutritional and functional properties of roselle (*Hibiscus sabdariffa* L.). *International Journal of Current Microbiology and Applied Sciences,* **6**(12), 2976-2982.

Simas, D.L.R., De-Amorim, S.H.B.M., Goulart, F.R.V., Alviano, C.S., Alviano, D.S., & Da-Silva, A.J.R. (2017). *Citrus* species essential oils and their components can inhibit or stimulate fungal growth in fruit. *Industrial Crops and Products,* **98**, 108- 115.

Sirin, M., & Aziz, K. (2017). Role of probiotics in gastrointestinal diseases. *EC Gastroenterology and Digestive System,* **4**(20), 94-100.

Soares, M.B., Brustolim, D., Santos, L.A., Bellintani, M.C., Paiva, F.P., Ribeiro, Y.M., Tossami, T.C., & Santos, R. (2015). Physalins B, F and G, Seco-steroids purified from *Physalis angulata* L., inhibit lymphocyte function and allogeneic transplant rejection. *Journal of International Immunopharmacology,* **6**, 408-414.

Suppakul, P., Thanathammathorn, T., Samerasut, O., & Khankaew, S. (2016). Shelf life extension of "fios de ovos", an intermediate-moisture egg-based dessert, by active and modified atmosphere packaging. *Food Control*, **70**, 58–63

Swain, M.R., Anandharaj, M., Ray, R.C., & Parveen, R.R. (2014). Fermented fruits and vegetables of Asia: A potential source of probiotics. *Biotechnology Research International*, **24**, 1–19.

Tavakoli, I., Patel, S., Sundarrao, M.A., & Veerabhadrappa, R.S. (2017). Effect of Redshank essential oil combined with ice seem for extending fish shelf-life. *Food Science and Technology,* **78**, 54–62.

Tucker, G.S. (2016). *Food Preservation and Biodeterioration*, 2nd Edition. John Wiley and Sons, 1-204.

Tyagi, A.K., Bukvicki, D., Gottardi, D., Tabanelli, G., Montanari, C., & Malik, A. (2014). Chemical composition, *in vitro* anti-yeast activity and fruit juice preservation potential of lemon grass oil. *Journal of Food Technology,* **3**, 89-94.

Udeozor, L.O., & Awonorin, S.O. (2014). Comparative microbial analysis and storage of tigernut soy milk extract. *Austin Journal Nutrition FoodScience*, **2**(5), 01-06.

Ugwuona, F.U. (2014). Effect of piper extracts on pork stability. *Journal of Food Processing and Preservation,* **34**(2), 235-246.

Umaru, G.A., Tukur, I.S., & Adamu, Z. (2014). Microflora of kunun-zaki and zobo drinks in relation to public health in Jalingo Metropolis, North-Eastern Nigeria. *International Journal of Food Research,* **1**, 16 – 21.

Umeh, S.O., Umerie, S.C., Emelugo, B.N., & Nwobi, S.C. (2014). Preliminary study of the antibacterial and analgesic effect of the leaf extract of *Pterocarpus santalinoides*L'Hér. Ex DC. *International Journal of Pharmaceutical Science Invention,* **3**(4), 17 – 22.

Unegbu, V., Nkwoemeka, N., Okey-Ndeche, F., & Obum-Nnadi, C. (2020). Phytochemical and antibacterial properties of *Moringa oleifera* leaf extracts on *Escherichia coli* and *Staphylococcus aureus. Nigerian Journal of Microbiology,* **34**(1), 5145 – 5152.

Ushie, O.A., Adamu, H.M., Abayeh, O.J., Chindo, I.Y., & Lawal, U. (2014). Phytochemical screening of *Chrysophyllum albidum* leaf extracts. *Journal of Applied Chemistry*, **2**(2), 40-46.

Uwimbabazi, F., Uwimana, J., &Rutanga, J.P. (2015). Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli. Journal of Medicinal Plants Studies*, **3**(4), 85-91.

Wan, J., Zhong, S., Schwarz, P., Chen, B., & Rao, J. (2019). Enhancement of antifungal and mycotoxin inhibitory activities of food-grade thyme oil nanoemulsions with natural emulsifiers. *Food Control*, **106**, 106-119.

Wang, J.H., Xu, J.L., Zhang, J.C., Liu, Y., Sun, H.J., & Zha, X. (2015). Physicochemical properties and antioxidant activities of polysaccharide from floral mushroom cultivated in Huangshan Mountain. *Carbohydrate Polymerization*, **131**, 240–247.

World Gastroenterology Organization (2016). *Probiotics and Prebiotics*. World Gastroenterology Organization Global Guidelines, Milwaukee, Wisconsin, United States, 1-245.

Yee, A. (2014). Food expiration dates: Sustainable America. *Food Science Technology,* **40**(4), 669-675.

Zemenu, L. (2014). Effect of ginger (*Zingiber officinale*) powder addition on pH, titratable acidity and total viable bacterial counts of minced meat under refrigerated storage. *InternationalJournal of Food Microbiology*, **111**(2), 164-169.

APPENDICES

Appendix A: Operational Definition of Terms

Biopreservatives: Biopreservatives are compounds from natural sources that are able to prevent or retard spoilage related with chemical or biological deterioration that prolong product shelf-life.

Efficacy: Efficacy is the ability to perform a task to a satisfactory or expected degree.

Essential oils: Essential oils are complex mixtures of volatile organic compounds (VOCs) produced as secondary metabolites in plants and frequently responsible for the characteristic odor of plants

Medicinal plants: Medicinal plants are those plants that are commonly used (parts, extract etc) in treating and preventing specific ailments and disease that are generally considered to be harmful to human.

Plant: A plant can be defined as a living thing that grows in the earth and has stem, leaves, and roots.

Probiotics: Probiotics are live microorganisms with believe to provide health benefits when consumed, generally by improving or restoring the gut flora.

Shelf-life extension: Shelf-life extension is an effort that is made to ensure that food products are safe for long period while maintaining their original quality.

Shelf-life: Shelf-life is defined as the length of time that a commodity such as; foods and drinks can be stored without spoilage or becoming unfit for use, consumption or sale.

Zobo: Zobo is an affordable and tasty drink that is made from the edible plant petals *Hibiscus sabdariffa*.

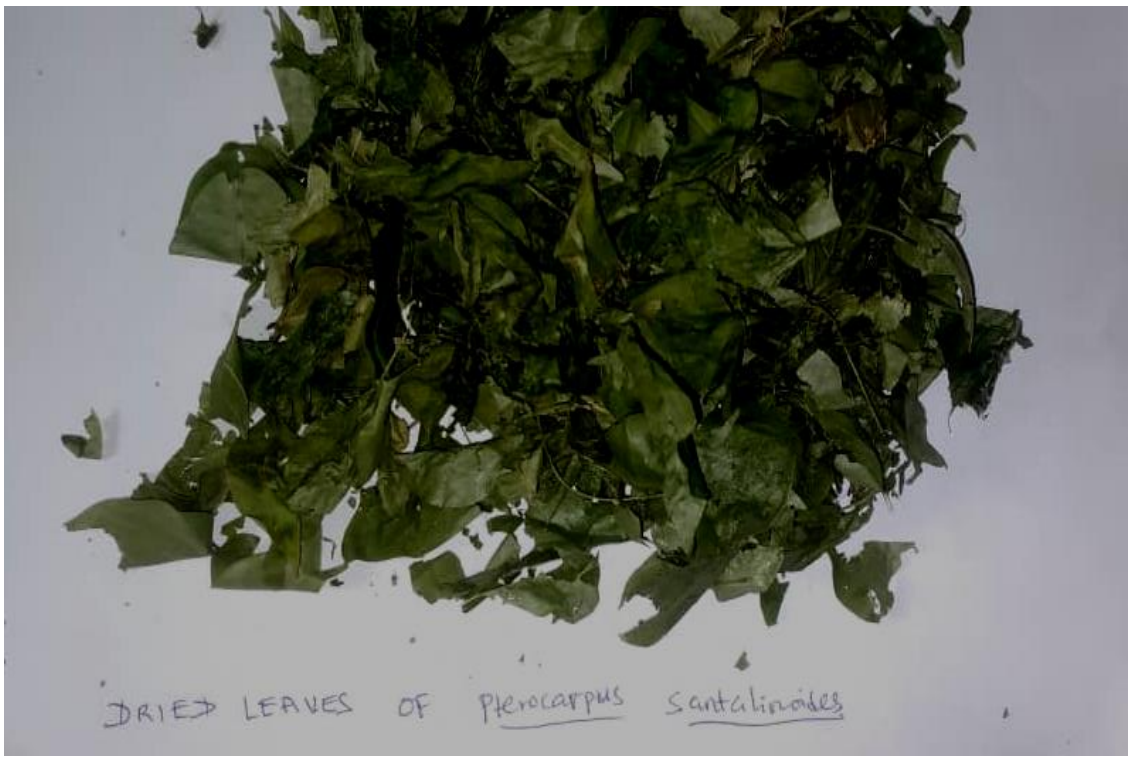
Tigernut milk: Tigernut milk is a drink made from tiger nut (C*yperus esculentus L*).

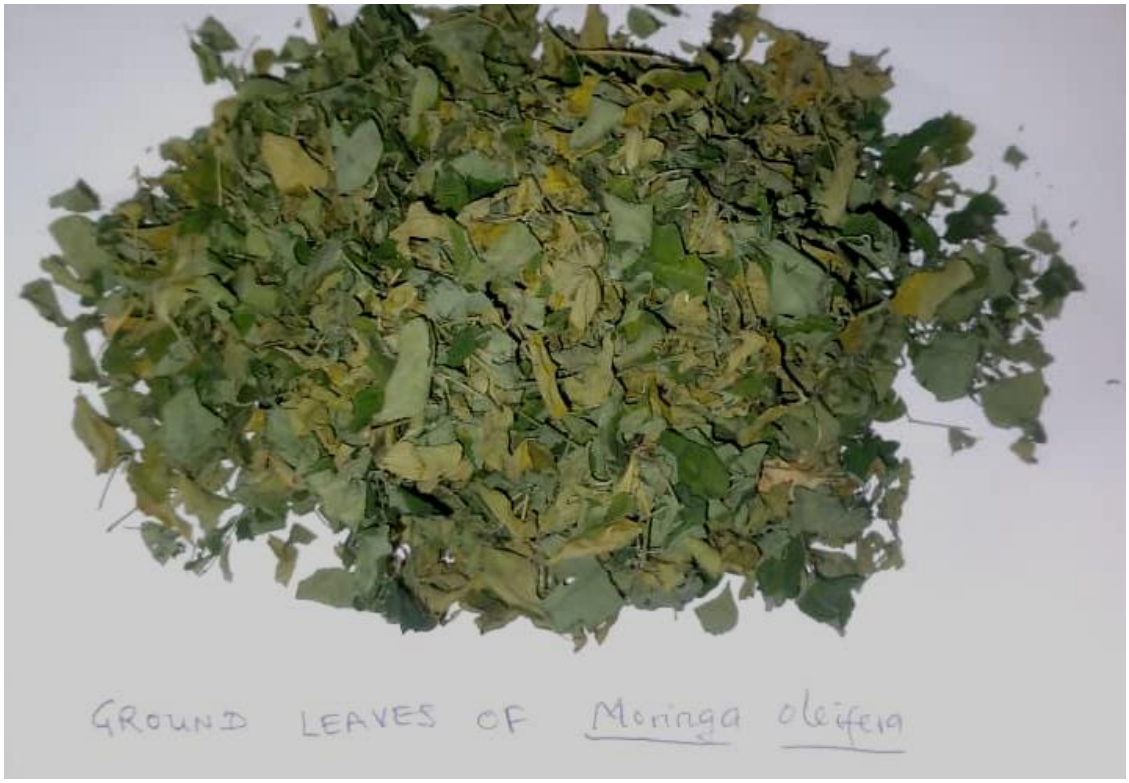
Organoleptic properties: Organoleptic properties are the aspects of food, water or other substances that create an individual experience through the senses – including taste, sight smell and touch.

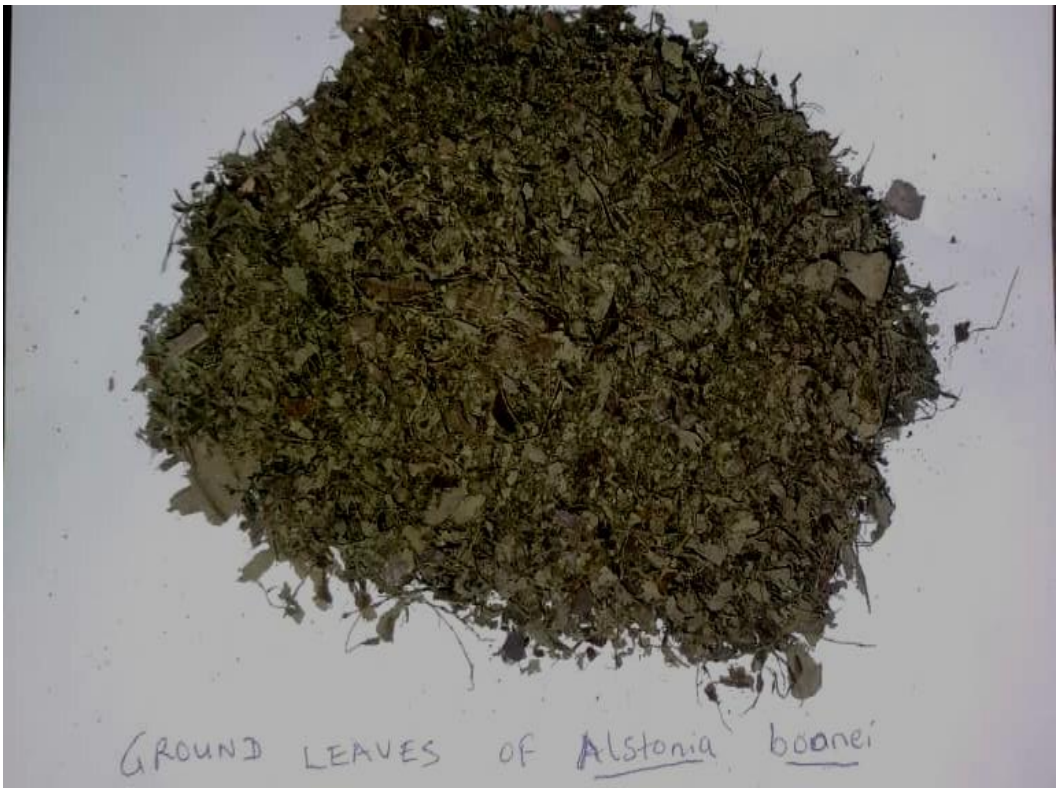
Preservation: Preservation is the act of keeping a substance in a stable condition in order to maintain its quality for an extended period.

Preservatives: Preservatives are substances used to preserve foods, drinks and other materials to prevent decomposition by microbial growth or by undesirable chemical changes.

Appendix B: Pictures of Medicinal Plants Used







Appendix C: Pictures of Produced Probiotic Drinks

Appendix D: Ethical Clearance/Informed Consent

CITI PROGRAM

Completion Date 26-Nov-2023
Expiration Date 26-Nov-2026
Record ID 59841291

This is to certify that:

Everestus chibuike Nwakire

Not valid for renewal of certification through CME.

Has completed the following CITI Program course:

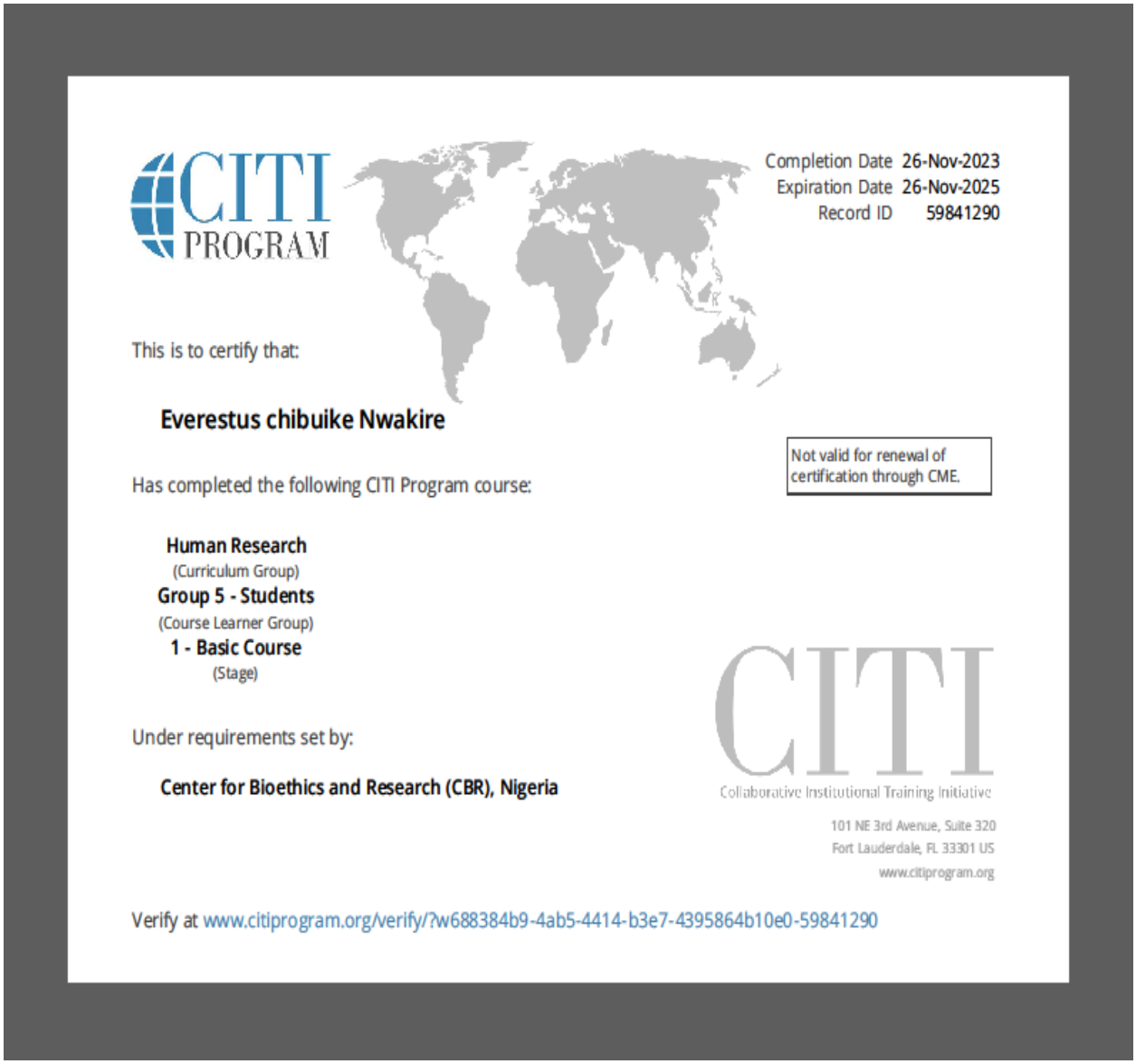
NIGERIAN NATIONAL CODE FOR HEALTH RESEARCH ETHICS
(Curriculum Group)
NIGERIAN NATIONAL CODE FOR HEALTH RESEARCH ETHICS
(Course Learner Group)
1 - Stage 1
(Stage)

Under requirements set by:

Center for Bioethics and Research (CBR), Nigeria

CITI
Collaborative Institutional Training Initiative
101 NE 3rd Avenue, Suite 320
Fort Lauderdale, FL 33301 US
www.citiprogram.org

Verify at www.citiprogram.org/verify/?wa7ce4ca1-7c84-491b-8226-6204754a1bde-59841291



CITI PROGRAM

Completion Date 26-Nov-2023
Expiration Date 26-Nov-2025
Record ID 59841290

This is to certify that:

Everestus chibuike Nwakire

Not valid for renewal of certification through CME.

Has completed the following CITI Program course:

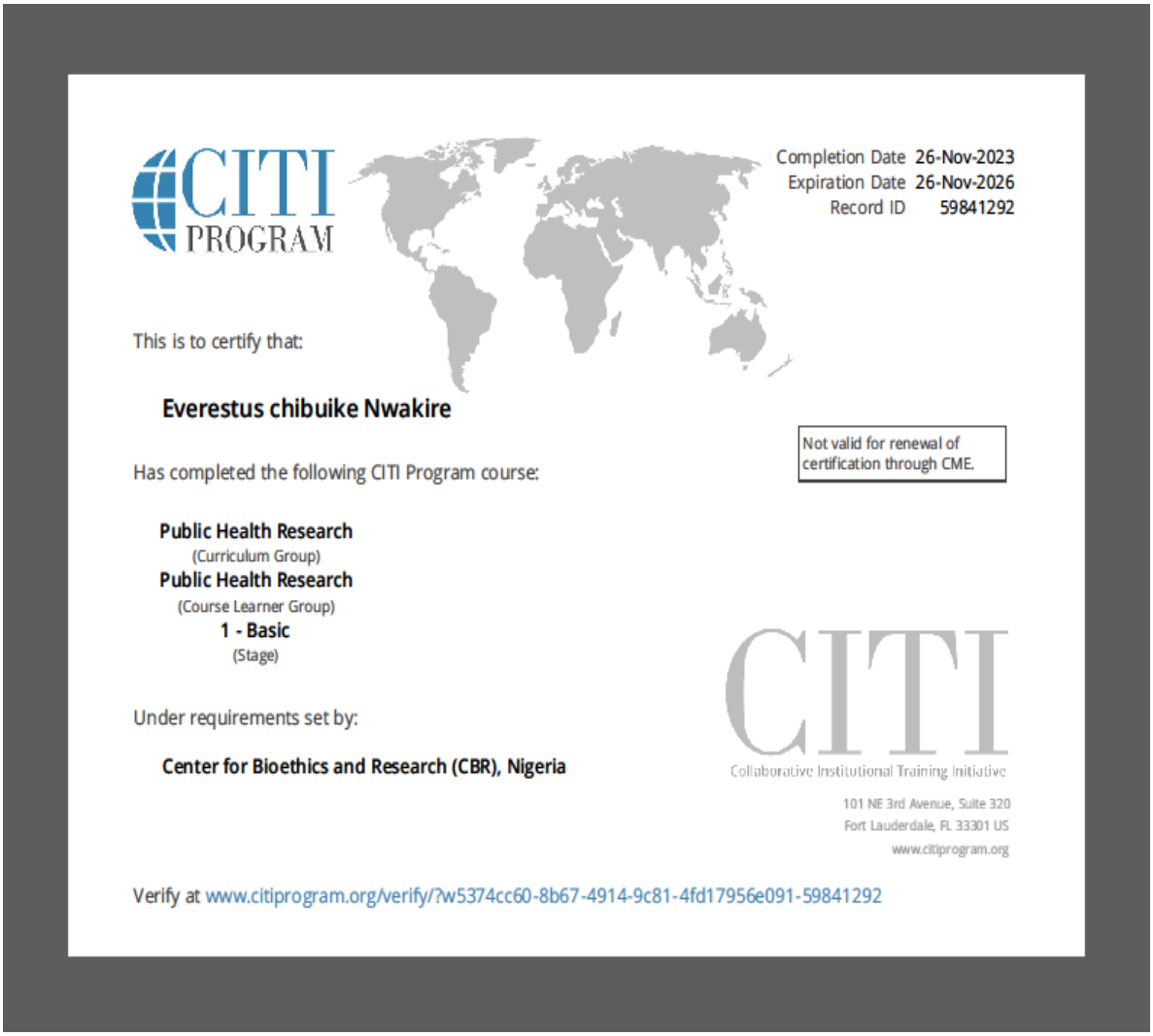
Human Research
(Curriculum Group)
Group 5 - Students
(Course Learner Group)
1 - Basic Course
(Stage)

Under requirements set by:

Center for Bioethics and Research (CBR), Nigeria

CITI
Collaborative Institutional Training Initiative
101 NE 3rd Avenue, Suite 320
Fort Lauderdale, FL 33301 US
www.citiprogram.org

Verify at www.citiprogram.org/verify/?w688384b9-4ab5-4414-b3e7-4395864b10e0-59841290



CITI PROGRAM

Completion Date 26-Nov-2023
Expiration Date 26-Nov-2026
Record ID 59841292

This is to certify that:

Everestus chibuike Nwakire

Not valid for renewal of certification through CME.

Has completed the following CITI Program course:

Public Health Research
(Curriculum Group)
Public Health Research
(Course Learner Group)
1 - Basic
(Stage)

Under requirements set by:

Center for Bioethics and Research (CBR), Nigeria

CITI
Collaborative Institutional Training Initiative
101 NE 3rd Avenue, Suite 320
Fort Lauderdale, FL 33301 US
www.citiprogram.org

Verify at www.citiprogram.org/verify/?w5374cc60-8b67-4914-9c81-4fd17956e091-59841292