EXTRACTION AND EVALUATION OF BIOACTIVE COMPOUNDS FROM TROPICAL SPICES*(Zingiber officinale, Tetrapluera tetraptera and Monodora myristica)* FOR FUNCTIONALBEVERAGE DEVELOPMENT.

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CERTIFICATION

I certify that this work, **"Extraction and Evaluation of Bioactive Compounds from Tropical Spices** *(Zingiber Officinale, Tetrapluera Tetraptera And Monodora Myristica)* **for Functional Beverage Development"**, was carried out by **Ofoedum, Arinze Francis (Reg. No. 20194199698)** in partial fulfilment for the award of Master of Science (M.Sc.) Food Science and Technology (Brewing and Beverage Technology, Option), in the Department of Food Science and Technology, School of Engineering and Engineering technology, Federal University of Technology, Owerri.

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DEDICATION

I dedicate this research work to my late mum, Mrs. Ofoedum, Mary-Cecilia who has been there for me in all dimensions.

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ABSTRACT

Crude extracts were extracted using a microwave technology from dried Ginger, *T. tetraptera*, and *M. myristica* and subsequently evaluated. Pineapple juice was extracted from freshly harvested pineapple fruits. Preliminary assays (quantitative phytochemicals and antioxidants activities) were conducted on the crude extracts generated. Beverages were produced with crude extracts from tropical spices and blended into pineapple juice as a carrier. Antioxidant activities, physico-chemical properties (pH, total solids, total acidity, brix, and viscosity), sensory evaluation and shelf life studies were conducted on the beverage formulated. The saponins were found to be highest in ginger extracts (2.71 mg/g) and differ significantly ($p \leq 0.05$) from other samples. Flavonoids, terpenoids, and tannins are highest in *Monodora myristica* with mean values of 14.33, 7.30 and 5.46 mg/g, respectively but had the least alkaloids (6.84 mg/g). *T. tetraptera* had the highest in alkaloids and steroids, with mean scores of 10.17 and 4.31, respectively, and significant differences ($p \leq 0.05$). Total phenolic compounds are highest in *M. myristica* (26.06 mg/g), followed by ginger (23.26) and *T. tetraptera* (20.93 mg/g). The crude extracts recorded higher antioxidant activities (using DPPH, FRAP, Lipid Peroxidation inhibition, and Nitric oxide scavenging tests). The crude extract from *M. myristica* gave the highest DPPH value (56.61), ginger extracts recorded the highest score for FRAP, while the control (Vit. C.) had the highest lipid Peroxidation inhibition. Among the formulated beverage samples, triple mix of ginger, *Tetrapleura* and *Monodora* had the highest DPPH value (68.20), likewise Nitric oxide scavenger (40.54) and with significant differences ($p \leq 0.05$) among the samples. FRAP is higher in sample AGT and AGY, while the control sample had the highest lipid peroxidation inhibition (2.86). Samples AGY, AMY, AGT and AGM showed no significant differences ($p \geq 0.05$). The sensory evaluation revealed that the colour of the control (Eviron Health Drink) is most preferred, followed by AGY and AMY, each recording mean scores of 8.40, 7.90 and 7.80, respectively. The taste was ranked highest for the control (8.20), followed by sample AGY (8.00) with no significant differences ($p \geq 0.05$). The flavour score for the control was 8.00, while sample AGM had the least (5.50). For the general acceptability, the control sample recorded the highest value of (8.00) followed by AGY (7.90) and with no significant differences ($p \geq 0.05$). The titrable acidity was highest in AGM (3.28 mg lactic acid) and lowest in control. The pH values ranged from 3.66 to 4.83, while TSS ranged from 2.93 mg/l to 5.38. The total sugars ranged from 2.94 to 7.17. Therefore, tropical spices can be used as additives to produce functional beverages.

Keywords: Tropical spices, Microwave assisted extraction, Pineapple juice, Functional beverages, Antioxidants, Phytochemicals, Sensory evaluation.

INTRODUCTION

1.0. Background of Study

A beverage is a drink that is designed for human consumption (Iwouno*et al.,* 2018). Drinks have major cultural roles in addition to their simple function of quenching thirst. Warm beverages have a long history, including coffee, tea, hot chocolate, and caffeinated drinks containing the stimulant caffeine (Amaravathil *et al.,* 2014).

Humans keep their water balance by drinking an equivalent amount of water as they excrete, because the body regulates water intake and excretion, beverages may contribute to the human water need. According to Spigno and Faveri, (2009), the global commercial beverage market is divided into four basic sectors: hot drinks, milk drinks, soft drinks, and alcoholic drinks. Tea and coffee are examples of hot beverages. Bottled water, carbonated soft drinks, fruit juices, ready-to-drink (RTD) teas, sports drinks, and other noncarbonated products are the five primary subcategories of soft drinks. Milk, soft drinks, and fruit juices are the most common and consumed in large quantities among the many types of beverages (Achumi *et al.,* 2018).

Soft drinks are pleasant beverages that are commonly made with 7 - 14% sugar content, 0.3 - 0.5% acid (mainly citric), flavoring, coloring, and chemical preservatives, and carbon dioxide. They may also contain caffeine, fruit juice, or both. Cola, cherry, lemon-lime, root beer, orange, grape, vanilla, ginger, fruit punch, and other additives that can be manufactured using minerals and some plant extracts (Achumi *et al.,* 2018). Furthermore, some of these soft drinks are sports drinks and/or nutraceutical beverages, which are frequently used to increase energy and stress management. A nutraceutical is defined as a food component, both nutritive and non-nutritive, that has good health benefits on disease prevention (Surth, 2003). These nutraceutical beverages may contain a variety of plant extracts, also known as bioactive substances (Achumi *et al.,* 2018), Because of their health benefits ranging from anti-stress, stimulants, antioxidant properties, anti-inflammatory, immuno-modulatory, anti-aging characteristics, and so on, these

bioactive chemicals are typically used as a single mix for pharmaceutical formulations or integrated into beverages due to their potencies in stress alleviation (Oka, 2015).

People utilize pills, medications, synthetic drinks (commonly referred to as energy drinks), and herbal preparations to treat the aforementioned stress-related issues. In addition to drugs, there are a variety of natural plant components that can be utilized to relieve stress in humans. Several plant-based foods and beverages, such as wines and fruit juices, contain key elements such as carbohydrates, amino acids, vitamins, minerals, lipids, essential oils, and so on, all of which play important health roles in the body (Farid and Shaheen, 2015). Nonetheless, due to some of the associated side effects of drugs, lots of people prefer natural substances derived from roots, herbs, and some tropical crops (spices) such as teas, coffee, ginger, turmeric, thyme, ginger, as well as some fruits and cereals (Martin, 2016). Some of these naturally occurring components have been used in pharmaceutical, cosmetics, food preparations, and food preparations due to their high concentrations of bioactive compounds/antioxidants and essential oils, which aid in stress management, body regulation, and illness prevention.

However, nutraceutical beverages came mostly from fruits and vegetables but also include those derived from other plants such as ginger, tea, turmeric, *Moringa oleifera*, coffee, chocolate, soybean, animal products such as milk and dairy-based products, and alcoholic beverages (Spigno & Faveri, 2009).

Phytochemicals present in plant foods, including fruits and vegetables, are responsible for the health benefits of such foods, and the method of action and chemical makeup of phytochemicals present vary. However, antioxidant and phytochemicals such as terpenes, isoflavones, phenolics, and polyphenolics appear to be important in health management (Bryer, 2005). These advancements have heightened interest in nutraceuticals and functional beverages (Kim *et al.*, 2005). Typically, these are employed in pharmaceutical dosage forms such pills, capsules, liquids, and others. Functional beverages, on the other hand, look like ordinary beverages and

contain physiologically active substances with health advantages above and beyond their basic nutrition. While the majority of customers may benefit from some nutraceuticals due to their diverse compositions (such as bioactives, phytochemicals, minerals, etc), however, individuals may also need to weigh the risk/benefit of such ingredients based on their genetic background and lifestyle. These bioactive beverages are used for a variety of reasons, the most important of which being therapeutic and/or health advantages (Surth, 2003).

Tropical spices such as ginger, thyme, curry, onion, garlic, and others are said to contain a variety of phytochemicals (bioactive compounds) such as carotenes, flavonoids, phenols, polyphenols, terpenes, myrcene, limonene, pinene, steroids, triterpenes, and numerous other antioxidants and essential oils which have lots of therapeutic benefits (Bone, *et al.,* 1999). However, due to their health and medicinal potentials, these tropical spices are sometimes employed in herbal remedies but largely in food preparations (Anon., 2014). However, little or no research has been conducted on the majority of these tropical spices in terms of their usage in beverage formulations; thus, the current investigation on extraction and phytochemical potentials.

1.2 Problems Statement

Some diseases and many ill health have become silent killers of several individuals all over the world. However, stress-induced health conditions such as ulcers, diabetes, cancers etc., are increasing globally. The fight against stress has assumed a wider dimension ranging from creating leisure activities and drugs and producing food and drinks to help individuals overcome stress. The use of synthetic drugs for health management also pose a long term risks, thus the need for alternative and safer means to preserve health, both in short and long term.

The extraction, quantification and utilization of bioactive compounds from tropical spices to produce beverage drinks have not been given sufficient scientific attention.

1.3 Objectives of Study

The main objective of this research is to extract and evaluate bioactive compounds from tropical spices (*Zingiber officinale, Tetrapluera tetraptera,* and *Monodora myristica*) for functional beverages development.

The specific objectives include:

(i) To extract bioactive substances from tropical ginger (*Zingiber officinale*),**Oshokirisho**(*Tetrapluera tetraptera)* and**Ehuru** (*Monodora myristica)*

(ii) To identify and evaluate the bioactive substances in the extracted tropical spices.

(iii) To extract pineapple juice from fresh pineapple fruits

(iv) To use the bioactive substances extracted for the development of functional beverages.

(v) To determine the antioxidant activity of the functional beverages

(vi) To determine the physicochemical compositions and sensory analysis of the functional beverages produced.

(vii) To determine the shelf life of the beverages developed.

1.4. Justification of Study

Many sports and nutraceutical/functional beverages are available in the market, but most are imported drinks containing substances that are not of natural origin. Understanding the types of bioactive compounds present in some tropical spices and incorporating them in the development of functional beverages will develop a new frontier in effectively utilizing these tropical spices. This research will explore tropical spices for producing functional beverages. It is expected that the findings of this study will help advance health care and benefit the farmers, as well as promote industry and commerce.

1.5. Scope of the Study

This study covers the sourcing, extraction and identification of the bioactive substances/phytochemicals in tropical spices such as ginger (*Zingiber officinale*), **Oshiokirisho** (*Tetrapluera tetraptera),* **Ehuru** (*Monodora myristica*) and extraction of pineapple juice from fresh pineapple fruits. It also entails using the extracted bioactive compounds to developfunctional beverages, conducting sensory evaluation and laboratory assays and shelf life studies on the developed beverages. Finally, the raw data obtained would be subjected to analysis of variance and the means would be separated using Fisher's least significant difference (LSD).

CHAPTER TWO

LITERATURE REVIEW

2.1. Bioactive Compounds

The term "bioactive" consists of two words: bio- and -active. Bio- is derived from the Greek words "bios" which means "life," and "active" is derived from the Latin "activus" which means "dynamic, full of energy, with energy, or involving an activity" (Islam *et al.,* 2014). Boots *et al.* (2008) defined this activity as "any phenomenon that manifests a type of life, a function, or a process." In a scientific sense, the term "bioactive" is an alternate term for "biologically active" (Valko *et al.,* 2005).

However, bioactive chemicals are a prominent component of nutraceutical and functional beverages. Furthermore, the creation of biomarkers for studying the synergistic and antagonistic effects of nutraceutical-drug interactions merits special consideration.

A chemical has biological activity if it makes direct contact with a living organism. Indeed, these molecules have a wide range of impacts, from good health maintenance and even healing to being dangerous and even death. The origins of these compounds, in addition to their activity, constitute a criterion in the definition of bioactive substances. Bioactive food components are those found in meals or dietary supplements that cause changes in health status in addition to providing basic nutritional demands eg: carotenoids, anthocyanins, and tocopherols. It is important to stress in this scenario that bioactive molecules, even if included in foods or their constituents, are not nutrients (Anokwuru *et al.,* 2011). Bioactive chemicals found in fruits and vegetables, as well as some tropical plants, including phenolics, carotenoids, anthocyanins, and tocopherols. Approximately 20% of all known plants have been used in pharmacological studies, benefitting the healthcare system by treating cancer and other harmful diseases (Nwozo *et al.,* 2006). Phytochemicals, which are abundant in fruits and vegetables, may protect against free

radical damage. Plants having beneficial phytochemicals may supplement human needs by functioning as natural antioxidants; for example, vitamins A, C, and E, as well as phenolic compounds present in plants such as flavonoids, tannins, and lignins, all work as antioxidants (Bagade *et al.,* 2008). Antioxidants reduce oxidative damage in foods by delaying or suppressing oxidation caused by reactive oxygen species (ROS), resulting in better shelf-life and quality (Cai, 2016). Anti-aging, inflammation reduction, and cancer prevention are all aided by beta carotene, ascorbic acid, and various phenolics (Ekeledo *et al.*, 2013). Many organizations and health-care systems around the world have campaigned for more fruit and vegetable consumption.

2.1.1 Types of Bioactive Compounds

Bioactive compounds are being studied thoroughly to discover their potential health benefits, and they appear to have beneficial physiological, behavioral, and immunological effects. To date, several bioactive compounds have been discovered. These compounds are categorised based on their chemical form and function. Carotenoids, flavonoids, phytosterols, phytoestrogens, glucosinolates, polyphenols, and taurine are examples of bioactive compounds. Because vitamins and minerals have pharmacological effects, they are also categorized as bioactive compounds.

Bioactive compounds can be found naturally in a number of foods. The majority of bioactive compounds have anti-carcinogenic, anti-inflammatory, and antibacterial properties. As a result, some epidemiologic studies suggest that some may have preventive advantages against cardiovascular diseases (Anokwuru *et al.,* 2011).

Phenolic compounds, particularly their subsection flavonoids, are found in almost all plants, including cereals, legumes, spices, nuts, olive oil, tea, red wine, vegetables, and fruits. They are largely antioxidants, and some studies have demonstrated that they have a positive impact on cardiovascular disease risk factors (Naczk and Shahidi, 2014).

Phytoestrogens and isoflavones can be found in soy and flaxseed oil, whole grains, fruits, and vegetables because, they are excellent free radical scavengers, carotenoids are one of the most potent antioxidant molecules (Boots *et al.,* 2008). Most fruits and vegetables contain them, including apricots, carrots, mangoes, tomatoes, and pumpkins. Although bioactive compounds are found naturally in many foods, they are also used as an additive and a processing aid. Bioactive chemicals are generally added to foods or food items to boost their health-promoting properties. The most well-known colorant bioactive compounds include carotenoids, anthocyanins, and curcumin, which are used to color a variety of food products. Ascorbic acid is a common food additive that prevents oxidation. The most common uses for cinnamon aldehyde and vanillin are to flavor sweet foods, chewing gums, and beverages (Ames *et al.,* 1993).

2.1.2 Plants' Phytochemicals and Phenolic Compounds as Antioxidants

2.1.2.1. Phenols and Phenolic Acids

Phenolic acids are carboxylic acid derivatives. Phenolic acids are mostly composed of hydroxycinnamic and hydroxybenzoic acids. Furthermore, scientists have revealed that p-coumaric, caffeic, ferulic, and sinapic acids are the primary components of hydroxycinnamic acids. These compounds are present in a wide range of fruits, vegetables, and tropical spices (Ames *et al.,* 1993). Figure 2.1a, depicts the molecular structure of phenol.

2.1.2.2. Flavonoids

Flavonoids have a low molecular weight (Ames *et al.,* 1993) (figure 2.1b). Flavone is a form of flavonoid containing two benzene rings in its chemical structure which are linked by a pyrane ring. Flavones, isoflavones, flavonoids, flavonols, flavanones, anthocyanins, and proanthocyanidins are all flavonoids, according to the flavonoid classification.

2.1.2.3. Anthocyanins

Anthocyanidins are the most basic type of anthocyanin. An aromatic ring is linked to a heterocyclic ring in anthocyanidins. A carbon bond additionally joins the heterocyclic ring to the third aromatic ring (Phang and Malek, 2013). Furthermore, due to the abundance of anthocyanins in nature, these phenolic compounds are highly complex. Anthocyanins, which may be found in a variety of fruits, have been discovered by scientists to be important compounds that can enrich and boost antioxidant activity. The structure is depicted in Figure 2.1c.

2.1.2.4. Tannins

Tannins are natural chemicals present in many plant families that include a large number of phenolic rings (figure 2.1d). Tannins are classified as hydrolyzable or condensed. Condensed tannins contain flavonoid units that have various degrees of condensation. Hydrolyzable tannins have a simple phenol structure with ester linkages. Tannins can be hydrolyzed by a range of factors, including alkaline compounds, mineral acids, and enzymes (Ren and Zhang, 2007).

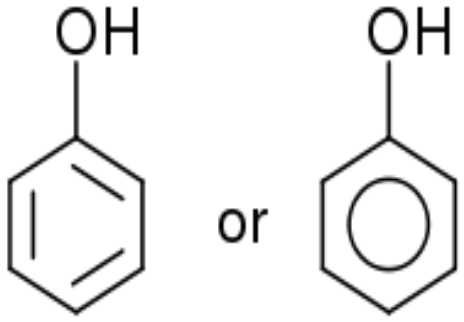

Figure 2.1 (a): Phenol structure

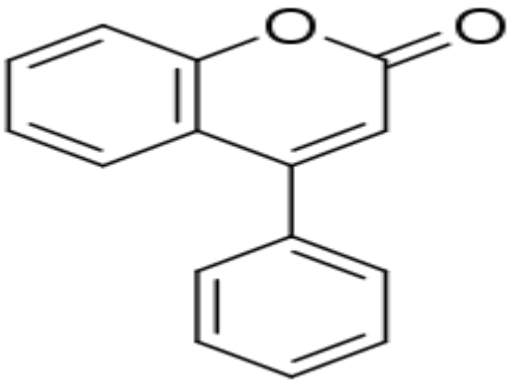

Figure 2.1 (b): Structure of flavonoids

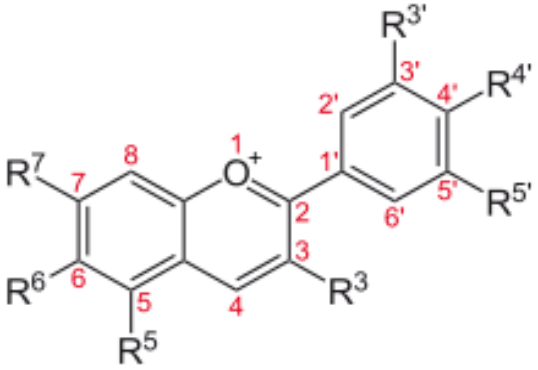

Figure 2.1 (c): Structure of Anthocyanin

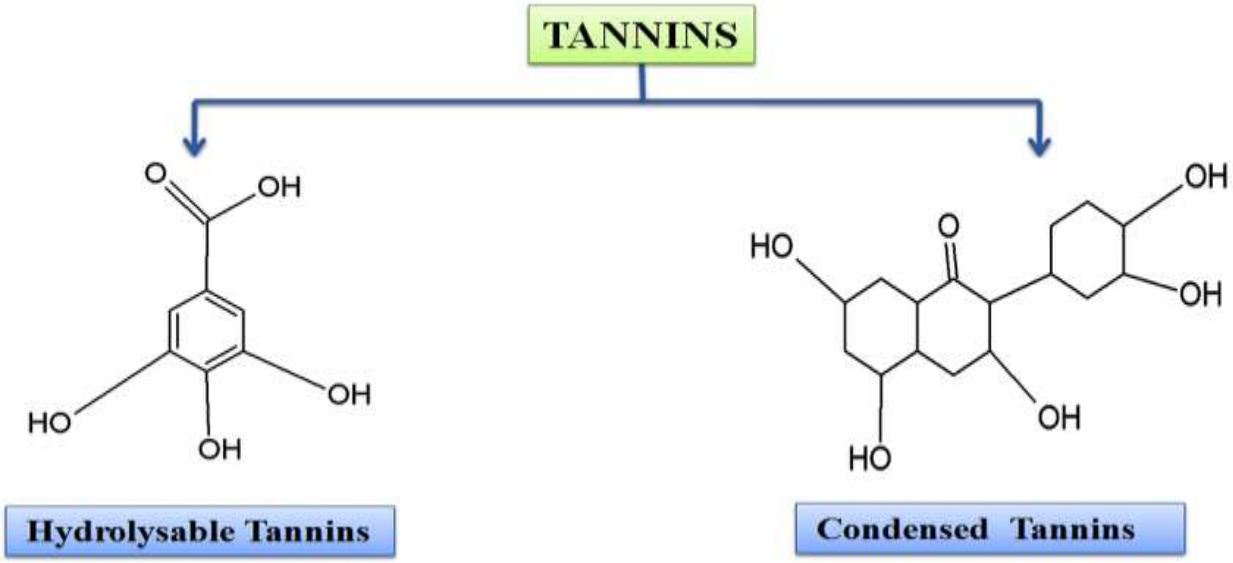

Figure 2.1 (d). The structure of Tannins.

[**Sources:** (Ames *et al.*, 1993)].

2.1.2.5. Other Potential Sources of Antioxidants and Bioactive Compounds

As previously stated, antioxidants are bioactive chemicals that block or delay the oxidation of molecules and can thus be natural or synthetic antioxidants. BHT, BHA, propyl gallate, and tertbutylhydroquinine are examples of synthetic antioxidants that are commonly used (Dastmalchi *et al.*, 2005). Many scientists are concerned about the safety of synthetic antioxidants, which have recently been shown to cause health problems such as liver damage due to toxicity and carcinogenicity. As a result, the development of safer antioxidants derived from natural sources has increased, and plants have been used as a good source of traditional

medicines to treat a variety of illnesses. Many of these medicinal plants are abundant in phytochemicals, which have antioxidant effects and are found in most spices and herbs (Ruberto *et al.,* 2000).

2.1.2.6. Antioxidant Activities.

- **DPPH (2,2-Diphenyl-1-picrylhydrazyl)Activity**

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) is a popular, quick, easy, and affordable approach for the measurement of antioxidant properties that includes the use of the free radicals used for assessing the potential of substances to serve as hydrogen providers or free-radical scavengers (FRS). The principle of the assay is based on the measurement of the scavenging capacity of antioxidants towards it (Gunathilake and Rupasinghe, 2014). The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Contreras-Guzman and Strong 1982). It is also a spectrophotometric technique based on a non-enzymatic method used to provide basic information on the reactivity of compounds for scavenging free radicals. As with the most antioxidant assays, DPPH$^{\bullet}$ method requires a spectrophotometer to measure absorbance at 517 nm. When antioxidant samples are mixed with DPPH$^{\bullet}$ reagent solution, the colour is turned to yellow from purple by time. The colour change is determined by measuring absorbance with a spectrophotometer at 517 nm.

- **Ferric Reducing Antioxidant Potential (FRAP) Activity**

As with the most antioxidant assays, DPPH$^{\bullet}$ method requires a spectrophotometer to measure absorbance at 517 nm. When antioxidant samples are mixed with DPPH$^{\bullet}$ reagent solution, the colour is turned to yellow from purple by time. The colour change is determined by measuring absorbance with a spectrophotometer at 517 nm. FRAP method compares antioxidants based on their ability to reduce ferric (Fe3+) to ferrous (Fe2+) ion through the donation of an electron, with the resulting ferrous ion (Fe2+). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to

the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. The ferric reducing antioxidant power (FRAP) mechanism is based on electron transfer rather than hydrogen atom transfer; thus, the FRAP assay is based on the ability of PH to reduce Fe^{3+} to Fe^{2+} (Prior *et al.,* 2005).

- **Lipid Peroxidation Inhibition Activities.**

The lipid peroxidation inhibition capacity (LPIC) method measures the ability of both lipophilic and hydrophilic antioxidants to protect a lipophilic fluorescent probe 4, 4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid, incorporated in the membrane, from 2,2'-azobis(2-amidinopropane)hydrochloride generated radicals in the surrounding aqueous solution (Jingli, *et al.,* 2006). The lipid peroxidation inhibition potentials confers the inhibitory effect on peroxidation of lipids, thereby preventing the production of radicals (Grigonis, *et al.,* 2005).

2.1.3. Antioxidants in Fruits

Fruit consumption has also been linked to a lower risk of a variety of diseases Plumb *et al.* (1999). Peaches (*Prunuspersica L.)* are an economically important fruit in many countries. According to study, phenolic compounds identified in several peach genotypes are an important source of potential antioxidants Block *et al.,* (1992). Peaches, curiously, have shown a considerable reduction in low-density lipoprotein (LDL) oxidation, with antioxidant activity ranging from 56 to 87%.

Furthermore, the antioxidant activity of the peach peel is restricted. Phenolic compounds are plentiful among fresh grapes and marketed grape juices. When calibrated at 10 mg gallic acid equivalents, the proportion of decreased LDL oxidation varied between 22% to 60% for fresh grapes and 68% to 75% for industrial grape juices. According to Plumb *et al.* (1999), grapes and their juices have a high oxygen radical absorption capacity, and its anthocyanin pigment

malvidin-3,5-diglucoside was an important constituent found from grapes. Polyphenols and carotenoids, as well as caffeic, quinic, and p-coumaric acids, are the primary phenolic elements of apples (Malusdomestica L.). These polyphenols can act as antioxidants. (Huanga *et al.,* 2017). The principal bioactive components discovered in this case were chlorogenic acid and phloretin glycosides; nevertheless, vitamin C was only a tiny fraction in apple juice (Takos, 2005). However, these bioactive compounds and phytochemicals are very critical in foods and pharmaceutical industries for alleviating stress and other related ailments.

2.2. Concept of Stress

Stress can be thought of as either an inherent or extrinsic stimulus that causes a biological response. It can be triggered by any event or thought that causes you to feel dissatisfied, furious, or nervous, resulting in emotional or physical tension (Oka, 2015). As earlier stated, stress is your body's reaction to a challenge or demand, and stress reactions are the compensatory responses to these stresses. In short spurts, stress can be beneficial, such as when it helps you avoid danger or meet a deadline (Habib *et al.,* 2017).

Stress is also defined as an emotional or physical tension, as well as an intrinsic/extrinsic stimuli that elicits a biological response. It can be triggered by any event or thought that causes you to feel dissatisfied, furious, or nervous. Your body's reaction to a challenge or demand is called stress.. Stress responses are the compensating responses to various pressures (Oka, 2015).

Stress, on the other hand, can have a variety of impacts on the body, ranging from changes in homeostasis to life-threatening effects and death, depending on the type, timing, and degree of the applied stimuli. Many pathophysiological consequences of disease are caused by stress, and persons exposed to stress, such as those who work or live in stressful circumstances, are more likely to develop a variety of disorders or ailments (Chorousos, 2009).

Stress can be a triggering or exacerbating factor for a wide range of diseases and pathological states; consequently, it can encompass acute stress, episodic acute stress, and chronic stress

(Hammen, 2009). When stress occurs, the hypothalamus in the brain sends a signal to the cerebrum, and hormones and chemical substances (adrenaline and cortisol) are released to counteract the consequences of the stress (Qin *et al.,* 2014). Adrenaline raises heart rate and glucose absorption while inhibiting insulin synthesis. In extreme circumstances of stress, however, a regular surge in adrenalin can cause a variety of problems like as blood vessel damage, high blood pressure/hyper pulse, migraines, cardiac arrest, sleeplessness, diabetes, energy loss, and mortality (Martin, 2016).

Stress can have a variety of impacts on the body, ranging from changes in homeostasis to life-threatening effects and death, depending on the nature, timing, and severity of the applied stimulus. Numerous pathophysiological complications of disease are caused by stress, and persons exposed to stress, such as those who work or live in stressful circumstances, have a higher risk of numerous disorders or ailments (Chorousos, 2009).Stress reactions are the voluntary responses to various pressuresWhen stressed, hormones and chemical substances (adrenaline and cortisol) are released to counteract the stress's effects (Qin *et al.,* 2014). Adrenaline works quickly to increase heart rate and glucose absorption, modify immune system response, increase blood glucose levels, increase breathing rate, and suppress insulin production.

2.2.1. Types of stress

Stress can be either a triggering or aggravating factor for many diseases and pathological conditions; thus, it may include; acute stress, episodic acute stress and chronic stress (Hammen, 2009).

2.2. 2. Causes and Symptoms of Stress

According to Qin *et al.* (2014), stress can be caused by a natural or man-made disaster, living with chronic illness, surviving a life-threatening accident or illness, being a victim of crime, living in poverty or being homeless, working in a dangerous profession, having little work-life balance, working long *hours, m*ilitary deployment, and other factors. Chronic pain, insomnia,

decreased sex drive, digestive problems, fatigue, lack of concentration, and other symptoms of stress include blood vessel damage, high blood pressure/high pretension, headaches, cardiac arrest, insomnia, diabetes, energy loss, and can result to death at extreme conditions (Martin, 2016).

2.2.3. Effects of Stress on Health

Several clinical data suggests that prolonged stress, which has reached uncontrolled levels, may produce several health and life-threatening diseases. According to one study, stress is extremely harmful to one's overall well-being and mental health (Hammen, 2009). However, Martin (2016) found in a clinical survey that extreme stress can cause degenerative and severe health conditions such as damage to body tissues and blood vessels, type 2 diabetes, increased risk of heart attack and stroke, high blood pressure and hypertension, peptic ulcers, brain fog (mental cloudiness) and memory loss, loss of energy, weakened immune systems and infections, and death.

2.2.4. Stress Management and Possible Remedies

People utilize pills, medications, synthetic drinks, and herbal preparations to treat the aforementioned stress-related ailments (Drugs.com, 2023). Some of their clinical integrity is questionable. When mild cases of stress are involved, some people participate in rest and other forms of amusement to reduce the impacts of stress. Despite this, most people prefer natural compounds such as roots, herbs, and some tropical crops (spices) such as teas, coffee, ginger, turmeric, thyme, ginger, as well as some fruits and cereals; and because of their high concentrations of bioactive compounds/antioxidants and essential oils, some of these natural components have been used in a variety of ways and in food preparations (Martin, 2016). Some beverages have recently been made by adding fruits and vegetable extracts containing bio-actives and antioxidants.

2.3. Beverages and their Sources of Bioactive Compounds

Table 2.1.Examples of Nutraceutical Beverages from different Fruits and their Bioactive Components

Beverages	Bioactive Components
Berries	Anthocyanins, other flavonoids, phenolics, and so on.
Citrus	Limonene, auraptene, Ascorbic acid and canthaxanthin
Milk	Biopeptides (such as caseinphosphopeptides), conjugated linoleic acid
Soy drinks	Isoflavones, phenolics, etc.
Grapes	Anthocyanins, etc
Beers, wines	Anthocyanins, oligomeric and polymeric anthocyanitin, etc.
Tea,	Catechins, thearubigins and theaflavins.
Coffee	Phenolics and caffeine.
Cocoa/chocolate	Procyanidins, (-)-epicatechin, etc.
Tomato juice	Carotenoids, Ascorbic acid, and so on.

Source: Ames *et al.*,(1993).

2.3.1. Additives added in the Production of Pineapple Beverages (Pineapple Jiuce).

There are sevral additives (preservatives used in the preservation of pineapple juicesso as to inhibit pathogenic microbes while extending the sghelflife of tge juices. Usually, two chemical preservatives are used to prevent juice contamination: Benzoic acid and Sulphurdioxide. But there are more available options that help extend the product's look and the shelf-life, and other essential nutrients (Khanom*et al.*, 2015). Of course, every juice, whether it is apple, orange, or tomato juice or a blend, requires its own

additive solution that will fit with the manufacturer's formulation and country's regulations. In this article, we have gathered the most universal and approved additives for fruit juices. These are safe-to-use preservatives and acidulants preventing sooner juice and beverage deteriorations while enhancing its other qualities.

- **Sodium Benzoate**

Sodium Benzoate is a common preservative in the form of white crystalline powder. Being an alternative to Benzoic acid and Citric acid, especially in beverage applications, it has a higher inhibitive property and prevents the growth of harmful bacteria, fungus, and other microbes. It is very efficient under acidic conditions. Thus, Sodium Benzoate prevents the contamination of bottled juices like apple or lemon juice. It has been used in the food processing industry for a long time, and it is still classified as Generally Recognized as Safe (GRAS).

- **Sorbic Acid.**

Sorbic acid is a recommended preservative for concentrated juices except for frozen concentrated orange juice. It is a white powdered additive that is also a natural organic compound. The solutions of Sorbic acid are rather low in concentration, but sufficient to prevent the growth of a wide specter of yeasts, molds, and bacteria. It is commonly used by food and beverage manufacturers worldwide because of its active properties to inhibit the growth of bacteria and other pathogens.

- **Sodium Carboxyl Methyl Cellulose (CMC)**

CMC has various applications in foods, it's good at thickening, emulsifying, suspending, retaining water, and keeping the product fresh. But these are its thickener/suspending properties that are unique. With a rather small solution of CMC (white fine powder form), juices obtain thick structure and suspended pulp, resulting in a rich taste and mouthfeel. This is the safe and approved additive to evenly disperse fruit particles in a beverage. In addition, CMC has a certain

preservation effect beside main application and it makes the juice color stay bright. Thus, it is a perfect additive for such juices like orange, pineapple, and coconut juices.

- **Potassium sorbate**

Potassium Sorbate is an acidic preservative used in apple or lemon juices to prevent the product from fermentation and keep the taste as fresh as possible. It is a well-known and popular additive due to its anti-microbial activity which reduces spoilage and contamination of natural juices.

- **Ascorbic Acid**

Ascorbic Acid is a powdered form of vitamin C. It falls into a group of acidulants more than preservatives. As an antioxidant, it has qualities of a natural preservative and is capable of preventing the fruit juice from browning, but it is recommended to use it in combination with other major acids. Ascorbic Acid is used in fruit juices, fruit-flavored drinks, sodas, and even beverage powder mixes because this is one of the most important vitamins that enrichens the nutritional value of juice (and other foodstuffs) after processing.

- **Citric acid.**

White crystals of Citric Acid Monohydrate are widely used as an acidity regulator in processed fruits, vegetables, and in juice. This is an acidulants and antioxidant as well, naturally present in lemons, limes, grapefruits and berries. That is why this is an excellent additive to enrich the flavor and maintain appearance (color, especially) of juice made of these citrus fruits and berries.

2.3.2. Mixing of Oil and Water based Mixtures

Oil and water are immiscible liquids, so they do not normally mix. However, by adding an emulsifier you can get them to mix. As a result, when you add oil to a cup of water the two don't mix with each other. Oil is less dense than water, thus, it will always float on top of water, creating a surface layer of oil. Oils, by contrast, are nonpolar, and as a result they’re not attracted

to the polarity of water molecules. In fact, oils are hydrophobic, or “water fearing”. Instead of being attracted to water molecules, oil molecules are repelled by them. As a result, when one add oil to a cup of water the two don’t mix with each other.

Unlike many other substances such as fruit juice, food dyes or even sugar and salt, oils do not mix with water based mixtures. The reason is related to the properties of oil and water. Water molecules are made up of one oxygen atom and two hydrogen atoms (Ekeanyanwu and Etienajirthevwe, 2012). In addition to having this very simple structure, water molecules are polar, which means there is an uneven distribution of charge across the water molecule. Water has a partial negative charge from its oxygen atom and partial positive charges on its hydrogen atoms. This polarity allows water molecules to form strong hydrogen bonds with each other, between the negatively charged oxygen atom on one water molecule and the positively charged hydrogen atoms of another. Other molecules such as salts and sugars are able to dissolve in water because of its polarity as well. The charges at either end of the water molecule help break up the chemical structures of other molecules.

2.3.3. Quality and Composition of Nutraceuticals Beverages

Nutraceutical bevearges have varying amounts of nutrients (such as terpenes, terpenoids, tocopherols, flavonoids, and other phytonutrients) and these components exist in different proportions and performing different health function (Singh, *et al.*, 2018). Vitamins, minerals, amino acids, fatty acids, prebiotic fibers, probiotic bacteria and antioxidant compounds are among the most common components used for the formulation of functional beverages such as nutraceuticals (Barder, *et al.,* 2019). Some studies have focused on the investigation of unconventional matrices as new sources of bioactive components, such as exotic fruits, succulent plants and cacti, envisaging their use to the development of new functional beverages (Gironés-Vilaplana, *et al.*, 2012).

Organic acids play an important role in taste, flavor and consumer acceptance of fruit beverages. Citric, succinic, malic and lactic acids were the organic acids found in the examined beverage formulations. Thus, Citric acid was the major organic acid.

2.3.4. Fruit Juice Beverage and Fruit Drink Beverages

“Fruit juice” drinks must contain more than 95 per cent juice. This means, it’s less likely to be a source of added sugar, and more likely to offer some nutrients of benefit, like vitamin c and potassium. It still pays to read the ingredients list though to make sure the juice hasn’t been sweetened. These include orange juice, cranberry juice, apple juice, and others. Different fruit juices offer various health benefits, but some risks are also associated with drinking large amounts of certain juices. Fruit juice comes from the flesh of the fruit or from the whole fruit itself.

“**Fruit drink”** contains a mixture of ingredients such as fruit juice, sugar, fruit puree, fruit juice concentrate, fruit puree concentrate, nectar, reconstituted fruit juice and water—in short, these all mean added sugars. Drinks labelled fruit drink, beverage, punch, cocktail (e.g. lemonade, mamalades, etc) are not real juice because they are made with added sugar. Fruit-flavoured drinks are made to look like juice, but are just added sugar and water with some flavours.

2.4. Essential Oils from Tropical Spices

Spice essential oils are concentrated oils extracted from various spices usually through the process of steam distillation or cold pressing. Spices have a unique aroma. These oils capture the aromatic compounds present in the spices, thereby providing a potent and highly concentrated form of fragrance. The most important spice crops from the tropical regions which contains essential oils include pepper, capsicums, nutmeg/mace, cardamom, allspice/pimento, vanilla, cloves, ginger, cinnamon and cassia, and turmeric. Coriander, cumin, curry, ginger, garlic, nutmegs and sesame seeds. Essential oils and oleoresins are derived from raw material plant

matter, such as flowers, spices, herbs, vegetables, and fruit peels. They are oil soluble substances, typically providing a stronger flavour than if you were to use dried ingredients.

2.4.1. Tea tree essential oil

Tea Tree oil is one of the most popular essential oils available. Other essential oils may include the following Rosemary essential oil, peppermint essential oil, Lavender essential oil, eucalyptus essential oil, lemon essential oil, roman chamomile oil. A macroscale direct method for steam distillation is used to extract the essential oil, which is composed primarily of trans-anethole, from the star anise spice. In this method, the steam is generated in situ by heating the ground dry spice material and water in the distillation flask.

2. 5. Ginger (*Zingiber officinale)*

Ginger, botanically referred to as *Zingiber officinale Roscoe*, is a member of the *Zingiberaceae* family and the *Zingiber* genus, and has been used for centuries as a spice and medicinal herb (Hinneburg *et al.*, 2013). Ginger root soothes and heals a wide range of common diseases, such as headaches, colds, vomiting, and emesis. Diverse bioactive components of ginger have been discovered, including phenolic and terpenes compounds. Ginger's various bioactivities are attributed to its phenolic compounds, particularly gingerols, shogaols, and paradols (Stoner, 2013). Ginger was recently found to contain biological qualities such as antioxidants, anti-inflammatory properties, antibacterial properties, and anticancer effects (Nile and Park, 2015). In addition, investigations have shown that ginger can prevent and control a wide range of illnesses, including neurological conditions, heart disease, obesity, type 2 diabetes, chemotherapy-induced indigestion, and respiratory problems (Walstab *et al.*, 2013). This research focuses on ginger's bioactive components and bioactivities, with a special emphasis on its therapeutic potential in

nutraceutical beverages. Figures 2.2(a) and 2.2(b) depict the ginger plant and rhizome, respectively.

Figure 2.2(a). A Ginger Plant. **[**Sources: (Walstab *et al.*, 2013)].

Figure 2.2(b): A Ginger Rhizome

Source: (Walstab *et al.*, 2013).

2.5.1 Agronomic Practices of Ginger

2.5.1.1 Climatic Requirement for Ginger Farming

Ginger grows in temperatures between 30°C and 35°C. It also requires a humid climate with an annual rainfall of around 1500mm and no rain a month prior to harvest. Ginger can be produced at altitudes ranging from 0 to 1500 meters above sea level in both rain-fed and irrigated conditions (Walstab *et al.,* 2013).

2.5.1.2. Ginger Land Preparation and Farming Operation

Ginger is a root crop, and the roots spread randomly across the land. To make crop harvesting easier, the soil should be adequately ploughed, with beds about 15 centimeters high, 1 meter wide, and a decent length, with at least 50 cm gap between beds. Ridges 40 cm apart should be used in irrigated farming (Walstab *et al.,* 2013). Ginger planting, on the contrary, should be done in the late dry season or early wet season, as in tropical countries such as Nigeria. Ginger sets should be planted 5-10cm beneath loose soil and 15-20cm apart, with the buds pointing upward. To avoid drying out, dig shallow trenches in rows, then put sets at the proper spacing in rows and pile up to form 15-20cm high ridges, and the field is softly irrigated and mulched shortly after sowing.

2. 5.1.3 Ginger Harvesting

Ginger is harvested at different times based on its intended purpose. It is frequently picked following 5 to 6 months to be used in food products. Meanwhile, fresh ginger is often collected 10 to 18 months once the leaves have gone off.

2.5.2. Chemical and Bioactive Components of Ginger

Ginger contains approximately 50% carbohydrate, 6 to 8% fats, and 9% crude protein, 1.81% minerals, 0.92% fibre and other active components including phenolic, terpene, gingerols, shogaols, and paradols compounds (Prasad and Tyagi, 2015). The most prevalent polyphenols in fresh ginger are gingerols such as 6-gingerol, 8-gingerol, and 10-gingerol. Heat treatment or long-term storage can convert gingerols to shogaols. After hydrogenation, shogaols can be transformed into paradols (Zhang *et al.,* 2007). Other phenolic chemicals found in ginger include quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione. Furthermore, ginger contains terpene components such as -bisabolene, -curcumene, -zingiberene, -farnesene, and -sesquiphellandrene, which are regarded to be the main components of ginger oil extracts (Yeh *et al.,* 2013).In addition to these, ginger contains polysaccharides, lipids, natural acids, and raw fibers.

2.5.3. Therapeutic Uses of Ginger (*Zingiber officinale*)

For millennia, ginger's anti-inflammatory and analgesic properties have been recognized and valued. 6-gingerol possesses analgesic and anti-inflammatory properties (Yeh *et al.* 2013). Furthermore, in the presence of soybean lipoxygenase, aqueous and alcoholic extracts of frequently utilized spices (garlic, ginger, onion, mint, cloves, cinnamon, and pepper) inhibited linoleic acid oxidation in a dose-dependent manner. The spice blends combined to reduce lipid peroxidation, revealing their synergistic antioxidant activity. Ginger species, on the other hand, serve an important economic role as medical herbs, food additives, and dietary supplements. Ginger products, such as aromatic oils and fresh and dried rhizomes, are able to treat malaria, asthma, and headaches, in addition to acting as anti-inflammatory and antimicrobial substances (Young *et al.*, 2005).

According to reports made by Kim *et al.,* (2005), Ginger possesses antifungal, antibacterial, and antiviral activities. It is also antitumorgenic, anticarcinogenic, antilipidemic, cardiotonic, cytotoxic, apoptotic, and immunomodulatory agents. In diabetic and non-diabetic rats, Shin *et*

al., (2005) revealed that ginger juice has a minor but significant blood glucose lowering effect. According to Kim *et al.,* (2005), 6-gingerol inhibits angiogenesis and may be useful for the management of tumors, malignancies, and other angiogenesis-dependent illnesses.

2.5.4. Antioxidant Activity of Ginger

Free radical overproduction, such as reactive oxygen species (ROS), has been linked to the development of several chronic diseases (Poprac *et al.*, 2017). A variety of natural things, including fruit, veggies, edible flowers, cereal grains, medicinal plants and herbal infusions, have been reported to have antioxidant potential. In accordance with Lien *et al.,* (2013), ginger possesses significant antioxidant activity.

In vitro antioxidant activity of ginger was assessed using ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS).Dried ginger demonstrated the greatest antioxidant activity, with 5.2-, 1.1-, and 2.4-fold higher phenolic components than fresh, stir-fried, and carbonized ginger. The antioxidant activity of different gingers varied as follows: dried ginger > stir-fried ginger > carbonized ginger > fresh ginger. This was owing to their high polyphenolic content. Because fresh ginger includes more moisture, roasted ginger has more antioxidant activity. The antioxidant activity of dried ginger was diminished when it was heated further to make stir-fried ginger and carbonized ginger because the technique could convert gingerols to shogaols. Furthermore, FRAP, oxygen radical absorbance capacity, and cellular antioxidant activity studies revealed that a polyphenol-rich fraction of dried ginger powder exhibited high antioxidant activity (Rau, 2015).Furthermore, the type of extraction solvent utilized may have an effect on ginger's antioxidant qualities. An ethanolic extract of ginger had high Trolox-equivalent antioxidant capacity and ferric-reducing ability, whereas an aqueous extract of ginger shown strong free radical scavenging activity and chelating ability. Furthermore, ethanolic, methanolic,

ethyl acetate, hexane, and water extracts of ginger inhibited 71%, 76%, 67%, 67%, and 43% of Cu2+-induced human low-density lipoprotein (LDL) oxidation (Gunathilake *et al.,* 2014).

An animal model was also used to study the antioxidant properties of ginger and its bioactive components in vivo. 6-shogaol demonstrated antioxidant effects in the colon of wild-type mice by increasing the expression of Nrf2 genes of interest (Chen *et al.,* 2015). Moreover, rats with diclofenac sodium-induced stomach ulcers were given ginger butanol extract. Furthermore, rats with diclofenac sodium-induced stomach ulcers were administered a ginger butanol extract. It has the ability to reduce MDA levels while also increasing enzyme activity and glutathione levels. Furthermore, in rats with oxidative damage produced by chlorpyrifos, the 6-gingerol-rich fraction of ginger may drop H2O2 and MDA levels, boost antioxidant enzyme activity, and elevate glutathione. Furthermore, therapy with ginger extract raised antioxidant and hormone levels in serum and protected rat testes from cyclophosphamide-induced damage (Saiah *et al.,* 2018).

2.6.Oshiokirisho (Tetrapleura tetraptera)

2.6.1. Botanical Description of *Tetrapleura tetraptera*

Tetrapleura tetraptera is a pea-family flowering plant native to West Africa, and hence a part of the order fabales and the family *fabaceae*. It is a deciduous tree known as the Aridan tree (Esseyin *et al.*, 2018). It is mostly found in Ghana, where it is known as "prekese" and is utilized in soup preparations due to its pleasant aroma and probable therapeutic effects. The fruit, seeds, and blooms are used in a variety of ways, including scents, pomades made from palm oil, the production of some alcoholic beverages, and as a flavoring for biscuits (Alara *et al.,* 2018). *Tetrapleura tetraptera* is mostly used in West Africa as a spice, medicine, and vitamin-rich food supplement. In some Igbo-speaking portions of Nigeria, the plant is known as

oshokirishi,Oshosho, or Ohiohio.Figures 2.3 (a) and 2.3 (b) depict the *Tetrapleura tetraptera* tree with mature (green) pods and dry pods.

Figure 2.3 (a): *Tetrapleura tetraptera* tree

Figure 2.3 (b):Dried *Tetrapleura tetraptera* pods.

Sources**:** (Alara *et al.*, 2018)

2. 6.2. Bioactive Components and Applications of *Tetrapluera tetraptera.*

Anti-inflammatory properties of *T. tetraptera* preparations promote their protective effects against certain human illnesses. According to Ertas *et al.* (2016), the plant is commonly utilized

for managing convulsions, leprosy, inflammation and arthritic pains, schistosomiasis, asthma, and high blood pressure, and it is also recommended for the speedy relief of diseases such as malaria fever. This plant's therapeutic potential stems from its bioactive phytochemical components, which produce certain physiological responses in the human body (Akinmoladun *et al.,* 2007). Tannins, alkaloids, sugars, triterpenoids, steroids, and flavonoids are examples of chemical substances found in medicinal plants that have a specific physiological role in the human body (Edogo *et al.,* 2005). These organic molecules, known as phytochemicals, defend plants from diseases and injury while also contributing to the plant's color, aroma, and flavor. Plant substances known as phytochemicals protect plant cells from environmental hazards such as pollution, stress, drought, ultraviolet (UV) exposure, and pathogenic attachment (Koche *et al.,* 2016).

2.6.3. *Tetrapluera tetraptera's* Therapeutic Properties and Other Economic Importance

T. tetraptera has a wide range of commercial and medicinal uses and because of their pleasant aroma, the fruits are frequently used in Nigeria to make culinary spices, pomades, and soaps (Okwu, 2004), and in Ghana as a vitamin. It is also frequently used in nursing mothers' soups to avoid post-partum contractions (Eseyin *et al.*, 2018). The soft portions of the fruit and the bark include sugars, tannins, traces of saponin, and amino acids. The herb has a number of traditional medicinal uses, including the treatment of convulsions, leprosy, inflammation, and rheumatic pains. The presence of caffeinic acid in the fruits has been shown to have anticancer, anti-inflammatory, and anti-HIV replication properties.

2.7. "Ehuru'' or Calabash nutmeg *(Monodora myristica)*

2.7.1 Origin of Ehuru *(Monodora myristica)*

2.7.2 Monodora myristica Botany

Monodora myristica, popularly known as "calabash nutmeg," is a tropical tree native to most West African countries, including Angola, Benin, Cameroon, Gabon, Ivory Coast, Kenya, Liberia, Nigeria, and the Republic of the Congo. However, its seeds were originally widely distributed as a low-cost substitute for nutmeg. This is becoming less frequent outside of its manufacturing zone. Calabash nutmeg is also known as Jamaican nutmeg, African nutmeg, ehuru, ariwo, awerewa, ehiri, airama, and African orchid nutmeg (Obame *et al.*, 2007).

• The tree and its leaves

The *Monodora myristica* tree can grow to be 35 meters tall and 2 meters in diameter at breast height (DBH). It has a distinct trunk and horizontal branches. The leaves are alternately arranged and drooping, with elliptical, oblong, or widest at the apex and tapering to the stalk. They are petiolate and can grow to be 45 x 20 cm in size (Nwozo andOrojobi 2010).

• Flower

The flower, which appears at the base of new shoots, is unique, pendant, huge, and fragrant. The pedicel is 20 cm long and has a leaf-like bract. The sepals of the flower are red-spotted, crisped, and 2.5 cm long. The corolla has six petals, and the stigmas of the flower become receptive before the stamens mature and discharge their pollen (protogynous). The flowers are pollinated by insects while the pollen is shed in the form of persistent tetrads (Nwozo and Orojobi, 2010).

- **Fruit and seeds**

The fruit is a smooth, green, spherical berry that grows to be woody. It is linked to a 60-cm-long stalk. The many oblongoid, pale brown, 1.5 cm long seeds within the fruit are surrounded by a whitish aromatic pulp. The seeds have 5-9% essential oil that is colorless (Uwakwe and Nwaoguikpe, 2008).

Figure 2.4 (a). Mature *Monodora myristica* fruits. Sources: [(Uwakwe and Nwaoguikpe, 2008)].

Figure 2.4 (b). Dried *Monodora myristica* seeds

Sources: [(Uwakwe and Nwaoguikpe, 2008)].

Monodora myristica seed has a nutmeg-like odor and flavor and is used as a spice in West African cuisine. The dried seeds are marketed whole or pulverized for use in stews, soups, breads, and desserts. They are used as stimulants, stomachics, pain relievers, and insect

repellents (Uwakwe and Nwaoguikpe, 2008). Seeds are also used to make necklaces). *Monodora myristica* fruits and seeds are depicted in Figures 2.2a and 2.2b.

• Bark and wood

Monodora myristica wood is strong but easy to work with, and it is widely used in carpentry, house fittings, and joinery. The bark is used in medicine to treat stomachaches, feverish pains, ocular issues, and hemorrhoids (Ekeanyanwu, *et al.*, 2010).

2.7.3. The Chemical Components and Therapeutic Properties of *Monodora myristica*

The essential oil produced from the leaves contains caryophyllene, humulene, and pinene. The essential oil derived from the seeds contains some of the following compounds: -phellandrene, -pinene, myrcene, limonene, and pinene (Iwu, 2002). According to phytochemical studies, *M. myristica* seeds have a high concentration of alkaloids, glycosides, flavonoids, tannins, saponin, and steroids (Ekeanyanwu and Etenajirhevwe 2012).

As previously stated, they are used as stimulants, stomachics, for headaches, sores, and as an insect repellant. When pulverized, the kernel is used to make pepper soup, which is utilized as a stimulant to relieve constipation and reduce passive uterine hemorrhage in women shortly after childbirth (Ekeanyawu *et al.*, 2010). *Monodora myristica* has been shown to be a source of potent antioxidants with metal binding and oxygen removing properties (Hamda *et al.*, 2008).

2.7.4. Ginseng Plant (*Panax ginseng*)

Ginseng*(Panax ginseng)* is traditionally used as a medicinal herb in Korea, Japan, China, and the United States (Attele, *et al.*, 1999). The reason for this long-established usage is that ginseng contains natural antioxidant compounds. These ginsenosides, which are extracted from the ginseng roots, leaves, stems, and fruit, have multiple pharmacological effects. They are subdivided into about 100 different categories (Kim, 2012). In many studies, ginsenosides have been presented as an effective treatment for organ damage and cell death, as well as for

immunological and metabolic diseases (Nguyen, *et al.*, 2015). In addition, these pharmacologically active constituents have been shown to support neurogenesis, synaptogenesis, neuronal growth, and neurotransmission, thus helping to protect the central nervous system from unexpected events; ginseng is also reported to be excellent for improving memory (Lee*et al*., 2006).

As a powerful natural antioxidant, ginseng effectively modulates apoptosis by reducing the excessive inflammatory response in acute or chronic inflammation (Thatte*et al.*, 2000). Abnormal apoptosis can result in functional impairment of organs. The human body contains many different protein types, and their interactions maintain the balance of mechanisms related to proliferation, differentiation, and apoptosis. When this homeostasis is disturbed, it can damage the immune system and lead to several fatal diseases (Leung*et al.*, 2007). Many studies conducted over the past decade have revealed that ginseng has a range of positive effects on the human body, but a systematic perspective on the efficacy of ginseng in the treatment of stress *in vivo* is not available. Therefore, this review will consider whether ginseng modulates human stress-related changes and diseases, and evaluate how ginseng could potentially act as a therapeutic agent for stress-induced diseases.

2.7.4.1. Ginseng and Stress

Ginseng has been used as an adaptogen to treat illness, both as a tonic and as a rejuvenator. In modern societies, we rarely depend on herbal remedies as the only treatment for critical and potentially fatal diseases. However, owing to an excessive amount of brain activity, overwork, and group living conditions, modern life involves constant exposure to stress. Moreover, the level of stress can be sustained over time because of the repetitive nature of some occupations; this can cause detrimental biological stress responses. When under certain kinds of stress, the human body secretes hormones and inflammatory cytokines, and chronic stress can promote the

development of anxiety, depression, and even panic disorders, in severe cases. Therefore, adaptogens are often used to cope with day-to-day and/or workplace stress. Ginseng shows superior regulation of stress, as compared with that shown by other adaptogens (Rege*et al.,* 1999). This efficacy as an antistress agent has been demonstrated using various behavioral conditioned stress tests, such as swimming and immobilization tests. *In vivo* studies have also shown that ginseng has excellent antistress effects, as compared to appropriate controls (Dang*et al.,* 2009).

2.7.4.2. Ginseng in Depression and Anxiety

Depression is a severe mental illness without any apparent physical symptoms. However, physical problems can emerge as depression becomes more advanced. About 10–30% of patients with depression are unable to overcome the initial stages, and eventually succumb to extreme physical harm; this includes committing suicide, inflicting self-harm, and developing drug dependence, which affects their quality of life. Furthermore, the prevalence of depression is increasing and this represents a major clinical challenge (Al-Harbi*et al.,* 2012).

Ginseng effectively suppresses stress, which is a major cause of depression. This activity has been demonstrated in depression tests using animal models. Ginseng demonstrated similar levels of efficacy as the commercially available antidepressant, fluoxetine (Xu*et al.,* 2010). In addition, depression can be associated with memory loss. This is because depression results in progressive damage to nerve cells. This neuronal cell damage, coupled with a neuroinflammation-induced reduction in neurogenesis, can result in hippocampal cell death (Dong *et al.*, 2016).

Ginseng is traditionally used to protect the nervous system. Ginseng is effective in memory improvement, and in the direct prevention of degenerative brain diseases such as Alzheimer's disease. The neuroprotective effect of ginseng may be useful in the prevention of depression. Indirectly, enhanced memory can ameliorate anxiety. In clinical studies, it was observed that

memory loss was attenuated in elderly patients treated with anxiolytics. These clinical studies may indicate that ginseng has the potential to improve anxiety (Churchill*et al.,* 2002).

Research studies can employ self-testing using the depression, anxiety, and stress scale to measure anxiety, depression, and stress levels induced by the environment, including emotional and physical factors (Lovibond*et al.,* 1995). Stress is closely related to psychological disorders such as depression and anxiety. Thus, ginseng is potentially an effective candidate for easing stress and can therefore improve the symptoms of depression and anxiety.

2.7.4.3. Use of ginseng in the prevention of stress-induced diseases

Based on the association between ginseng and the various diseases caused by stress, several studies on cytokines and receptors involved in this activity are being conducted. The treatment of anxiety and depression caused by stress could reduce the prevalence of inflammatory diseases. Thus, the effects of ginseng on the anxiety and depression associated with the initial stage of chronic inflammation should also be studied.

Patients with anxiety and depression can develop a variety of diseases, as discussed above. This is because anxiety and depression can promote inflammatory responses. First, the proinflammatory cytokines such as IL-1, IL-6, interferon-γ, and TNF-α play a role. Second, oxidative or nitrosative stress can occur owing to the increased levels of reactive oxygen species and reactive nitrogen species. As a result, anxiety and depression can predispose patients to the development of cancers, neurodegenerative conditions, and inflammatory diseases (Maes*et al.,* 2012).

In addition to defending against the increase in proinflammatory cytokines induced by anxiety and depression, ginseng can defend effectively against oxidative or nitrosative stress (Hong and Lyu, 2011). Although not yet fully clinically tested, ginseng effectively suppresses the chronic inflammation caused by stress-induced anxiety and depression and could therefore contribute to

the prevention of secondary diseases. When ginsenosides are ingested, a number of biological effects occur. These include the prevention of tissue damage, as well as cellular regeneration and repair effects.

2.7.4.4. Ginseng for disease treatment

Chronic stress can trigger such diseases because of abnormal immune responses and hormonal disorders. However, regular ingestion of ginseng has both preventive and therapeutic effects on several human diseases, including heart disease, stroke, diabetes mellitus (DM), rheumatoid arthritis (RA), osteoporosis, erectile dysfunction (ED), and allergic asthma. These diseases can be more prevalent in patients with depression and anxiety, in comparison to healthy individuals (Clarke, and Currie, 2009). This may reflect an increase in depression and anxiety in patients with a physical illness, because of their physical pain, or indicate that depression and anxiety predispose to secondary physical illnesses. The extracts from ginseng plants have been confirmed to be applied in the treatment of the following diseases: Vascular diseases, Osteoporosis, Aethritis, Erectile dysfunctioz, Diabetes mellitus, and Allergic Asthma.

2.8. Isolation, activity, stability and effects of bioactive compounds

Bioactives can be found in a wide range of beverages and foods. Many of the bioactives found, among other things, may be antioxidative or contain bioactive alkaloids. Various reactions, most notably the Maillard reaction, can affect beverages and their constituents. These products may have an effect on a range of biomolecules, including genetic material (DNA). Bioactives isolation for further research or use as nutraceuticals could be of interest. Because countercurrent chromatography is now available, this isolation/purification method has gained popularity in recent decades (Degenhardt *et al.*, 2000).

2.8.1 Methods Used for Bioactive Compound Extraction

2.8.1.1. Extraction of Phenolic Compounds Using Solvent

Scientists researched and evaluated the effects of several solvents, such as methanol, hexane, and ethyl alcohol, on antioxidant extraction from various plant parts, such as leaves and seeds. To extract distinct phenolic compounds from plants with great precision, various solvents with varied polarity must be used (Wong and Kitts, 2006). Scientists have also discovered that highly polar solvents, such as methanol, are exceptionally effective antioxidants. Anokwuru *et al.*(2011) discovered that acetone and dimethylformamide (DMF) are highly effective in extracting antioxidants, however methanol was more effective than ethanol in extracting phenolic components from walnut fruits. Ethanolic extracts of Ivorian plants extracted higher concentrations/amounts of phenolics than acetone, water, and methanol.Scientists traditionally employed a dry powder of plants to extract bioactive components while removing the influence of water (Wong and Kitts, 2006).

The polarity of the solute of interest is utilized to select solvents for plant biomolecule extraction. A solvent having the same polarity as the solute will dissolve it properly. Multiple solvents may be used sequentially to reduce the number of comparable compounds in the target yield. The polarity of a few common solvents is as follows, from least polar to most polar: Hexane, chloroform, ethylacetate, acetone, methanol, and water are all examples of solvents.

2.8.1.2. Supercritical fluid extraction

Supercritical fluid extraction is an environmentally friendly process that is frequently used to extract bioactive chemicals from natural sources including plants, food waste, algae, and microalgae. Supercritical carbon dioxide (SC-CO_2) is an enticing alternative to organic solvents since it is non-explosive, non-toxic, and inexpensive. It may easily be removed from the final products and can solubilize lipophilic chemicals (Wang and Weller 2006). The raw material is placed in an extraction container equipped with temperature and pressure controllers to ensure

optimal extraction conditions.A pump then fills the extraction container with fluid. After transferring the fluid and dissolved chemicals to the separators, the products are collected via a tap positioned in the separators' lower section. Lastly, the fluid is recycled, cycled, or released back into the environment. The choice of supercritical fluids is crucial for the proper operation of this process, and a variety of substances can be used as solvents in this procedure (Wang and Weller 2006).

Giannuzzo *et al.* (2003) reported that SC-CO_2 treated with ethanol provided higher extraction yields of naringin (flavonoid) from citrus garbage than pure SC-CO_2 at 9.5 MPa and 58.6 °C.Ashraf-Khorassani and Taylor (2004) used SFE to extract polyphenols and procyanidins from grape seeds, and methanol enhanced CO_2 (40%) released more than 79% of catechin and epicatechin from grape seed.Liza *et al.* (2010) investigated the feasibility of the SFE method for extracting lipophilic substances from sorghum, such as tocopherols, phytosterols, policosanols, and free fatty acids, as well as the preventative value of these substances in a number of disorders (skin, cardiovascular, cardiovascular disease, and cancer). SC-CO_2 and Soxhlet extraction were also employed to obtain antioxidant-rich wheat bran oil. Pressure and temperature during SC-CO_2 extraction ranged from 10 to 30 MPa and 313.15-333.15 K, respectively. SC-CO_2 extracted oil outperformed hexane extracted oil in terms of oxidation resistance and radical scavenging activity.Ahmadian-Kouchaksaraie and Niazmand (2017) used SC-CO_2 to extract antioxiant compounds from Crocus sativus petals for 47 minutes at 62 °C and 164 bar pressure. Under these ideal conditions, the total phenolic content was 1423 mg/100 g, the total flavonoid content was 180 mg/100 g, and the overall concentration of anthocyanin was 103.4 mg/100 g. Wang and Weller (2006) emphasized the supercritical method as a viable alternative to traditional organic solvent extraction methods for acquiring biologically active compounds.

2.8.1.3. Extraction using ultrasounds

Ultrasound-assisted extraction is claimed to be a simpler and more successful approach than normal extraction methods for extracting bioactive compounds from natural commodities. Ultrasound increases solvent penetration into cellular materials, increasing mass transfer, and also tears down cell walls, allowing bioactive components to be released. Depending on the type of the plant material to be extracted, ultrasonic frequency has a substantial impact on extraction yield (Lim*et al.,* 2011) used ultrasound-assisted extraction to extract three dibenzylbutyrolactone lignans (tracheloside, hemislienoside, and arctiin) from Hemistepta lyrata. High-performance liquid chromatography was employed to determine the target components in the extracts simultaneously.Rostagno *et al.* (2003) used a mix-stirring method and different extraction times and solvents to study the extraction efficiency of four isoflavone derivatives from soybean, namely glycitin, daidzin, genistin, and malonyl genistin. Ultrasound was discovered to increase extraction yield depending on the solvent employed. Lim*et al.* (2011) extracted anthocyanins and phenolic compounds from grape peel with ultrasound-assisted extraction. Bimakr *et al.* (2013) extracted bioactive value components from winter melon (Benincasa hispida) seeds using ultrasound-assisted extraction.

2.8.1.4. Microwave Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) for bioactive compound extraction, is an innovative extraction technique that combines microwave and traditional solvent extraction methods. When compared to typical methods of compound extraction, it is a more advantageous technique owing to its shorter recovery time, greater recovery rate, reduced solvent demand, and lower cost (Delazar *et al.* 2012). MAE extracts plant metabolites in less time than ultrasonic aided extraction and Soxhlet method (Afoakwah *et al.* 2012). In accordance with reports, the mangiferin content was boosted up to 500 W power before decreasing as microwave power was

raised further. The optimal mangiferin yield was 41 g/ml after an extraction period of 15.32 s at the microwave power of 500 W. Kerem *et al.* (2005) discovered that MAE was superior to Soxhlet extraction in terms of solvent amounts, time, and energy spent for extracting saponins from chickpea (*Cicer arietinum*). Mangiferin was obtained from the leaves of *Mangifera indica* using microwave-assisted extraction protocols and water as a solvent, providing a maximum extraction yield of 55 mg/g after 5 minutes of extraction, a solid-to-solvent ratio of 1:20, and a microwave power of 272 W. In compared to successive batch extraction as well as Soxhlet extraction, MAE increased extraction yield in a short period of time while using less solvent (Kerem *et al.* 2005).

MAE and pressured liquid extraction were examined by Smiderle *et al.* (2017) as improved approaches for extracting macromolecules (especially physiologically active -glucans) from Pleurotus ostreatus and Ganoderma lucidum fruiting bodies as well as heteropolysaccharides, in all extracts. Filip *et al.* (2017) employed a response surface technique to optimize MAE for the extraction of polyphenols from basil (*Ocimum basilicum L*) in an exciting study. 50% ethanol, 442 W microwave power, and a 15-minute extraction period were the optimal extraction conditions. Basil liquid extract contained 4.299 g GAE/100 g overall polyphenols and 0.849 g catechin equivalents/100 g DW in total flavonoids under these circumstances.

In recent years, microwave extractors have been used to extract bioactive compounds from plant samples. This is because typical extraction processes use more solvent, harm thermally sensitive bioactive compounds, and take some time to extract. Because of its lower solvents consumption, shorter operation time, reproducibility, higher recovery output, good specificity, and less sample manipulation, MAE has received the most attention of all modern extraction techniques (Alara *et al.,* 2019).Rau, (2015) was the initial researcher to describe the utilization of microwave energies

for the synthesis of chemicals, which was also applied in biological extraction of specimens for organic component analysis.

The MAE method is used in a wide range of samples, such as geological, ecological, and biological specimens. MAE is increasingly widely used to extract bioactive compounds from plant samples, sparking significant interest in the invention and research sectors. When compared to traditional methods, this method allows for faster solute recovery from plant samples while retaining high extraction efficiency. MAE is a modern technique that uses a shorter extraction time, less solvent, and safe thermo-labile compounds. It is a green approach which efficiently extracts bioactive compounds from plant samples (Raut, 2015). Because of the importance of MAE, it has been separated into two sub-classes: microwaves solvent-free extraction (MSFE) and microwave-assisted solvent extraction (MASE).

Microwave irradiation employs a certain frequency of electromagnetic field in a manner similar to photochemical processes activation; the frequency ranges between 300 MHz and 300 GHz (Perino-Issartier *et al.,* 2013). Nonetheless, only a few frequencies are approved for medical, scientific, and industrial uses; these frequencies vary between 0.915 and 2.45 GHz worldwide; hence, heat-sensitive bioactive compounds can benefit from dielectric heating from MAE (Chee *et al.,* 1996). It has been reported that employing water for extracting phenolic compounds is unsuccessful when compared to conventional approaches due to the lower dissipation factor and greater dielectric constants associated with water when compared to other solvents; therefore, using solvents with increased dissipation and dielectric constants is recommended in MAE. Furthermore, the extractability of bioactive compounds from plants is related to the type of plant sample and the solvent used in the extraction. When ionic species or polar molecules are used in MAE, they quickly heat up, resulting in collisions with molecules in the surrounding environment that do not require higher pressure. In most instances, the extraction time and

microwave power range between 30 seconds and 10 minutes, respectively (Kaufmann and Christen, 2002). Several studies on the use of MAE to recover phenolics from plant samples such as bitter leaf, purple fleabane, roselle, coffee leaf, vanilla, radix, flax seeds, scent leaf, siam weed, and others have been published (Alara *et al.*, 2019).

2.8.2. Operating Principle and Working Mechanisms of MAE

2.8.2.1 Operating principle of MAE.

MAE technology differs from conventional processes in that it is based on electromagnetic waves that cause cell structure to change. Microwave-assisted extraction works on the premise that polarizable compounds and dipoles of polar solvent interact with microwave radiation, causing magnetic and electric interactions to rapidly change direction. Polar solvent molecules are heated when they orient in the changing field direction. Non-polar solvents with no polarizable groups require little heating. This molecular heat impact is rapid but restricted to a small proportion of the samples and depth surrounding the surface while other part of the samples is heated by conduction.As a result, the MAE has a substantial drawback in that large samples or agglomeration of small samples are not able to heat uniformly. High power sources could be employed for increasing infiltration depth, but electromagnetic radiation has an exponentially drop once within a microwave-absorbing material (Handa *et al.*, 2008).

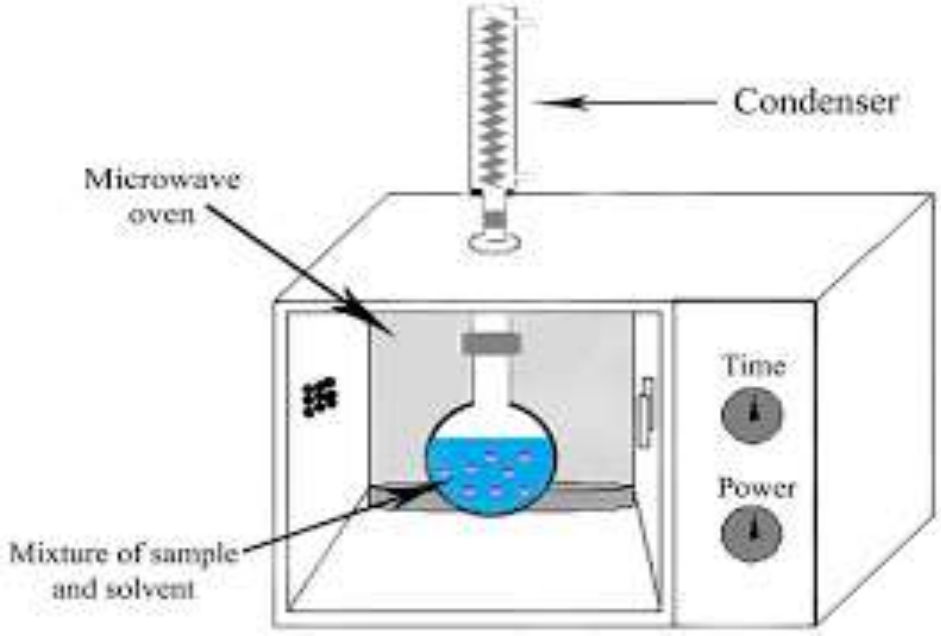

Figure 2.5. Microwave assisted extraction.

Source: (Handa*et al.*, 2008).

2.8.2.2. Working mechanism of MAE

Microwave-assisted extraction is distinct from conventional extraction methods in that it occurs as a result of changes in cell structure caused by electromagnetic waves. This extraction process, as shown in Figure 2.5, involves a synergistic combination of mass and heat transfers that work in the same direction, whereas mass transfer in traditional processes occurs from the interior to the exterior of the substrates and heat transfer occurs exterior to the interior of the substrate (Azwanida, 2015). During the MAE process, the following experiential phases occur:

a. Without absorption, microwave irradiation heat is transmitted to the solid via the microwave-transparent solvent.

b. The intense heating of the (a) above results in residual microwave-absorbing in the heated solid;

c. The heated moisture evaporates and creates a high vapor pressure;

d. The high vapor pressure breaks the cells of the substrate; and

e. Cell wall breakage increases the release of the extract from the samples (Raut, 2015).

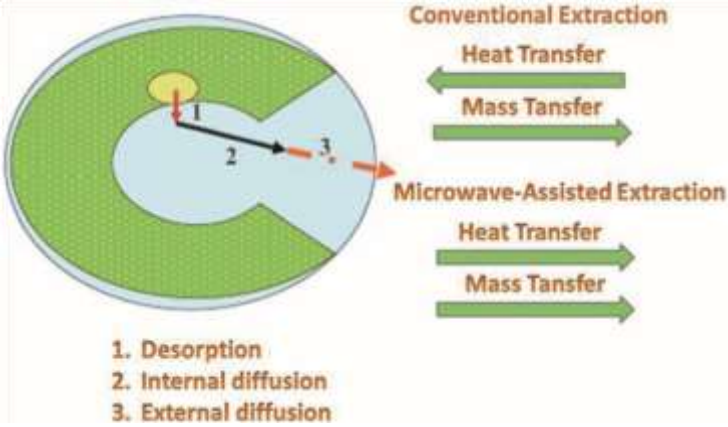

Figure 2.6(a).*Heat and mass transfer mechanisms in conventional and microwave extraction.*

[Source: (Perino-Issartier *et al.*, 2013)].

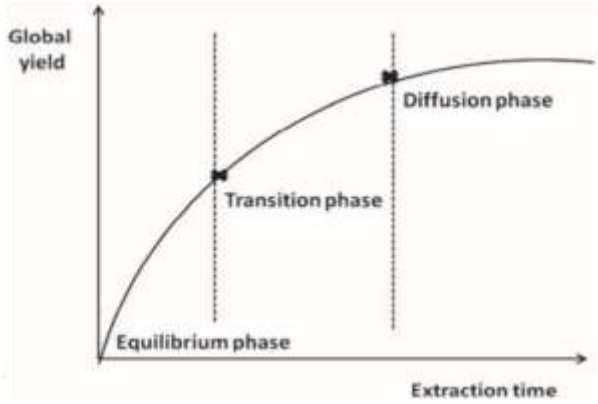

Figure 2.6(b). Pictorial diagram of yield against time in the extraction

[Source: (Perino-Issartier*et al.*, 2013)].

Furthermore, the extraction solvent is diffusely absorbed into the plant sample, causing solute dissolution into the solvent until saturation. This solution diffuses to the plant's surface via effective diffusion and then transfers to the bulk solution. Several forces, including physicochemical interactions, can be noticed during the process (chemical interactions, driving forces, interstitial diffusion, and dispersion forces), and the magnitude and persistence of

properties can be related to the extraction solvent's properties (polarity, solubility in water, purity, dissolution, and so on) (Alara *et al.,* 2018).

2.8.2.3. The Critical Factors influencing MAE and mechanism of action.

Several studies on optimizing MAE parameters to obtain optimum yields from the plant samples studied had been carried out. All operational parameters that effect MAE include the solvent-to-feed ratio, solvent composition, plant sample properties and water content, microwave power, irradiation length, stirring impact, microwave energy density, and extraction temperature. These operational variables determine MAE's efficiency. As a result, it is vital to understand the impacts and interactions of these parameters on the extraction process.

(i) Solvent-to-feed ratio

The solvent employed has the greatest influence on microwave-aided extraction. A correct solvent selection will result in an efficient extraction process. The dissolution rate of the molecule of interest, the process's mass transfer kinetics, and solvent absorption induced by interaction between the dielectric effect and the sample matrix are all inevitable parameters (Spigno and De Faveri, 2009). According to Chan *et al.* (2011), the extraction solvent chosen is governed by its ability to absorb microwave energy. The solvent's ability to absorb microwave radiation will be enhanced if it has a high dielectric constant and dielectric loss (Spigno and De Faveri, 2009). According to Tatke and Jaiswal (2011), solvents such as alcohol and water are efficient microwave-absorbing solvents having sufficient polarity to be heated by microwave power.

Adding a tiny amount of water to a polar solvent increased water diffusion into matrix cells, resulting in effective heating and thereby promoting chemical transport into the solvent at faster mass transfer rates (Spigno and De Faveri, 2009). According to Veggi *et al.* (2013), the concentration of the extraction solution must not exceed 30-34% (w/v); hence, earlier studies found that a solvent-to-feed ratio of 10:1 (mL/g) to 20:1 (mL/g) provided the greatest yields.

Another critical factor is the volume of extracting solvent; a large volume of solvents requires more energy and time to condense extraction solution throughout the purifying process, and MAE may result in lower recoveries due to non-uniform dispersion and microwave exposure.

(ii) Irradiation Time

The irradiation period is another important factor that affects microwave-assisted extraction. One advantage of MAE over traditional techniques is the short extraction time. Depending on the plant matrix, the normal duration ranges from a few minutes to half an hour to avoid oxidation and heat damage (Spigno and De Faveri, 2009). The irradiation period is affected by the dielectric property of the solvent used. After extended exposure, solvents such as ethanol, water, and methanol may rapidly heat up, resulting in the destruction of thermo-labile compounds in the extracts. Longer irradiation time may boost recovery yield; however, higher irradiation time can reduce recovery yield (Alara *et al.*, 2018).

If the extraction will take a long time, the plant materials will be removed in stages utilizing a sequential extraction cycle. A new solvent is added to the residues, and the procedure is repeated to ensure that the plant sample has been depleted. This approach improves recovery yield while preventing overheating (Chan *et al.,* 2011). The nature of the plant sample and solute determines the number of extraction cycles. According to one study, three 7-minute cycles of MAE extraction of triterpene saponins from yellow horn were sufficient (Xiao *et al.*, 2008). The optimized MAE for extracting triterpenoids saponins from Ganoderma atrum, on the other hand, took 5 minutes each cycle.

(iii) Effect of Stirring

Stirring is widely utilized in the solvent phase to optimize mass transfer procedures. The gaseous-aqueous phase equilibrium is reached faster. The use of a stirrer in MAE speeds up the

extraction process by increasing the solubility and desorption of bioactive components in the sample matrix. By lowering the mass transfer barrier, thorough stirring can overcome the drawbacks of using a low solvent-to-solid ratio (Rau *et al.,* 2015).

(v) Microwave Power and Temperature

Microwave power and temperature are crucial factors that influence extraction yield when using MAE. Higher microwave power can boost system temperature, increasing extraction yield until it becomes insignificant or declines (Rau *et al.,* 2015).Temperature increases can improve solvent power by lowering surface tension and viscosity, which strengthens the solvent's capacity to solubilize solutes and improves matrix wetting and penetration. Spigno and De Faveri (2009) discovered that the efficiency of MAE increases with elevated temperatures until the ideal temperature is reached. Microwave power is proportional to sample volume and extraction time. The power, on the other hand, causes localized heating in the plant matrix, which drives MAE to breakdown the plant matrix and allow the solute to diffuse and dissolve in the solvent. As a result, raising the microwave power frequently improves extraction yield while decreasing extraction time.However, excessive microwave power can result in poor extraction yield and the loss of thermally sensitive compounds in the plant matrix (Veggi *et al.,* 2013). The proper microwave power must then be selected to shorten the extraction time required to attain the desired temperature and avoid a "bumping" problem (Raut *et al.,* 2015).

(v) Characteristic of plant sample and its water content

MAE could be modified by the plant sample's features and water content. As the contact surface area of the plant sample increases, so does the extraction efficiency. Furthermore, finer samples enable for greater absorption of microwave irradiation (Huie, 2002). However, too fine a plant sample can pose technical challenges; hence, filtering or centrifugation is used in the treatment of plant samples (Tatke and Jaiswal, 2011). During preparation of the sample, the ground sample

is homogenized to promote interaction between the solvent and the plant matrix. Plant particle sizes range between 2 and 100 mm. To boost yield, the plant matrix is sometimes steeped before extraction; this is known as pre-leaching.In general, moisture acting as a solvent increases the recovery of bioactive compounds from plant matrix. This moisture is heated, evaporated, and dispenses the solutes by rupturing the cell wall, enhancing the generation of bioactive molecules. The addition of water has a good effect on microwave-absorbing capacities, which helps the heating operation as the polarity of the solvent increases (Chan *et al.*, 2011). Extra water causes hydrolysis and slows bioactive ingredient oxidation.

(vi). Density of Microwave Energy

Three heating operating modes are used in the performance evaluation of microwave-assisted extraction (Chan *et al.*, 2015). Among these are the constant-power heating mode, intermittent heating mode, and constant temperature heating mode.Terigar *et al.* (2010) state that the continuous power heating method is standard practice in the extraction of thermally sensitive active components from plant matrix. It should be noted that microwave power alone does not explain how energy gets absorbed during biological medium extraction. Perino-Issartier *et al.* (2013) evaluated the link between microwave energy density and extraction yield and determined that microwave energy density is the most important factor impacting extraction efficiency in a microwave-assisted extraction for a unit of extracting solvent.

Gao *et al.* (2006) discovered an accelerated effect on ionic conduction and dipole rotation, resulting in a higher extraction yield. As microwave power increases, additional microwave radiation is discharged into the biological medium. Polar solvent absorption rates increase with increased power, resulting in higher heating and extraction rates.

(vii) Influence of Stirring

The mass transfer process in a solvent is affected by stirring, which leads to convention. It is possible to quickly develop stability between the vapor and water phases as a result. In the plant sample, agitation increases the dissolution and adsorption of bioactive components by accelerating the process (Mandal *et al.,* 2007). The mass transfer barrier resulting from solutes in a constrained area produced by insufficient solvent can be reduced by using a low solvent-to-feed ratio.

2.8.2.4. Previously utilized microwave assisted extraction to extract bioactive substances from plants.

In a number of ways, MAE has been used to separate bioactive compounds from different plant samples; these isolates are then used in pharmaceutical and nutraceutical applications.Most often, microwave irradiation is used to solve some of the drawbacks of earlier methods. The use of microwave-assisted extraction technique results in higher global yields, phenolic compound diversity, and biologically active compound recovery. These findings proved that MAE was the best extraction technique. MAE is a potential technique for extracting substantial bioactive compounds from plant sources due to its superiority over other processes. However, it can be said that the MAE is favoured for the extraction of bioactive compounds due to its greater extraction efficiency, shorter extraction time, less labor, and higher extraction selectivity compared to other methods (Cai, 2016).

2.8.2.5. Enzyme Assisted Extraction

Enzymes are frequently employed to remove bioactive substances from food waste. In addition to containing polysaccharides like cellulose, hemicellulose, and pectins that serve as barriers to the release of intracellular molecules, plant tissues and cell walls are the main sources of antioxidants. Enzymes used for extraction include cellulases, -glucosidases, xylanases, -gluconases, and pectinases help breakdown the structure of plant cell walls and depolymerize

polysaccharides, releasing associated compounds (Martino *et al.*, 2006). Since enzyme aided extraction employs water as a solvent rather than synthetic solvents, it is recognized as a more environmentally friendly method of extracting bioactive chemicals and essences (Puri *et al.*, 2012).

The use of blended enzyme preparations with cellulolytic and pectinolytic activities, as well as the comparably low cost of commercial food-grade enzyme preparations, could significantly improve lycopene recovery on an industrial scale, according to Singh *et al,*. (2016) who studied the enzyme-assisted extraction of lycopene from the peel fraction of tomato processing waste. With an emphasis on food and nutraceutical applications, Puri *et al.*, (2012) explored the enzyme-assisted extraction of the bioactive compounds stevioside from Stevia rebaudiana from plant sources. Traditional solvent-based extraction techniques can be effectively replaced by enzyme-assisted extraction. According to Grigonis *et al.* (2005), it is based on enzymes' capacity to catalyze reactions in water-based solutions under gentle processing conditions.

2.8.1.6 Solvent Extraction Method

The right amount of raw material is exposed to a variety of organic solvents, which absorb any soluble components that are of interest as well as additional flavoring and coloring ingredients like anthocyanins, which have anti-inflammatory and anti-cancer properties. Before being used as an addition, nutritional supplement, or in the production of functional foods, samples usually get centrifuged and filtered to remove solid residue (Herrero *et al.*, 2012). Solvent extraction is superior to other techniques due to its low processing cost and simplicity of use.

However, this method uses hazardous solvents, calls for an evaporation/concentration phase for recovery, and often requires enormous amounts of solvent and a protracted period of time. Furthermore, owing to the high temperatures of the solvents during the prolonged extraction, the likelihood of thermal destruction of natural bioactive cannot be overlooked.Other methods have

improved solvent extraction yields, including Soxhlet's, ultrasonic, microwave, and SFE (Herrero *et al.,* 2012).

Using ethanol, Baysal *et al.* (2000) were able to recover up to 50% of the lycopene and beta-carotene from tomato pomace that contained dried, crushed, and carotenoid-rich skins and seeds. They came to the conclusion that 50% acetone produced the highest quantity of polyphenols when compared to methanol, ethyl alcohol, ethyl-acetate, and hexane. In their analysis, Cakir *et al.* (2003) found that ethanol was the most effective organic solvent and produced the best yield, whereas hexane produced the lowest yield when bioactive compounds were extracted using these techniques and they also discovered that extending the extraction period increased the yield of extracts.

2.8.2.7. Identification of Chemical Compounds Using Mass Spectrometry

In mass spectrometry, organic molecules are blasted with either electrons or lasers, resulting in very energetic charged ions. A mass spectrum is a visualization of a fragmented ion's relative abundance versus its mass/charge ratio. With mass spectrometry, relative molecular mass (molecular weight) may be estimated with high accuracy, and a precise molecular formula can be established with knowledge of where the molecule has been broken. Bioactive compounds from pith were identified and purified before using bioactivity-guided solvent extraction, column chromatography, and HPLC (Cakir *et al.,* 2003). UV-visible, infrared, nuclear magnetic resonance, and mass spectroscopy were used to characterize the structure of the bioactive chemical. Furthermore, compounds can be hydrolyzed and their derivatives described. When tandem mass spectrometry (MS) is used, mass spectrometry gives a wealth of information for the structural elucidation of substances. Since a pure standard is not always accessible, the combination of HPLC and MS enables the quick and accurate identification of chemical components in medicinal herbs (Alara *et al.,* 2020). For studying phenolic compounds, LC/MS

has recently been used extensively. Electrospray ionization (ESI) is a suggested source due to its great efficiency at ionizing phenolic compounds.

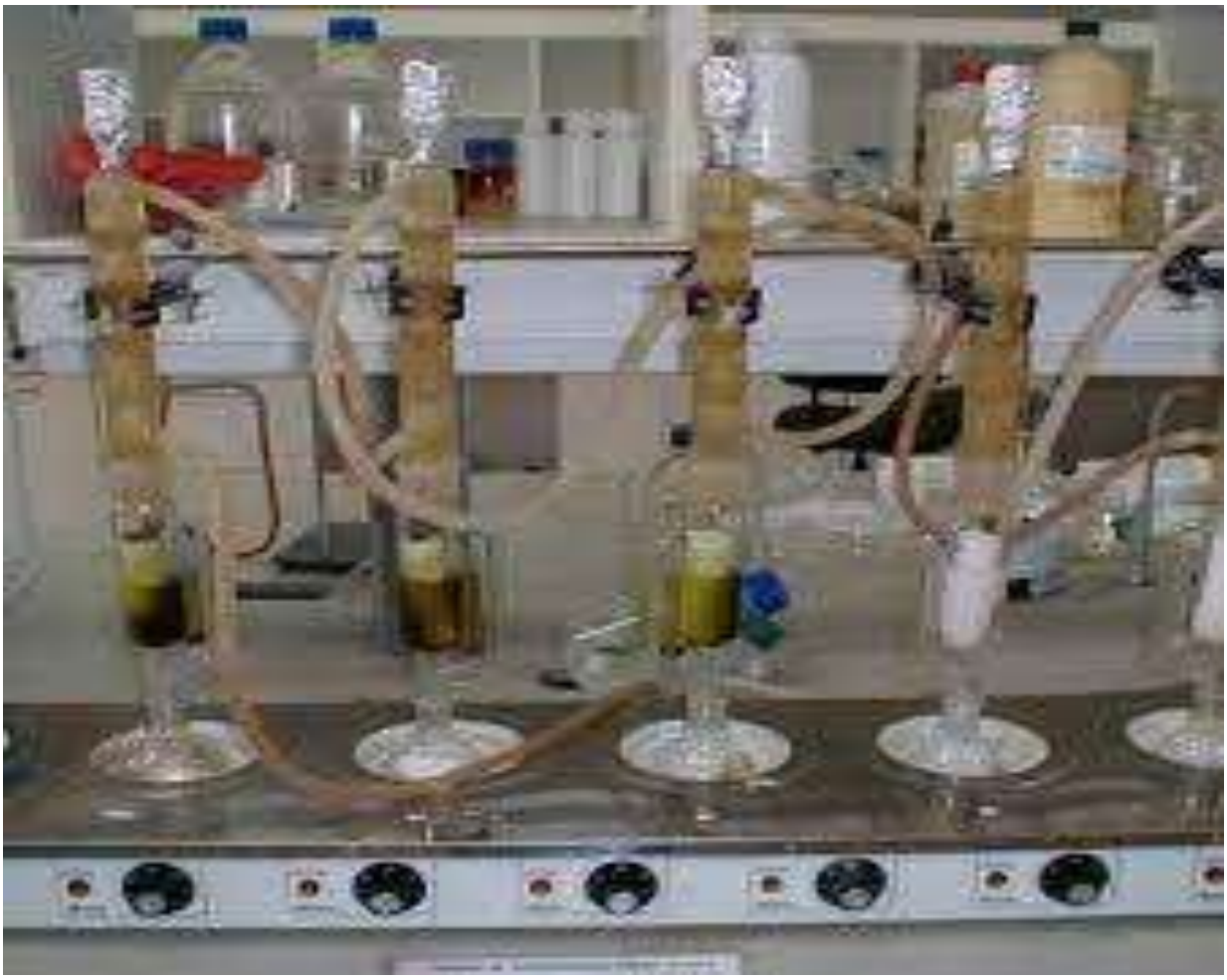

Figure 2.7. Soxhlet extraction units. [Source: (Szentmihályi *et al.,* 2002)].

2.9. Pineapple (*Ananas comosus*) products and Processing:

2.9.1 History and Botanical Descriptions of Pineapple (*Ananas comosus*).

An exotic fruit belonging to the *Bromeliaceae* family, the pineapple (*Ananas comosus*) is grown in tropical and subtropical climates. India, the second-largest producer of fruits in the world after Brazil, has a significant amount of this plant grown there. Large-scale producers of it include the Philippines, Thailand, China, Brazil, India, Mexico, and South Africa.With about 9% of worldwide output, India ranks fourth in the world for fresh pineapple production. The majority of

the remaining fruit is produced by Nigeria, Kenya, Indonesia, and Costa Rica (Caiqin and Wanlin 2010). In addition to canned pineapple slices, chunks, and dice, pineapple juice, fruit salads, sugar syrup, booze, citric acid, pineapple chips, and puree are also popular worldwide. In addition to having a pleasant sugar-acid balance, a high concentration of ascorbic acid, and organic acids, it is essentially made up of water, carbohydrates, sugars, vitamins A, C, and carotene (Bartolomew *et al.*, 1995).

Figure 2.8(a): A Green mature pineapple plant. Source: (Farid *et al.*, 2015).

Figure 2.8 (b): A cut section of ripped pineapple

Source: (Farid *et al.*, 2015).

Squash, syrup, and jelly are just a few of the dishes made using pineapple. Vinegar, alcohol, citric acid, calcium citrate, and other items are also made from pineapple juice. Additionally, pineapple is recommended as part of some sick people's medical diets. The National Library of Medicine classifies bromelain as a digestive enzyme that is proteolytic (Farid *et al.,* 2015). By disintegrating proteins into amino acids when taken with food, bromelain promotes the digestion of proteins. Among the 13–19% total solids and 81.2–86.2% moisture found in pineapple, sucrose, glucose, and fructose are its main sugars. Up to 85% of all solids are composed of carbohydrates, whereas only 2% are made up of fiber. The most prevalent organic acid is citric acid.A small amount of ash, nitrogenous compounds, and lipids (0.1%) are present in the pulp. 25–30% of true proteins are nitrogenous compounds. A protease called bromelain is responsible for around 80% of the proteolytic activity in this region. Minerals like calcium, chlorine, phosphorus, and salt are present in fresh pineapple (Farid *et al.,* 2015).

Pineapple fruits feature high levels of moisture, sugar, soluble solids, ascorbic acid, and low levels of crude fiber. Therefore, while the fresh fruits are normally ingested, pineapple can be used as a nutritional supplement for good personal health. They come in a range of dishes including dessert, fruit salad, jam, yogurt, ice cream, sweets, and as a side dish to meat meals, and they can be consumed fresh, tinned, or juiced.Additionally, pickles are made with green pineapple. The leftovers and tender leaves are both used as livestock feed once the juice has been extracted.

2.9.2 Nutritional Content of Pineapple *(Ananas comosus)*.

The pineapple is a wonderful tropical fruit with lots of health benefits, tremendous juiciness, and a distinct tropical flavor. Pineapple is a rich source of calcium, potassium, vitamin C, carbs, crude fiber, water, and a number of other minerals that are good for the digestive system, support healthy weight management, and promote balanced nutrition. Fresh pineapples contain a lot of bromelain, an anti-inflammatory that reduces swelling in inflammatory diseases like gout, acute sinusitis, sore throats, and arthritis. Foods created include pickles, jam, and jelly (Hossain *et al.*, 2015). The less-fattening and lower-sodium pineapple is a well-liked fruit in Bangladesh (Saad, 2004).The edible part of pineapple composition has mostly been examined. Pineapple has a moisture content of 81.2 to 86.2% and a total solids content of 13 to 19%, the bulk of which are sugar, glucose, and fructose. Up to 85% of all solids are made up of carbohydrates, but just 2% to 3% are made up of fiber. The organic acid that is most prevalent in it is citric acid. Ash, nitrogenous compounds, and lipids are relatively seldom components of pulp (0.1%). A quarter to thirty percent of nitrogenous compounds are true proteins. A protease called bromelain is responsible for around 80% of the proteolytic activity in this percentage. Minerals included in fresh pineapple include calcium, chlorine, potassium, phosphorus, and sodium (Idise, 2012). Ascorbic acid, a component of pineapple juice, aids in the absorption of iron while preventing

bacterial and viral infections. It is also a powerful antioxidant.An adult needs 50% of their daily vitamin C intake in one half cup of pineapple juice. Manganese, a trace mineral necessary for the synthesis and activation of particular enzymes as well as bone growth, is found in pineapples, which also include other essential minerals. Copper, a trace mineral, can also be found in pineapples. Additionally, it lowers blood pressure and slows the heart rate (Debnath, 2012), and it aids in iron absorption.

2.9.3 Utilization and Economic Importance of Pineapples

While citrus is primarily considered a subtropical fruit, pineapple ranks third in terms of importance behind bananas and mangoes. Although it is typically grown between 30N and 30S, it is planted in all tropical and subtropical countries. However, small plantations can be found in warm environments outside of these latitudes, sometimes with protective (Rohrbach *et al.* 2003).Worldwide pineapple production increased from 3,833,137 tons in 1961 to 15,287,413 tons (FAO 2004). In 2004, Thailand (17,000,000 t), the Philippines (1,650,000 t), Brazil (1,435,600 t), China (1,475,000), and India (1,300,000) accounted for more than half of global output. Processed goods such as canned slices, chilled fresh cut chunks and spears, juice and juice concentrates dominate global pineapple business. Worldwide concentrated juice exports, for example, surpass US$ 250 million, while canned pineapple exports exceed US$ 600 million. Nonetheless, the fresh fruit business, particularly the chilled, fresh-cut fruit market, is rapidly growing in value (Rohrbach *et al.* 2003). Pineapple is also high in fiber and bromelain, an enzyme used to tenderize meat, and its byproducts are utilized as animal feeds.

2.9.4. Processing of Pineapple into Different Products.

Tropical fruits, which are now underutilized, can help address the growing demand for nutritious, beautifully flavored, and appealing natural foods with high medicinal properties. They are widely recognized to be abundant in vitamins, minerals, and dietary fiber, making them an important

component of a well-balanced diet. Aside from their nutritional, therapeutic, and medicinal characteristics, several of these tropical fruits have a delicious flavor and a vibrant color. Pineapple is a complex food to eat on its own. The pineapple can be transformed into a range of commodities, with pineapple slices being the primary necessity for all types of products (Hossain, et al., 2015).Because the fruit's storage quality cannot be maintained for an extended period of time, advances in post-harvest processing will boost the fruit's effective utilization. Because of its hard shell, mucilaginous texture, and abundance of seeds, it is rarely eaten fresh.The fruit has a strong aroma that lingers even after cooking. Therefore, there is a huge potential for producing a variety of goods from this fruit. Typically, it is processed into jam, syrup, powder, leather, squash, nectars, and refreshing drinks. These products can easily become well-known in both domestic and foreign markets due to their high nutritional value and therapeutic importance (Idise, 2012). Around 80% of pineapple production worldwide is available in processed form on the market, with 30% going into canned goods and 48% going into single or concentrated juice (Saad, 2004). Tinned slices, juice, squash, dehydrated slices, and jam are examples of processed pineapple products. Confectionery can also be made from fruit core.Worldwide consumption of processed pineapples has prompted processing companies to experiment with or use cutting-edge technologies to maintain the fruit's nutritional content.

2.9.4.1 Pineapple Beverages

With artificial sweeteners, (Achumi *et al.,* 2018) produced a Pineapple RTS beverage with few calories. Combine the necessary amounts of juice, sugar, sweeteners, citric acid, and water to produce this. An RTS beverage with 15% juice, 14% sugar (sucrose), and 0.3% citric acid was the standard control recipe. However, while making RTS beverages, sugar substitutes were used in place of sugar (based on sugar equivalents). RTS drinks with aspartame, sucralose, 50% sucrose+50% sucralose, and 50% sucrose+50% sucralose are included in the treatments. Finally,

it can be said that with 50% sucrose + 50% sucralose, a high quality, organoleptically acceptable low calorie pineapple RTS beverage can be produced. The prepared low-calorie pineapple RTS beverage has about half as many calories as the control sample. Ready-to-drink beverages with spiced pineapple were developed by (Amaravathi *et al.,* 2014). The pineapple RTS drinks had extracts of ginger, green chiles, pepper, cardamom, and nutmeg. Spice extracts like ginger + pepper, ginger + cardamom, and ginger + nutmeg were combined to produce the RTS drinks. Organoleptic analysis was used to standardize the spiced pineapple RTS. The following parameters were measured: TSS, pH, acidity, reducing sugar, total sugar, tannin, -carotene, ascorbic acid, and non-enzymatic browning. All of the beverages exhibited good sensory qualities, with the exception of B2 and B3 beverages, according to the sensory analysis. (Ekeledo *et al.,* 2013) created pineapple drinks with a ginger basis. To make a spice for fried rice, turmeric and ginger were pounded into flours and combined in the ratios 9:1, 4:6, and 7:3 (w/w).

To create a drink with a turmeric-pineapple flavor, ginger and turmeric extracts were made and mixed in the ratios 13:6:1, 6:3:1, and 4:3:3 (v/v). The liquids' pH and total soluble solid contents were evaluated, and samples of the drinks and fried rice were sensory-evaluated. The drinks created with pineapple, ginger, and turmeric extracts in the ratios of 6:3:1 and 4:3:3 were rated on par with the commercial pineapple drink by the Taste Panelists. The acidity and total solids levels of the drinks were likewise superior to those of the typical beverage.

Pine apple juice:

Nevertheless, another important pineapple beverage is pineapple juice. However, processing pineapple is critical for keeping juice fresh. The effects of pasteurization and other preservation techniques on overall juice quality were discussed because the processing method utilized affects the quality of pineapple juice (Islam *et al.,* 2014). Juice processing causes chemical changes and the death of microorganisms. Although data on the kinetics of these events, the degradation of

amino acids and vitamin C, and the change in sugar levels during pineapple juice pasteurization are currently missing, this knowledge is necessary to optimize processing settings. Furthermore, a precise method like high-performance liquid chromatography should be used to study the kinetics of hydroxyl methyl furfural production. (Hounhouigan *et al*, 2014).

Pineapple Wine:

Wine is an alcoholic beverage made s fermented fruit juice (Okafor, 2007). Any fruit that contains a lot of sugar can be used to make wine, and the finished product is typically named after the fruit. The fruit and yeast strain employed are determined by the type of wine produced (Amerine and Kunkee 2005). Among the preservatives used in wine production are sulphur dioxide, potassium sorbate, sorbic acid, and metabisulphites (Idise and Emmanuel, 2012). When these preservatives are present in wine in excessive concentrations, it can lead to a number of systemic illnesses, including gastrointestinal irregularities in allergic persons and breathing difficulties in asthmatic patients. The effects of these drugs' bioaccumulation could make the issue worse (Okafor, 2007).

2.9.4.2. Pineapple Jam

Jam is a wet food made of pectin, sugar, acid, and fruit pulp. The impact of sugar and pectin concentrations, pH, shear rate, and temperature on the time-dependent rheological characteristics of pineapple jam was examined using a rheometer. Thixotropic pineapple jam was used. Temperature, content, and pineapple jam's shear stress at a specific shear rate all played a role (Okafor, 2007). The time-dependent flow characteristics of pineapple jam were described using models created by Weltman, Hahn, Figoni, and Shoemaker. The Hahn model was found to adequately explain the rheological characteristics of pineapple jam by (Bhavasgar *et al.,* 2010).

Banana, pineapple, and watermelon pulp blends with a fruit pulp: sugar ratio of 0.45: 0.55 were used to make jam by (Achumi *et al.,* 2018). The formulation therefore had 713 g of sugar and

584 g of fruit pulp. 2.9 mL of pectin, 0.03 g of citric acid, and 0.06 g of sodium benzoate were additional ingredients. Banana (*Musa paradisiaca*), pineapple (*Ananas comosus*), and watermelon (*Citrullus lanatus*) flesh were combined to make the pulp. For blending, each fruit was cleaned, dried, peeled, and sliced into smaller pieces. After blending, they were promptly refrigerated till use. The fruit pulp mixture was simmered for 10 minutes to pasteurize and soften the bits of fruit. The boiling pulp was mixed for ten minutes before sugar (713 g) and pectin solution were added for thickening. The mixture was then supplemented with preservatives (citric acid and sodium benzoate). Colorant was added and mixed for a further 55 minutes to produce the desired hue and gelation. Directly into a clean, covered jar, the liquid was transferred before being refrigerated with ice water.

2.9.4.3. Pineapple Candy

Candy is characterized as a mixture of sugar, honey, or other natural or artificial sweeteners with additional ingredients or flavorings, such as chocolate, fruits, nuts, or other ingredients (Achumi *et al.,* 2018). Producing ready-to-eat foods, particularly compacted bars, has been demonstrated to be one way to provide food security and reduce poverty (Pee and Bloem, 2009). The bar is described as a combination of ingredients that gives the food power and a low water content, providing a source of nutrients, in contrast to candies that are consumed as sweetened foods. The goal of the current study was to make gummy candies using pineapple and carrot juice. This high-value, nutrient-rich gummy candy will offer consumers countless nutritional benefits due to its potent beta-carotene content. According to research by (Achumi *et al.,* 2018), gummy candies can be successfully manufactured by combining pineapple juice, carrot juice, agar-agar, and sugar. The experimental gummy candy in treatment T3 had the highest organoleptic evaluation score and was determined to have the finest organoleptic attributes. Using 40, 50, and 60% sugar solutions with fresh pineapple, Hamid (2007) created pineapple candy, which was then sun-

dried. The acceptance of the product was also looked at. The thickness of the pineapple slices varied from 0.5 to 1.0 cm.

2.9.4.4. Pineapple Leather

Preparing the fruit puree, either with or without the addition of other ingredients, is a general step in the production of fruit leather. Depending on the fruit used, the type of ingredients used, the drying technique and technology employed, and other factors, these processes may vary. The advantages and disadvantages of different fruit leather processing techniques. The findings show that while most fruit leathers have certain drawbacks, the majority of them are brought on by the absence of color-protecting preservatives (Diamante *et al.* 2014).

2.9.4.5. Pineapple Powder

Due to its long shelf life at ambient temperature, simplicity of use, and low transportation costs, pineapple powder is a fascinating product. Pineapple powder can be used as a flavoring agent or as an instant juice powder. There haven't been many studies done yet on how pineapple powder is made. According to certain experiments, drying fruit juice could produce fruit powder that quickly reconstitutes into a fine product that closely resembles the original juice. It is as a result of the fact that during the drying process, the product temperature is infrequently raised above 100^{o}C (Adhikari *et al.,* 2004). Due to their thermoplasticity and hygroscopicity at high temperatures and humilities, fruit juice with a high sugar content, like pineapple, presents a number of obstacles while drying, leading to packaging and use issues (Chauca *et al.*, 2005). Pineapple powder specimens were produced by Jittanit *et al.*,(2010) using a spray drier with various drying settings. At rates of 0.020, 0.022, and 0.035 liter per minute, respectively, fresh pineapple juices were treated with maltodextrin at concentrations of 15, 20, and 25% before being dried at temperatures of 130, 150, and 170oC. Then, pineapple powders and reconstituted

pineapple powders' moisture content, solubility, color, pH, and consumer acceptance were investigated. The results indicate that 15% MD should be added to pineapple juice before drying at 150°C. Additionally, the pineapple powder generated under these circumstances contained 5.1% moisture and was soluble for 6.2 minutes while its solution had a lightness of 58.8, a redness of 5.2, a yellowness of 25.1, and a pH of 3.5. Foam mat drying is a more economical process than drum drying, spray drying, and freeze drying for the production of fruit powders. This study was conducted by Shaari *et al.* (2017) to investigate the effects of foaming agent concentration and whipping time on the properties of pineapple powder. Egg albumen (EA) was utilized to foam pineapple juice at different concentrations and whipping times (10, 20, and 30 minutes).The concentrations used were 5, 10, and 20% wt/wt. Foam density fell but foam expansion increased when the concentration of the foaming ingredient increased while the whipping time remained constant. Pineapple juice and puree that were 11 to 12 °Brix were dried in an oven to create pineapple powder. Maltodextrin was added in treatments 2 and 4 to produce high-quality powder, however treatments 1 and 3 produced a sticky substance that was later processed to create pineapple leather. The recovery of powder was much higher with treatment 2 (pineapple puree and maltodextrin) than with treatment 4 (pineapple juice and maltodextrin).

Pineapple powder solubility increased as maltodextrin content rose from 40% to 60%. The final product's stickiness was also decreased by the addition of maltodextrin. An instant pineapple powder with a moisture content of between 5.47 and 5.33% could be produced by oven drying. This amount of moisture content stops bacteria from growing in pineapple powder, but if the product is stored in high humidity for a long time, it may also allow mold or yeast to thrive. According to moisture sorption isotherm data, molds were found on the 17th day at a relative humidity of 89%. This suggests that pineapple powder should be stored properly with a moisture barrier.

2.9.4.6. Pineapple Vinegar

A great approach to turn wasteful cores, peels, and trimmings into income is to turn overripe, imperfect, or extra fruit into vinegar. Despite being less well-known than coconut vinegar, pineapple vinegar is already exported in small quantities. Pineapple vinegar can be produced by fermenting alcohol and acetic acid. Pineapple wastes can be turned into vinegar to lessen pollution while also creating a product with extra value. Pineapple peel fermentation is used to make vinegar (Raji *et al.,* 2012). With the aid of additional ingredients and baker yeast (Saccharomyces cerevisae), the fermentation was carried out in two stages. 48 hours were spent fermenting pineapple peel to turn sugar into ethanol. Acetic acid bacteria (Acetobacter aceti) used a chance strategy with the ethanol to convert it to vinegar using a chance technique with constant aeration for nine days. The data also showed that pineapple peel provided the desired and ideal vinegar output, and it was noted that vinegar yield increased as acidity increased. The pH, density, refractive index, viscosity, percentage of acetic acid, and acid value were therefore calculated and recorded as 2.80, 1.08 g/ml, 1.390, 0.94cp, 4.77, and 0.0477, respectively. Vijaya *et al.* (2017) produced pineapple vinegar by mixed yeast fermentation. The pineapple bran was juiced, and the leftovers were mixed with yeast and lactic acid bacteria for an alcoholic fermentation at 22 ^{o}C.

Acetic acid bacteria were added for acetic fermentation at 30 ^{o}C with full ventilation once the alcohol concentration stopped increasing. The acetic fermentation process was terminated when the acid content stopped increasing, and the vinegar was then aged for 30 days at 18 to 20 ^{o}C. Such brewed pineapple vinegar's physical and chemical characteristics comprised a total acid of 4.52 g/100 mL (in acetic acid) and a reducing sugar of 1.58 g/100 ml. The vinegar was centrifuged, filtered, and combined at a concentration of 2% using gas chromatography and mass spectrometry for testing and analysis. According to the findings, the vinegar possessed 36 flavors, including 13 volatile and 23 nonvolatile ones. These substances combined to give

pineapple vinegar a unique flavor. Vijaya *et al.* (2017) looked into the fermentation of pineapple bran into vinegar using a semi-solid medium. This method used pineapple bran as its main raw material, fruit wine yeast and Hennaing 1.01 acetic bacteria powder as its microbiological strains, and semi-solid fermentation and secondary sugar supplementation as its processes to find its best brewing process parameters. Finally, a semi-solid combination of pineapple vinegar with a total acid (acetate) concentration of up to 6.78g/100g was produced with an acetic acid conversion rate of 82.5%. The final result of pineapple vinegar, with a total acid content (acetic acid) of 3.672g/100ml, emerged golden, clear, and transparent, with both acidic aroma and pineapple's fruity aroma, as well as soft and refreshing flavor, after 12 months of soaking, filtering, and aging.

2.9.5. Utilization of Pineapple Wastes

The by-product ratios of tropical and subtropical fruits are much higher than those of temperate fruits (Schieber *et al.,* 2001). The by-products from pineapples are no different, and they mostly include leftover pulp, peels, stems, and leaves. Rejected fruit and waste materials are anticipated to be utilized in upcoming industrial processes including fermentation, the extraction of bioactive components, and others. The properties of *Saccharomyces cerevisiae* and *Zymomonasmobiles* grown on pineapple waste were studied by Dacera *et al.,* (2009). The pineapple waste contained 53% cell soluble components, including 5.2% sucrose, 3.1% glucose, and 3.4% fructose, and only 19% cellulose, 22% hemicellulose, 5% lignin, and 22% soluble sugars. As a result, substrate pretreatment was required. In two days, pineapple waste that had been pretreated with cellulose and hemicellulose and then fermented with *S. cerevisiae* or *Z. mobiles* produced about 8% ethanol. Because of its rich vitamin and amino acid content, pineapple bran is perfect for creating fruit vinegar. China has an abundance of pineapple resources, and turning pineapple bran into vinegar can boost the value of processing pineapple fruit by 10% or more. This not only increases the use and conversion rate of pineapple bran, but also realizes the full utilization

of finite natural resources by turning waste into profit, achieves the effective utilization of pineapple by-products, lowers resource waste, and significantly lowers the environmental pollution caused by pineapple by-products. As a result, it offers enormous social and economic benefits as well as a large commercial potential (Huanga *et al.*, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sources of Materials:

3.1.1Raw Material Collection

Mature and freshly harvested ginger (*Zingiber officinale*) and dried Ehuru (*Monodora myristica*) seeds and fresh ripe pineapple fruits were purchased directly from the farmers in Owerri, Imo State.

Mature dry *Oshiokirisho (Tetrapluera tetraptera)* fruits were obtained from the Federal University of Technology, Owerri, among the trees planted as wind-breaks and ornamental plants within the university.

The ginger *(Z. officinale), Oshiokirisho (T. tetraptera*) fruits, and *Ehuru (M. myristica)* were identified by a crop scientist at the Department of Crop Science FUTO and then taken to the Department of Food Science and Technology Laboratory, Federal University of Technology, Owerri where the sample preparations were carried out. The pictorial views of the tropical materials were shown in plates 3.1 and 3.1.

Plate 3.1. Freshly harvested Ginger and *Tetrapleura tetraptera.*

Plate 3.2. Left: (Roasted seeds of *Monodora myristica*); Right: (Deshelled roasted *Monodora myristica*).

3.1.2. Reagents, Laboratory Wares and Equipment

The various chemical reagents and laboratory wares/equipment used were of analytical grades. They were obtained from the Department of Food Science and Technology, Department of Biochemistry, and Department of Chemistry in the Federal University of Technology, Owerri, Nigeria.

3.2 Methods:

Sample Preparations and Phytochemical Screening for the bioactive compounds

3.2.1. Processing and Extraction of Ginger (*Z. officinale*) Extracts

The processing and extraction of wild ginger (*Z. officinale*) extract was done according to the method described by Jayashree*et al.* (2014). About 10 kg of freshly harvested ginger rhizomes were processed for extraction. The ginger was cleaned and washed with alcohol to decontaminate the surfaces from microbial loads. The washed ginger rhizomes were peeled to remove the barks.

The peeled fingers of ginger rhizomes were sliced into smaller sizes 5, 10, 15 20 and 30 mm long and dried under the sun for about five days before finish-drying in an oven at initial temperature of 55^{o}C before it was elevated to 65^{o}C for 10 hours which brought the moisture content from 88.7% down to 11.56%. The dried materials were milled into powdered forms (37µm) and stored in air-tight containers for subsequent processing. Afterwards, it was followed by weighing 400 g of the milled samples into a flask and microwave-assisted extraction to generate the essential oils (crude extracts) required for the beverage formulation. The flow chart is represented in Figure 3.1.

3.2.2. Processing and Extraction of Bioactive Compounds from Ehuru *(Monodora Myristica)*.

The method described by Onwuka (2005) was adopted with slight modification. A 2 kg of matured and dried Ehuru (*Monodora myristica*) seeds were sorted and gently roasted to enhance ease of dehulling. The dehulled seeds were further dried using a drying oven (at 105^{o}C) to ensure that the moisture contents were drastically reduced. The dried seeds were ground, stored in airtight containers and subsequently, 400 g of the dried milled samples were prepared to extract the bioactive components/essential oils (crude extracts). The flow chart for the process is shown in Figure 3.2.

3.2.3. Processing and Extraction of Crude Extracts from Oshiokirisho *(Tetrapluera tetraptera)* Fruits

The method described by Jayashree *et al.,* (2014) was adopted for this extraction, with slight modifications. About 20 pieces of matured and dried fruits of Oshiokirisho (*Tetrapluera tetraptera*) were used for this process. However, the fruits were cleaned and washed with an alcohol solution to disinfect the surface microorganisms. The washed fruits were dried in an electric oven (EUROSONIC; Model No. ES-9080, China) at 60^{o}C. The dried pods were cut into pieces for easy grinding into powders. The ground samples were stored in an air-tight contained and subsequently 400 g of the *T. tetrapluetra* powder subjected to microwave-assisted extraction to generate the bioactive component in the *T. tetraptera* fruits. The flow chart for the process is given in Figure 3.3.

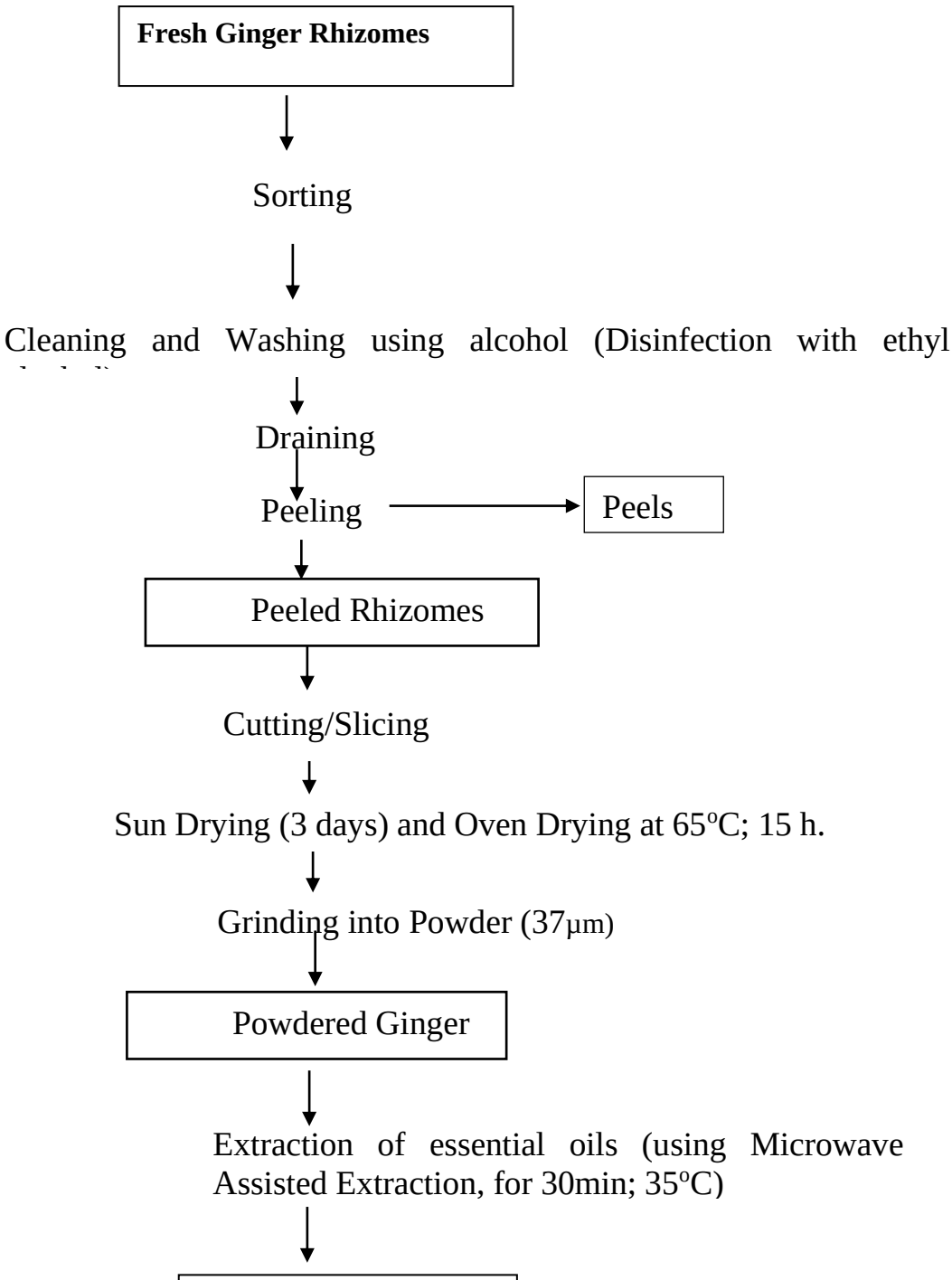
Fresh Ginger Rhizomes
Sorting
Cleaning and Washing using alcohol (Disinfection with ethyl
Draining
Peeling
Peels
Peeled Rhizomes
Cutting/Slicing
Sun Drying (3 days) and Oven Drying at 65ºC; 15 h.
Grinding into Powder (37μm)
Powdered Ginger
Extraction of essential oils (using Microwave Assisted Extraction, for 30min; 35ºC)

Figure 3.1. Processing/Extraction of Ginger Essential oils.

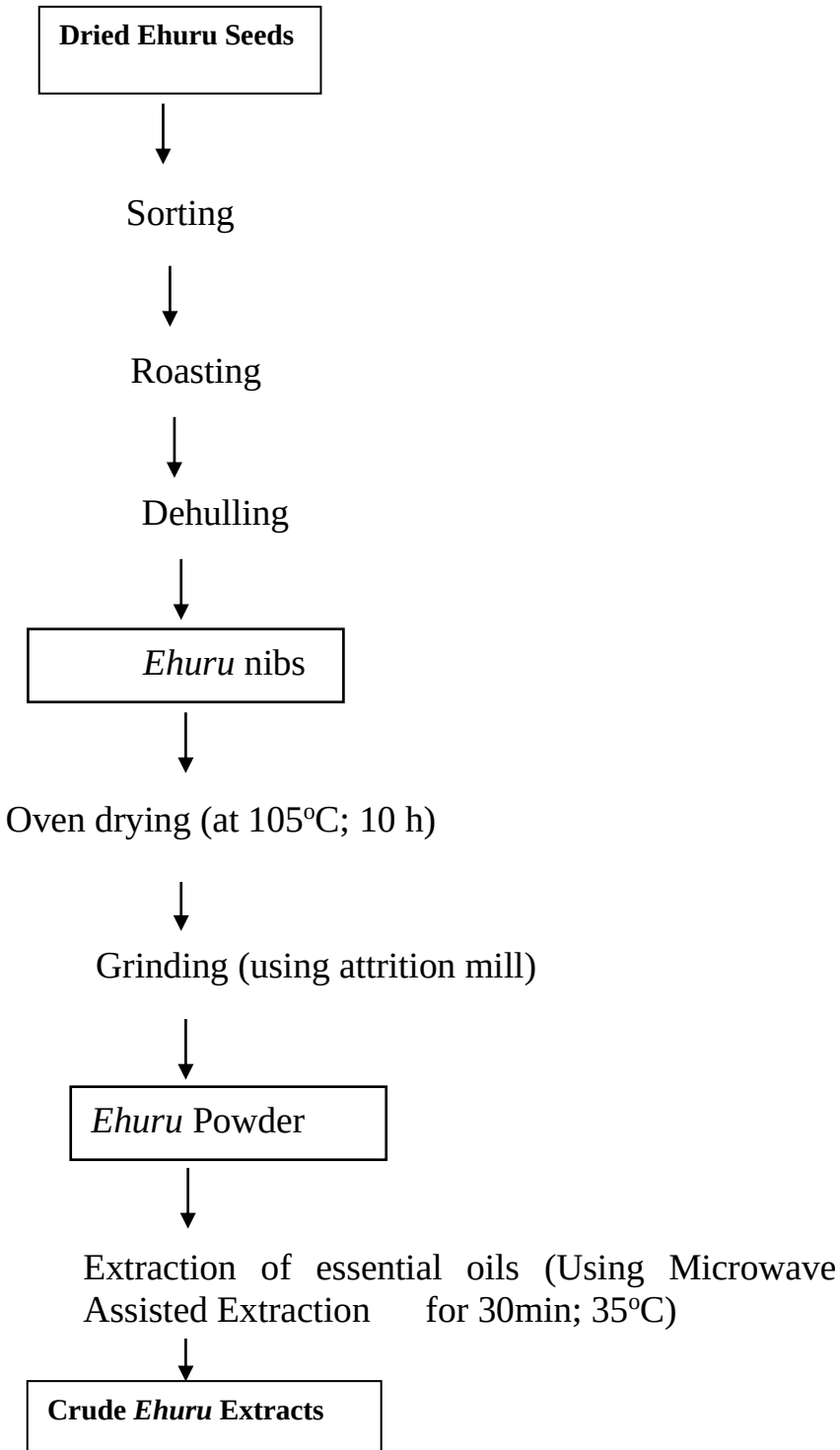

Figure 3.2. Processing/Extraction of Ehuru (*Monodoramyristica*) essential oils.

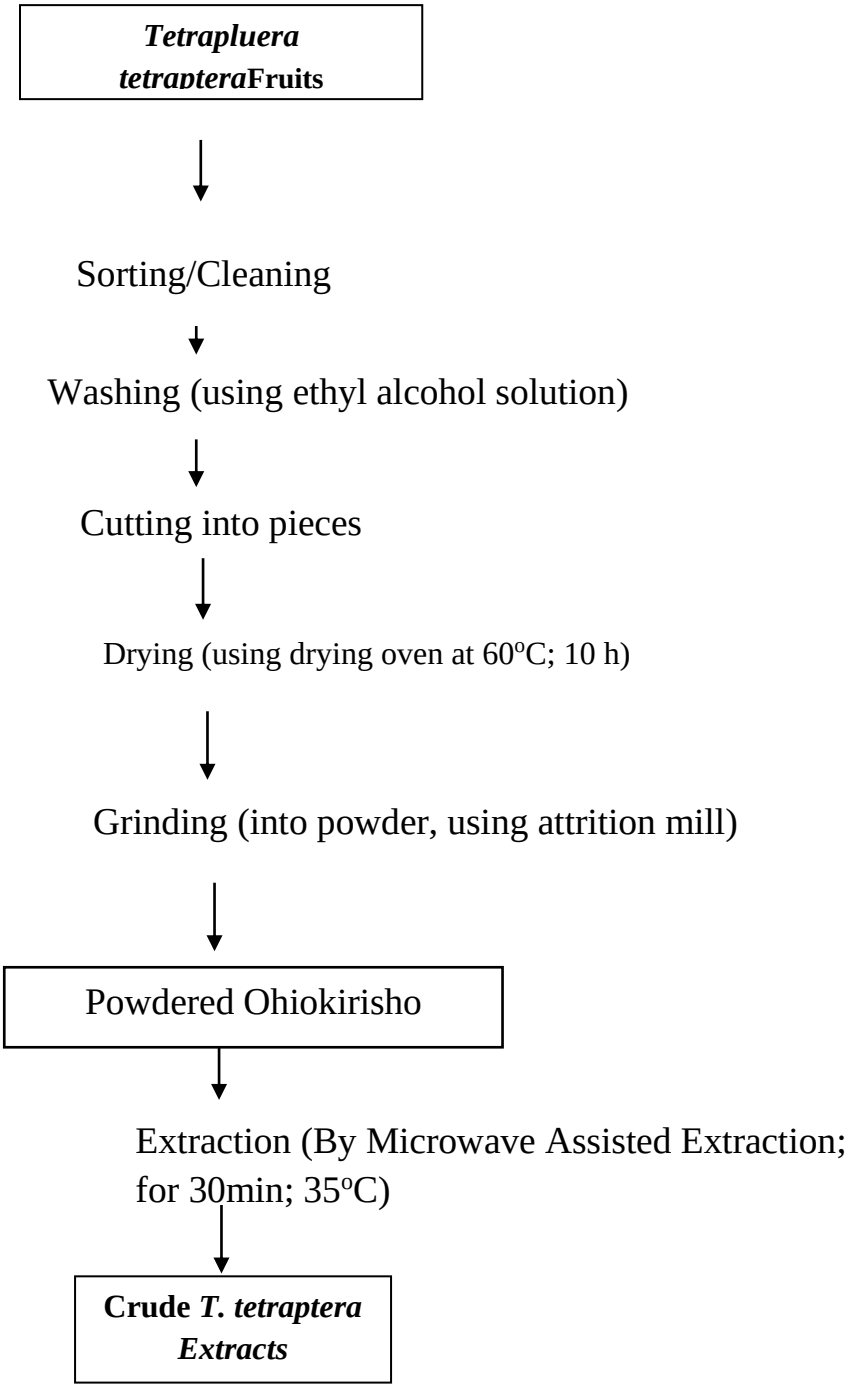

Figure 3.3. Processing/Extraction of *Tetrapluera tetraptera* crude extracts

3.2.4. Microwave-assisted extraction of the Crude Extracts

Spigno and Faveri (2009) described the method to extract the bioactive compounds. A 200g of the milled, dried sample of the plant materials in each case was transferred into a flat-bottomed flask of 500 ml capacity and 250ml of appropriate organic solvent (ethyl ether) was transferred into the flask containing the sample to be extracted. The flask was placed inside the microwave, stoppered and fitted to a condenser (a cooling system designed with a water inlet and outlet). The microwave

Figure 3.3.A Microwave assisted extraction set-up (containing, retort stands, condensers, and flat-bottomed flask holding the samples under extraction).

is electric powered, taking into cognizance the predetermined temperature of the solvent, extraction time (30 minutes) and power (300W), the basic operating conditions for the MAE. The microwave generated from the magnetron is directed by the waveguide onto the sample/solvent system, thus causing the solvent to boil and rise within the vessel. However, the evaporating solvent was cooled by the water-cooled reflux condenser. This made the solvent condense and return to the holding vessel. This process was repeated for a short time between 30

minutes, enabling the organic compounds to be desorbed from the sample matrix into the organic solvent. As the boiling process continued for about 30 minutes, the crude extracts (essential oil) was recovered by separating the solvent from the pool.

This procedure was repeated for all the different dried (powdered) samples of the plant materials (*Zingiber officinale*, *M. Myristica* and *T. tetraplegia*) as shown in figure 3.3. The extract yield was determined and recorded in each of the cases.

3.3. Production of Pineapple Juice

The pineapple juice production process used for the functional beverage formulation was prepared according to a method described by Ojukwu*et al.* (2015) and in line with FDA (2022) guidelines with slight modifications. A 20 kg of fully ripe and matured pineapples were graded, washed, and peeled. Then they were crushed in the crusher (juice extractor) to obtain the juice, and the juice extracted was transferred to kettle and boiled. The extracted fruit juices were filled in clean plastic cans and pasteurized at 68^{o}C for 30 minutes. The cans containing the pasteurized juice were cooled quickly after sealing and stored for subsequent use. The process flow chart is shown in figure 3.5.

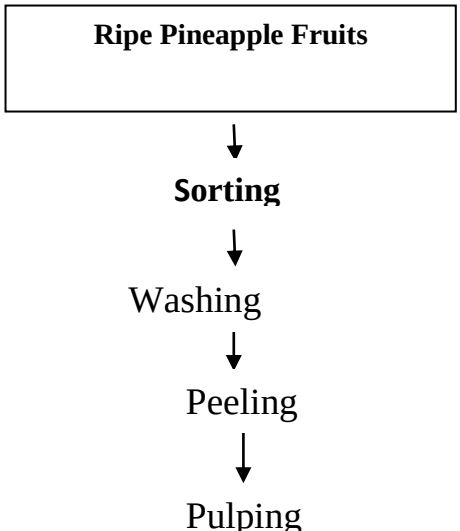

Figure 3.5: The production of Pineapple Juice.

3.4. Extract Yield, Phytochemical Screening/Evaluation of the bioactive compounds in the crude Extracts.

3.4.1 Determination of extraction yield:

The method described by AOAC. (2019) was used for this determination. The yield of evaporated dried extracts based on dry weight basis was calculated using the equation shown below:

$$(\%) Extract\ Yield = \frac{W1}{W2} \times 100 \qquad (3.1)$$

Where W_1 = weight of extract after evaporation of the solvent.

W_2 = dry weight of the sample

3.4.2. Phytochemical screening of the Presence (Relative abundance) of the different Phytochemicals/Bioactive compounds Present in the Crude extracts.

The methods of AOAC (2005) was adopted for the determination of the relative abundance and presence of the different phytochemicals/bioactive compounds (saponins, alkaloids, terpenoids, flavonoids, cardiac glycosides, steroids and phenols) in the crude extracts. The procedures for each of the tests were stated as follows;

Test for Saponins

About 2 g of each of the studied plant extracts was weighed and dissolved in 5 ml of distilled water. Thereafter, aliquots of 2 ml were taken from each plant extract solution, stirred for 30 seconds, and briskly agitated. The setups were then allowed to settle for 15 minutes. The presence of frothing, which persisted for over 15 minutes, was an indication of the presence of saponins in the tested sample.

Test for Alkaloids

About 2 g of each of the studied plant extracts was added to 10 ml of 0.1 M hydrochloric acid, warmed in a water bath (50°C) for 5 minutes, and filtered through Whatman filter paper No. 1. After cooling, 3 drops of Dragendorff's reagent were added and mixed. The appearance of a reddish-brown colour is a positive indication for the presence of alkaloids in the sample.

Test for Terpenoids

Into clean test tubes, 2 ml of alcoholic extracts were mixed with 5 drops of acetic anhydride. Thereafter, 5 drops of concentrated sulphuric were carefully added through the side of the test

tube. The formation of a blue ring at the interface showed the presence of terpenoids in the tested sample.

Test for Flavonoids

To 2 ml of alcoholic extracts of the studied plants and 5 drops of concentrated hydrochloric acid were added. The formation of a red colour indicates the presence of flavonoids. To another portion of the alcoholic extracts (2 ml), 1 ml of dilute ammonia was added and gently mixed. A greenish-yellow colour indicated the presence of flavonoids.

Test for Cardiac Glycosides

To test for cardiac glycosides presence, 0.5 g of the extract was dissolved in 2 ml glacial acetic acid containing 2 drops of 10% ferric chloride solution. One milliliter of concentrated H_2SO_4 was then slowly introduced into the underlying mixture. Appearance of either a violet band at the boundary is a positive test for the deoxy-sugars (cardenolides).

Test for Steroids

The presence of steroids in the studied plant extracts were determined in this study. About 0.5 g of each extract was dissolved in 2 ml of chloroform. This was followed by addition of 3 drops of the Liebermann–Burchard reagent and gently agitated. The presence of reddish-purple colour indicated the presence of steroids.

Test for Phenols

About 0.5 g of each of the studied plant extracts were boiled in 5 ml of 70% ethanol in a water bath for 5 minutes and then filtered through Whatman filter paper No. 1. After cooling, 5 drops of 5% ferric chloride were added and mixed. The appearance of a green precipitate indicates the presence of phenols in the sample.

3.4.3. Quantitative Evaluation of the Bioactive Compounds and Phytochemicals in the Crude Extracts.

Phytochemical tests were done to determine the presence or absence of chemical substances, including the presence of bioactive components (such as flavonioid, phenols, gingerols, carotenoids, terpenes, etc) in the crude plant extracts. The tests were conducted using standard procedures to identify the preliminary phytochemical screening following the methodology of Savithramma *et al.* (2011) by GC-MS techniques.

3.5. Development of the Functional Beverage

The method, as described by FDA (2022), was used for the beverage formulation with slight modifications. A 5 ml of the crude extracts of Ginger (*Zingiber officinale*), Oshiokirisho (*Tetrapluera tetraptera*) and Ehuru (*Monodora myristica*) were accurately measured out, respectively, using a pipette. A 500 ml of the extracted pineapple juice was measured and transferred to each crude extract alongside other ingredients such as 500 ml water, 3 drops of flavourings, 1 gram of acidity regulator (Sodium citrate), 1 gram of binders (carboxyl methyl cellulose; CMC) etc. However, for the beverage containing the blends of the extracts, the ration of formulation was 50:50 (i.e. 50% of each sample of crude extract, mixed with 50% of another, with combination of pineapple juice that was extracted). The mixture was continuously stirred until a homogenous solution was obtained. The resultant mixture was transferred into a kettle and sterilized at 65^{o}C for 30 minutes. The resultant beverages were filled and packaged in clean, PET bottles and pasteurized at 60^{o}C for 30 minutes before cooling. The crude extracts were also used in different combinations (proportions/ratios) to produce beverages using the same protocols. The beverage developed was stored at room or refrigeration temperature. See Figure 3.6 for the production steps of the beverage.

Pineapple juice, Crude Extracts from tropical spices, water, CMC, + other ingredients (Flavours, etc)

Figure 3.6. The production of Functional Beverage

3.6. Quality Evaluation of Formulated Beverage Samples

Determination of Physicochemical Properties of the Formulated Beverage

3.6.1 Determination of Total Solids

The method of Iwouno*et al.*, (2019) was used for this determination. A 25ml sample was weighed into a silica dish of known weight using an electronic balance (Gold Tech Precision Electronic Instrument Co. G.TET024, Delhi, India) and the value was recorded. The silica dish containing the sample was placed over a boiling water bath to evaporate excess water, and the evaporated sample was dried in an oven (EUROSONIC; Model No. ES-9080, China) at 100^{o}C for 2hours. The dried residue was cooled and weighed, and returned to the oven. It was continuously dried until a constant weight was obtained. The percentage of dried matter was calculated as the percentage of total solids as follows:

$$Percentage\ Total\ solids\ (TSS) = \frac{Mass\ of\ dried\ residue}{Mass\ of sample} X\ 100 \qquad 3.2$$

3.6.2Determination of Specific Gravity (S.G)

AOAC (2017) method was used to determine the specific gravity of the beverage samples. Thus, the specific gravity of the beverage samples was determined using a density bottle as stated below:

A clean and previously dried specific gravity bottle of 50ml capacity was weighed with the stopper, and the readings obtained were recorded. The distilled water of 50ml volume was weighed, and 50ml of the test sample was measured using a measuring cylinder and substituted with water after drying the bottle. It was then weighed afterwards. The weight of the sample and the bottle was determined and recorded. The specific gravity of the beverage samples was calculated using the density of a reference sample (water). Thus, the Specific gravity was calculated as follows:

$$Specific\ Gravity = \frac{Density\ of\ Sample}{Density of equal\ volume of\ water} \qquad 3.3$$

3.6.3. Determination of pH

The beverage pH was determined by AOAC (2019) procedure. The analysis was conducted using a portable (hand-held) pH meter or potentiometer (Tecnal model TEC-3MP, China) calibrated in pH 4.0 and pH 7.0 as indicated below:

The pH meter electrode was thoroughly rinsed with distilled water, and the reading was adjusted to zero mark. A50ml test sample was poured into a beaker, and the pH meter (previously calibrated) was immersed into the test samples. The sample was allowed to cover the pH meter electrode (probe), and the readings were taken and recorded.

3.6.4. Determination of Total Titratable Acidity

The total titratable acidity was determined by the method of AOAC (2017). The acidity was determined by titrimetric analysis using a sample of 1 ml of the formulated beverage diluted in 49 ml of distilled water. This diluted solution was neutralized with 0.1 N NaOH, using a solution of 1% phenolphthalein as an indicator.

3.6.5. Determination of Brix (Sugar content)

The apparent degrees brix (^{o}B) of the beverage samples was determined according to the method described by Iwouno *et al.* (2019) using a Milwaukee Digital refractometer. The glass prism of the digital Milwaukee refractometer was cleaned with distilled water and blotted with a clean wiper. A few drops of the test sample at 20^{o}C were placed on the refractometer's optical (sensitive) region using a micro-pipette, and the readings were noted from the visual display and, thus, recorded.

3.6.6. Viscosity Determination

The method used for this determination is described by Onwuka (2005) using a viscometer. The Ostwald viscometer model-LVT (Brook field Engrg. Lab. Inc., M.A.0217 U.S.A) was used to check the viscosities of the various beverage samples. That was achieved by measuring 200 ml

of the sample in a beaker of 250 ml capacity. The viscometer was set to revolve at 250 revolution per minute (rpm), and the readings were determined Sand recorded.

3.7. Evaluation of Bioactive Compounds in the Formulated Beverage

3.7.1. Antioxidant Activity

The spectrophotometric technique described by AOAC (2019) was used to determine antioxidant activity. The protocols involved the reduction of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, with some modifications. Briefly, five different concentrations of the studied plant extracts (50, 100, 200, and 400µg/ml) were prepared in methanol (analytical grade). The same concentrations were also prepared for L-ascorbic acid, which was used as a standard antioxidant. 5 ml of each studied extract was transferred into a clean test tube into which 5 ml of 0.3 mM DPPH in methanol was added in a tube and covered with aluminium. The absorbance was spectrophotometrically determined after 30 minutes of reaction ("plateau"). The colour turned from purple to yellow as the molar absorbance of the DDPH radical at 517 nm reduced when the odd electron of the DPPH radical became paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. Vitamin C was used as a reference compound. The Spectrophotometer (DR/2000 Hach Company, China) and 1 cm path length cuvettes were used to determine the antioxidant activity. It used mixtures of 2 ml solution of DPPH and 2 ml of the antioxidant. The same protocols were repeated for Ferric Reducing Antioxidant potential (FRAP), Lipid inhibition activities of the crude extracts and formulated beverages.

3.8. Shelf-life Study

Chukwu *et al.* (2017) reported the method to determine product shelf-life and it was also used in this research. The samples were placed in Scotch flasks with screw lids and stored at refrigeration condition (3-4^{o}C) and ambient temperature for a period of 60 days and were taken out at intervals; 0 day, 7th day, 14th day, 21st day, 28th day, 35th day, 42nd day, 56th day, 63rd

day, 70th day, 80thand 90th day and the changes in the beverage samples (eg. colour, odour, pH, precipitates, phase separations, and sedimentations) were determined.

However, analysis of liquid phase separation of the beverages was carried out according to Baccouche *et al.* (2013) with little modifications. In brief, the samples in 10 mL screw caped glass tubes were kept at 4 °C to assess liquid separation under the natural gravity force. The sample tubes were regularly inspected several times at one week interval for 56 days. A layer of clear supernatant was found at the top of the tube when the sedimentation occurred, and the volume recorded as an indication of the instability of the product. The liquid separated was represented as percent volume. The colour, phase separations, and off flavours were determines subjectively.

Sedimentation was determined according to the method of Gad *et al.* (2013) with few modifications. An aliquot of 5 mL of the beverage samples were centrifuged at 3000×*g* for 30 min at 25 °C. The sedimentation value was indicated as a percentage of the total fluid weight by using the following equation according to Baba, *et al.*, (2016):

$$\text{Sediment}\,\% = \frac{\text{Sediment formed}}{\text{Fluid total weight}} \times 100 \qquad 3.4$$

3.9. Sensory Evaluation

3.9.1. Sensorial tests on the final beverage developed and during shelf-life

The evaluated samples were the formulated beverage, and the control sample (Eviron health drink)contained extracts from ginseng plant was presented for sensory evaluation.

The samples were presented to each person and placed in 50 ml plastic disposable cups. However, 30 panelists that evaluated the samples were drawn from the labs, students and colleagues who take soft drinks (beverages) frequently. They were told about the aim of the research and were given a little orientation on how to evaluate the samples using a 9-point hedonic scale provided. The various attributes to be determined are colour/appearance, flavour, taste, after taste and general acceptability.

A 9-point hedonic scale was adopted for the rating to determine the sensory qualities, with scores ranging from:

9 = like extremely

8 = Like very much

7 = like moderately

6 = like slightly

5 = no difference

4 = Dislike slightly

3 = Dislike moderately

2 = dislike very much

1 = dislike extremely.

The higher ratings indicated good quality attributes ranging from 1 signifying dislike extremely to 9 signifying like extremely (Chukwu *et al.* 2017).

Meanwhile, necessary precautions were taken to prevent the carryover of flavours and overlap during the tasting, respectively. This was achieved by ensuring that the panelists rinsed their mouths and washed their hands with bottled water after each stage of sample evaluation.

3.10. Experimental Designs

Three spices, *Zingiber officinale*, *Monodora myristica* and *Tetrapluera tetraptera*, were subjected to extraction of their essential oils for laboratory analyses.

The extracts were used to produce a single strength beverages and in different proportions (50:50 for each of the extracts). In all, seven beverage samples were produced and subjected to various analyses, alongside the control sample, a commercial drink obtained from the market.

3.11. Statistical Analysis

The experimental design and statistical analysis were that of a randomized complete block design. All determinations were in triplicate. The data generated were analyzed using IBM SPSS software version 20.0 (SPSS Inc.) and Matlab 2015, and Ms Excel, version 2013, was also used. The mean comparison was carried out using a one-way ANOVA while the sample means were separated using the Fisher's least significant difference (LSD) at $P<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Results

4.1.1 Extract Yield

Table 4.1. The Results of the Extract Yields of the Samples

Samples	Extract Yield (g)	Percentage (%)
AGX (Ginger)	21.30	10.65
ATX (Ohiokirihio)	25.60	12.80
AMX (Ehuru)	30.14	15.07

Keys:

AGX = Crude Ginger extracts
ATX = Crude *T. tetrapluera* extracts
AMX = Crude *M. myristica* extracts

The result of the yield of extracts is presented in Table 4.1. The *Monodora. myristica gave the highest extract yield of* 30.14 g (15.07 %*), while* Ginger (*Zingiber officinale*) had the least yield of 21.30 g (or 10.65) %.

4.1.2. Phytochemical Analysis/Qualitative Tests

Table 4.2 Phytochemical Screening of the Presence of the Different Bioactive Compounds in the Crude Extracts.

Phytochemicals	AGX	ATX	AMX
Saponins	+	+	+
Flavonoids	++	++	++
Terpenoids	+	+	++
Gingerols	+++	++	++
Alkaloids	+	++	+
Phenolics	+++	+++	+++
Steroids	+	+	+
Tannins	+	+	+
Cardiac glycosides	+	+	+

Keys: AGX = Crude Ginger extracts
ATX = Crude *T. tetrapluera* extracts
AMX = Crude *M. myristica* extracts
\+ = Moderately Present (High)
++ = Very much present (Relatively higher)
+++ = Heavily Present.

The result of the presence of the phytochemicals and bioactive compounds in the crude extracts is shown in Table 4.2. Phenolic compounds were found to be heavily present in all the crude extracts (*Zingiber officinale, T. tetraptera, and M. myristica*), while flavonoids were relatively high in all at the same degree. Saponins, Steroids and cardiac glycosides are also present to the same degree, while only gingerols were found to be abundant in ginger extracts.

Table 4.3: The Mean Scores of the Quantitative Phytochemicals in the Crude Extract (mg/g)

Samples	Saponin	Flavonoid	Terpenoid	Tannins	Alkaloids	Phenolics	Steroids	Cardiac Glycoside
AGX (Ginger)	2.71^{a}±0.06	11.84^{b}±0.12	5.87^{b}±0.05	4.84^{c}±0.14	7.47^{b}±0.09	23.26^{b}±0.55	3.36^{b}±0.06	2.37^{c} ±0.15
ATX (Ohiokirihio)	2.53^{b}±0.04	10.68^{c}±0.12	5.36^{c}±0.14	5.29^{ab}±0.03	10.17^{a}±0.03	20.93^{c}±0.27	4.31^{a}±0.06	3.83^{a} ±0.06
AMX (Ehuru)	2.48^{b}±0.03	14.33^{a}±0.38	7.30^{a}±0.05	5.46^{a}±0.06	6.84^{c}±0.09	26.06^{a}±0.57	2.02^{c}±0.05	3.56^{b}±0.05
LSD	**0.1162**	**0.5833**	**0.2295**	**0.3882**	**0.1916**	**1.1864**	**0.1396**	**0.2454**

*The means 'abc…' with **different** superscripts within the same column are significantly different ($p \leq 0.05$).*

Keys: **AGX** = Crude Ginger extracts

ATX = Crude *T. tetrapluera* extracts

AMX = Crude *M. myristica* extracts

The results of the quantitative photochemistry is shown in Table 4.3. Phenolics were highest in *M. myristica* extracts (26.06) and least in ginger extracts (23.6). Saponin is highest in ginger and lowest in *M. myristica* but highest in flavonoids, Tepenoids and tannins, with values of 14.33, 7.30 and 5.46 mg/g, respectively. Ginger gave the least cardiac glycoside (2.37), while *M. myristica* had the highest value (3.56) mg/g.

4.1.3. Antioxidant Assays

Table 4.4: Mean Scores of DPPH Radical Inhibitory Activities of the Crude Extracts at Different Concentrations (%).

Samples	50(µg/ml)	100(µg/ml)	200(µg/ml)	400(µg/ml)
Control	41.07^{d}±0.63	41.07^{d}±0.82	41.07^{d}±0.28	41.07^{d}±0.19
AGX (Ginger)	49.2^{b}±0.33	66.38^{b}±0.50	80.79^{b}±0.32	87.14^{b}±0.14
ATX(Ohiokirihio)	44.64^{c}±0.24	60.06^{c}±0.14	77.60^{c}±0.47	84.65^{c}±0.17
AMX (Ehuru)	56.61^{a}±0.06	71.27^{a}±0.21	85.54^{a}±0.47	88.02^{a}±0.23
LSD	**1.2998**	**1.1486**	**0.9102**	**0.4264**

*The means 'abc…' with **different** superscripts within the same column are significantly different ($p \leq 0.05$).*

Keys: **Control** = Vitamin C
AGX = Crude Ginger extracts
ATX = Crude *T. tetrapluera* extracts
AMX = Crude *M. myristica* extracts

The result of the DPPH radical inhibitory activities of the crude extracts at different concentrations is described in Table 4.4. The *M. myristica* extracts recorded the highest mean scores for all the concentrations, while the control (Vitamin C) had the least value. The trend followed the same pattern as the concentration increased.

Table 4.5: The Mean Scores of the Percentage FRAP Activities of the Crude Extracts at Different Concentrations.

Samples	50(µg/ml)	100(µg/ml)	200(µg/ml)	400(µg/ml)
Control	18.20 [d] ±0.5050	18.20[d] ±0.2250	18.20[d]±0.2501	18.20[d]±0.3132
AGX (Ginger)	73.39 [a] ±0.4613	82.42 [a] ±0.2212	88.51[a]±0.2001	89.93[a]±0.1504
ATX(Ohiokirihio)	34.42 [c] ±0.076	61.72[b]±0.4225	77.22[b]±0.0113	79.53[b]±0.0802
AMX (Ehuru)	40.84 [b] ±0.226	50.18[c]±0.1550	58.30[c] ±0.1450	76.80[c]±0.2179
LSD	**0.8352**	**0.6338**	**0.4256**	**0.4819**

The means 'abc…' with differentsuperscripts within the same column are significantly different (p≤0.05).

Keys: **Control** = Vitamin C
AGX = Crude Ginger extracts
ATX = Crude *T. tetrapluera* extracts
AMX = Crude *M. myristica* extracts

Table 4.5 displays the mean values for the Ferric reducing antioxidant potentials of the crude extracts at different concentrations. Sample AGX (Ginger extracts) gave the highest mean scores at all concentrations, while the control recorded the least values for FRAP activities. The means for all the samples ranged from 18.20 to 40.48% at 50(µg/ml) and 62.69 to 89.93% at 50(µg/ml).

Table 4.6: The Mean Scores of the Lipid Peroxidation Inhibition Activities of the Extracts at Different Concentrations.

Samples	50(µg/ml)	100(µg/ml)	200(µg/ml)	400(µg/ml)
Control	2.86^{a} ±0.0529	2.85^{b} ±0.0153	2.86^{b} ±0.02	2.88^{c}±0.0838
AGX (Ginger)	2.01^{c}±0.0737	2.63±0.0702^{c}	2.88^{b}±0.0721	3.48^{b}±0.0173
ATX (Ohiokirihio)	1.73^{d} ±0.0850	1.97^{d} ±0.0737	2.29^{c} ±0.0361	2.91^{c}±0.0666
AMX (Ehuru)	2.23^{b} ±0.0361	3.26^{a} ±0.03606	3.50^{a} ±0.0500	4.55^{a}±0.0473
LSD	**0.1493**	**0.1258**	**0.1118**	**0.1364**

*The means 'abc…' with **different** superscripts within the same column are significantly different (p≤0.05)*

Keys: Control =Vitamin C
AGX = Crude Ginger extracts
ATX = Crude *T. tetrapluera* extracts
AMX =Crude *M. myristica* extracts

The lipid peroxidation inhibition activity at different concentrations is presented in Table 4.6. The control recorded higher mean values than all other samples, which is 2.86 µg/mg at 50(µg/ml) t at 400 (µg/ml). Sample ATX *(M. myristica*) recorded the least mean scores ranging from 1.73 to 2.91 **(**µg/ml)for the same concentrations.

Table 4.7: The Mean Scores of the Tocopherols, Carotenoids, Gingerols, Terpenes, Vitamins C and B Vitamins contents of the Extracts (µg/ml).

Samples	Tocopherol	Carotenoids	Gingerols	Terpenes	Vit. C	Vit. B_6	Vit. B_{12}
AGX (Ginger)	0.029^{ab}±0.01	15.50^{a}±0.30	18.79^{a}±0.49	9.46^{a}±0.252	9.04^{a}±0.28	8.56^{a}±0.30	15.00^{a}±0.10
ATX (Ohiokirihio)	0.041^{a}±0.01	7.55^{c}±0.05	13.26^{b}±0.08	8.64^{b}±0.14	6.21^{b}±0.08	7.26^{c}±0.07	12.09^{c}±0.11
AMX (Ehuru)	0.024^{b}±0.00	17.66^{a}±0.52	12.07^{c}±0.17	5.49^{c}±0.15	9.33^{a}±0.11	7.88^{b}±0.12	13.89^{b}±0.22
LSD	**0.0145**	**0.8550**	**0.7411**	**0.4642**	**0.4328**	**0.4703**	**0.3746**

*The means 'abc…' with different superscripts within the same column are significantly ($p \leq 0.05$), while the means with the **same superscripts** are not significantly different ($p \geq 0.05$)*

Keys: **AGX** = Crude Ginger extracts

ATX = Crude *T. tetrapluera* extracts

AMX = *Crude M. myristica* extracts

The mean scores for the tocopherol, carotenoids, gingerols, terpenes, Vitamin C and other B-Vitamins in the crude extracts are shown in Table 4.7. *Tetrapleura* is the sample with the highest means (0.041 µg/ml), while *M. myristica* extracts had the least mean score (0.024 µg/ml). Ginger extract was the highest for carotenoids, gingerol, terpenes, vitamin C, Vit B_6 and B_{12} with the means of 15.50, 18.79, 9.46, 9.04, 8.56 and 15.00 (µg/ml), .respectively. *T. tetraptera* had the lowest value for carotenoids (7.55 µg/ml) and the lowest for Vit. B_6 and B_{12} (7.26 and 12.09 µg/ml, respectively). *M. myristica* was the least for gingerols, and terpenes, having mean scores of 12.07 and 5.49 µg/ml, respectively.

4.1.4. Results of the Formulated Beverage Samples produced from the different Extracts from the Tropical Spices.

Table 4.8 (a). The Mean Scores of the Percentage DPPH radical inhibitory activities of the Beverage Samples at different concentrations.

Samples	1.00 ml	2.00 ml	5.00 ml	10.00 ml
Control	61.07^{c}±0.63	61.07^{g}±0.07	61.07^{f}±0.28	61.07^{f}±0.19
AGY	56.16^{e}±0.07	77.96^{a}±0.07	86.49^{d}±0.83	88.90^{c}±0.16
ATY	54.85^{f}±0.03	64.39^{h}±0.67	80.74^{e}±0.61	85.98^{e}±0.16
AMY	59.66^{d}±0.12	73.23^{d}±0.28	88.55^{bc}±0.45	89.55^{c}±0.38
AGT	61.90^{c}±0.05	71.09^{e}±0.50	87.54^{c}±0.87	88.49^{d}±0.41
AGM	66.78^{b}±0.00	76.57^{b}±0.08	88.87^{b}±0.19	89.70^{bc}±0.28
ATM	61.30^{c}±0.24	68.23^{f}±0.23	85.53^{d}±0.57	90.37^{b}±0.59
GTM	68.20^{a}±0.13	74.70^{c}±0.36	92.15^{a}±0.25	91.88^{a}±0.19
LSD	**0.85**	**0.76**	**0.99**	**0.69**

*The 'abc… means' with **different** superscripts within the same column are significantly different (p≤0.05), while the means with the **same superscripts** are not significantly different (p≥0.05)*

Keys:

Control = Commercial Health drink

AGY=100 % Crude Ginger extracts; ATY= 100 % *T. tetraptera* Crude Extracts

AMY= 100 % *M. myristica Crude* Extracts; AGT= *50:50 Ginger and T. tetraptera* Crude Extracts.

AGM= *50:50 Crude Ginger and M. myristica* Extracts; ATM= *50:50 T. tetraptera and M. myristica Crude* Extracts

GTM= 1:1:1 Crude Ginger, *T. tetraptera and M.myristica* CrudeExtracts.

The results presented in Table 4.8(a) show the values for the DPPH antioxidant for the different samples of the developed beverage. GTM recorded the highest mean score of 68.20 and 91.88% at 1 ml and 10 ml concentrations, respectively. The control sample, (Eviron Health Drink) recorded the scores of 61.07% at all levels of concentrations (5 ml and 10 ml concentrations). The values for other samples, except the control were found to increase with an increase in concentrations.

Table 4.8 (b) The Mean scores of the Percentage Nitric Oxide Scavenging Activities in the Beverage Samples at Different Concentrations.

Samples	1.00 ml	2.00 ml	5.00 ml	10.00 ml
Control	39.61^{b}±.27	39.61^{e} ±0.22	39.61^{g}±0.34	39.61^{g}±0.20
AGY	33.79^{f}±0.19	44.89^{e} ±0.02	65.25^{f}±0.42	83.46^{f}±0.11
ATY	35.72^{e}±0.14	58.94^{d}±0.53	70.77^{e}±0.47	87.83^{e}±0.57
AMY	36.03^{d}±0.07	58.85^{d}±0.39	73.61^{c}±0.41	89.15^{d}±0.31
AGT	36.49^{d}±0.71	58.29^{d}±0.27	65.73^{f}±0.31	90.75^{c}±0.28
AGM	38.19^{c}±0.28	59.70^{c}±0.27	72.84^{d} ±0.38	90.63^{c}±0.33
ATM	38.59^{c}±0.17	62.86^{b}±0.38	75.12^{b} ±0.43	93.78^{b}±0.25
GTM	40.54^{a}±0.41	64.87^{a}±0.39	77.61^{a}±0.51	95.20^{a}±0.28
LSD	**0.71**	**0.72**	**0.88**	**0.67**

*The means 'abc…' with **different** superscripts within the same column are significantly different (p≤0.05), while the means with the **same superscripts** are not significantly different (p≥0.05).*

Keys:

Control = Commercial Health Drink.

AGY=100 % Crude Ginger extracts; ATY= 100 % *T. tetraptera* Crude Extracts

AMY= 100 % *M. myristica Crude* Extracts; AGT= *50:50 Ginger and T. tetraptera* Crude Extracts.

AGM= *50:50 Crude Ginger and M. myristica* Extracts; ATM= *50:50 T. tetraptera and M. myristica Crude* Extracts

GTM= 1:1:1 Crude Ginger, *T. tetraptera and M. myristica* CrudeExtracts.

The means for the Nitric Oxide Scavenging Activities for the formulated beverage are shown in Table 4.8(b). The control (Eviron Health Drink) was the least at both 5 ml and 10 ml respectively with corresponding values of 39.61g. Sample GTM comprising equal volumes of the three extracts records the highest means at all concentrations: 1 ml, 2 ml, 5 ml, and 10ml with corresponding values of 40.54, 68.87, 77.61 and 95.20 %. AGY recorded the lowest values (33.79 and 65.29 for 1 ml and 5 ml, respectively) while the control gave 44.70 and 67.39 µg/ml for 2 ml and 10 ml, respectively, the lowest mean scores.

Table 4.8(c). The Mean Scores of the Percentage FRAP activities of the Beverage Samples at different Concentrations.

Samples	1.00 ml	2.00 ml	5.00 ml	10.00 ml
Control	38.09^{h}±0.22	38.09^{g}±0.23	38.09^{h}±0.25	38.09^{f}±0.31
AGY	75.50^{b}±0.55	86.28^{b}±0.44	89.50^{b}±0.19	90.13^{b}±0.05
ATY	44.56^{g}±0.16	71.72^{c}±0.42	79.99^{c}±0.25	83.42^{d}±0.40
AMY	50.84^{e}±0.23	54.75^{f}±0.66	68.30^{d}±0.15	86.80^{c}±0.22
AGT	76.00^{a}±0.84	88.57^{a}±0.10	90.49^{a}±0.16	91.41^{a}±0.08
AGM	66.45^{c}±0.61	71.14^{c}±0.70	75.78^{e}±0.10	86.22^{c}±0.36
ATM	48.58^{f}±0.35	58.25^{e}±0.24	67.26^{f}±0.46	76.06^{e}±0.22
GTM	55.53^{d}±0.24	60.52^{d}±0.30	65.69^{g}±0.44	68.94^{g}±0.14
LSD	**0.97**	**0.92**	**0.59**	**0.54**

*The means 'abc…' with **different** superscripts within the same column are significantly different ($p \leq 0.05$), while the means with the **same superscripts** are not significantly different ($p \geq 0.05$).*

Keys:

Control = Commercial Health Drink.

AGY=100 % Crude Ginger extracts; ATY= 100 % *T. tetraptera* Crude Extracts

AMY= 100 % *M. myristica Crude* Extracts; AGT= *50:50 Ginger and T. tetraptera* Crude Extracts.

AGM= *50:50 Crude Ginger and M. myristica* Extracts; ATM= *50:50 T. tetraptera and M. myristica Crude* Extracts

GTM= 1:1:1 Ginger, *T. tetraptera and M. myristica* CrudeExtracts.

The FRAP activity values for the formulated beverage are given in Table 4.8(c). The control showed consistency in values of FRAP at all levels of concentrations with mean score of 38.09 g; thus among the least scores. Sample AGT gave the highest mean score in all concentrations; thus:-76.00%, 88.57%, 90.49 and 91.41 for the respective concentrations. At 1 ml and 5 ml, sample ATM recorded 48.58 and 67.26%, the lowest likewise the control with the values of 49.64 and 72.69% for 2ml and 10ml, respectively. The value of the FRAP is directly proportional to the concentration.

Table 4.8 (d). The Mean scores of the Lipid Peroxidation Inhibition Activities of the Beverage Samples at Different Concentrations (µg/ml)

Samples	1.00 ml	2.00 ml	5.00 ml	10.00 ml
CONTROL	2.86^{a}±0.05	2.86^{c}±0.02	2.86^{e}±0.02	2.86^{e}±0.08
AGY	2.37^{b}±0.26	2.82^{d}±0.03	3.01^{c}±0.04	3.84^{c}±0.08
ATY	1.81^{c}±0.06	2.03^{f}±0.04	2.52^{d}±0.03	3.02^{e}±0.05
AMY	2.33^{b}±0.04	3.36^{a}±0.04	3.75^{a}±0.05	4.63^{a}±0.03
AGT	2.32^{b}±0.20	2.92^{d}±0.05	2.68^{e}±0.07	3.24^{e}±0.04
AGM	2.34^{b}±0.15	3.12^{b}±0.05	3.22^{b}±0.05	4.12^{b}±0.05
ATM	2.07^{bc}±0.10	2.22^{e}±0.07	2.96^{d}±0.10	3.73^{d}±0.08
GTM	2.20^{c}±0.09	2.81^{d}±0.15	3.03^{c}±0.11	4.15^{b}±0.23
LSD	**0.29**	**0.14**	**0.14**	**0.21**

The means 'abc…' with different superscripts within the same column are significantly different (p≤0.05), while the means with the ***same superscripts*** *are not significantly different (p≥0.05).*

Keys:

Control = Commercial Health drink

AGY=100 % Crude Ginger extracts; ATY= 100 % *T. tetraptera* Crude Extracts

AMY= 100 % *M. myristica Crude* Extracts; AGT= *50:50 Ginger and T. tetraptera* Crude Extracts.

AGM= *50:50 Crude Ginger and M. myristica* Extracts; ATM= *50:50 T. tetraptera and M. myristica Crude* Extracts

GTM= 1:1:1 Ginger, *T. tetraptera and M. myristica* Crude Extracts.

Table 4.8(d) showed the lipid peroxidation inhibition for the nutraceutical beverages formulated. The control gave the highest means at the 1 ml concentration and thus, showed consistent value at other levels of concentrations (2, 5 and 10 ml respectively) with a mean score of 2.86 µg/ml. while sample ATY gave the t mean scores for all the concentrations, which ranged from 1.81 to 3.0 µg/ml for 1 ml and 10 ml concentration respectively while AMY recorded mean scores ranging from2.33 to 4.63 across all levels of concentrations.

Table 4.9. The Mean scores of Sensory Evaluation of the Beverage Samples.

Samples	Colour	Taste	After Taste	Flavour	General Acceptability
CTY	$8.40^{a}\pm0.07$	$8.20^{a}\pm0.15$	$8.50^{a}\pm0.42$	$8.00^{a}\pm0.38$	$8.00^{a}\pm0.32$
AGY	$7.80^{bc}\pm0.21$	$8.00^{a}\pm0.07$	$7.50^{b}\pm0.08$	$7.58^{a}\pm0.09$	$7.90^{ab}\pm0.08$
ATY	$7.90^{b}\pm0.16$	$7.50^{b}\pm0.04$	$8.00^{a}\pm0.25$	$7.80^{a}\pm0.08$	$7.70^{b}\pm0.12$
AMY	$7.80^{b}\pm0.08$	$6.55^{c}\pm0.03$	$5.50^{d}\pm0.32$	$6.80^{c}\pm0.12$	$6.50^{d}\pm0.05$
AGT	$7.50^{c}\pm0.06$	$7.80^{b}\pm0.22$	$7.00^{bc}\pm0.09$	$7.10^{bc}\pm0.04$	$7.50^{b}\pm0.12$
AGM	$6.50^{e}\pm0.27$	$6.00^{e}\pm0.09$	$5.20^{d}\pm0.28$	$5.50^{d}\pm0.23$	$5.80^{e}\pm0.03$
ATM	$7.75^{bc}\pm0.15$	$6.20^{e}\pm0.09$	$6.50^{c}\pm0.07$	$6.68^{c}\pm0.09$	$6.70^{c}\pm0.22$
GTM	$7.00^{d}\pm0.08$	$6.50^{d}\pm0.03$	$5.00^{d}\pm0.35$	$7.40^{b}\pm0.07$	$6.30^{d}\pm0.15$
LSD	**0.38**	**0.24**	**0.67**	**0.48**	**0.29**

The means 'abc…' with different superscripts within the same column are significantly different ($p\leq0.05$), while the means with the same superscripts are not significantly different ($p\leq0.05$).

Keys:

Control = Eviron Health drink

AGY=100 % Crude Ginger extracts; ATY= 100 % *T. tetraptera* Crude Extracts

AMY= 100 % *M. myristica Crude* Extracts; AGT= *50:50 Ginger and T. tetraptera* Crude Extracts.

AGM= *50:50 Crude Ginger and M. myristica* Extracts; ATM= *50:50 T. tetraptera and M. myristica Crude* Extracts

GTM= 1:1:1 Ginger, *T. tetraptera and M. myristica* Crude Extracts.

The result of the sensory evaluation is stipulated in Table 4.9. The control sample recorded the highest values in all the attributes evaluated, while AGM recorded the least mean value for all the attributes. The control (Eviron Health drink) was generally preferred among all the samples, having recorded a value of 8.00, followed by sample AGY with a mean score of 7.90, as it was generally preferred among all the formulated beverages.

Table 4.10. The mean scores of Physicochemical Properties of the Beverages Produced

Samples	TTA(mg lactic acid)	pH	Viscosity (mPas)	TSS (mg/l)	Total Sugars (°Brix)
CTY	1.92^{c}±0.001	3.66^{d}±0.04	69.45^{e}±0.66	2.93^{f} ±0.04	7.17^{a}±0.25
AGY	2.60^{b}±0.02	4.50^{b} ±0.19	70.29^{e}±0.04	5.38^{a}±0.06	6.22^{b}±0.19
ATY	3.21^{a}±0.01	4.23^{c} ±0.03	60.71^{f}±0.52	4.60^{b}±0.03	4.20^{cd}±0.00
AMY	2.90^{ab}±0.58	4.28^{c} ±0.02	77.47^{d}±0.51	2.94^{f}±0.02	4.10^{cd} ±0.10
AGM	3.28^{a}±0.00	4.83^{a} ±0.02	77.71^{d}±0.84	4.48^{c}±0.04	3.40^{e} ±0.10
ATM	2.56^{b}±0.00	4.25^{c} ±0.03	85.62^{b}±0.42	4.33^{d} ±0.03	2.94^{f}±0.04
AGT	2.92^{ab}±0.00	4.16^{c} ±0.03	90.41^{a}±0.24	4.38^{d}±0.01	3.94^{d}±0.04
GTM	2.08^{c}±0.010	4.18^{c} ±0.03	81.45^{c}±0.68	3.46^{e}±0.02	3.46^{e}±0.02
LSD	**0.43**	**0.15**	**1.15**	**0.07**	**0.26**

The means 'abc…' with different superscripts within the same column are significantly different (p≤0.05), while the means with the ***same superscripts*** *are not significantly different (p≤0.05).*

Keys:

CTY= Control (Eviron Health drink)

AGY=100 % Crude Ginger extracts; ATY= 100 % *T. tetraptera* Crude Extracts

AMY= 100 % *M. myristica Crude* Extracts; AGT= *50:50 Ginger and T. tetraptera* crude Extracts.

AGM= *50:50 Crude Ginger and M. myristica* Extracts; ATM= *50:50 T. tetraptera and M. myristica Crude* Extracts

GTM= 1:1:1 Ginger, *T. tetraptera and M. myristica* Crude Extracts.

Table 4.10 states the mean scores for the physicochemical properties of the beverage samples formulated and the control sample. The TTA values ranged from 1.92 to 3.28 and were recorded by the control and AGM, respectively. The pH values ranged from 3.66 to 4.83 and were recorded by the control and sample AGM. The viscosity values also ranged from 69.45 to 90.41, and values were ascribed to the control and sample AGT. The control had the lowest TSS value while ATY gave the highest mean score of 4.60 for the TSS; the total sugars (brix) is highest for the control (7.17°B) and least for sample ATM with a mean score of 2.94°B.

Table 4.11. Results of the shelf life Studies for the formulated Beverage.

Parameters	**Days**										
	0	**7**	**14**	**21**	**35**	**42**	**56**	**63**	**70**	**80**	**90**
pH	4.25	4.23	4.20	4.15	4.00	3.97	3.92	3.87	3.82	3.78	3.60
Sedimentation (%)	7.01	7.12	7.57	7.04	7.02	7.52	7.09	7.88	7.9	8.54	8.60
Off flavours	-	-	-	-	+	+	+	++	++	++	+++
Off colour	-	-	-	-	-	-	-	+	+	+	++
Precipitate	-	-	-	-	-	+	+	++	++	+++	+++
Phase Separation	-	-	-	-	-	+	+	++	++	+++	+++

Keys:

pH = Acidity/Alkalinity

- = Absent

+ = Present

4.2. DISCUSSIONS

4.2.1. Extract Yield.

The result of the percentage extract yields for the different tropical spices was stated in Table 4.1. From the values obtained, it revealed that sample *Monodora myristica* extracts gave the highest yield of 30.14 g (15.07 %), followed by sample *Tetrapleura tetraptera* extracts with 25.60 g (12.80%) yield, and lastly, sample *Zingiber officinale* extracts which yielded 21.30 g (10.65 %). However, from the related work done by Yang *et al*. (2003), ginger yields relatively smaller amounts of essential oils (extracts) when compared to other tropical spices such as *Monodora myristica* and *Tetrapluera tetraptera.* Thus, the low extract yield found in ginger compared to other samples *(M. Myristica and T. Tetraptera*) could be due to the intrinsic factors and/or the nature and chemical composition of the plant materials. By implications, the higher the extract yield, might not translate higher potencies of phytochemicals and antioxidants as they may vary in chemical constituents. However, low extracts yields could be attributed to the nature of the samples, and owing to the facts that essential oils have abundant of oils. However, the higher extract yield of the samples may also be translated to the potency of the spices in relation to their bioactive compounds. Alara and Abdurahman (2018), reported a 10.30 gram yield of ginger extracts using steam distillation, this value is related to the score obtained in this research.

4.2.2. Phytochemical Screening and Relative Abundance Bioactive Compounds Present in the Crude Extracts

Table 4.2 shows the different phytochemicals in the crude extracts generated from the different plant materials and their relative abundance. The evaluated bio-actives and Phytochemicals are saponins, flavonoids, terpenoids, gingerols, alkaloids, phenolics, steroids, tannins, and cardiac glycosides. The laboratory assay showed that saponins are moderately present in all the samples (AGX, ATX and AMX, respectively). Flavonoids are very much present in all three respective samples, whereas terpenoids were moderately present in both AGX and ATX but relatively

higher in sample AMX. Gingerols were found to be heavily present in sample AGX but relatively high in samples ATX and AMX, respectively. Prasad and Tyagi (2015) also reported that ginger contains high amounts of terpenes and 6-gingerols, and other essential oils.

However, alkaloids were moderately present in samples AGX and AMX but relatively higher in sample ATX (*T. tetraptera*). Phenolics were found to be heavily present in all the samples, while steroids were moderately present in all the samples. Tannins and cardiac glycosides were also moderately present in all the samples of the plant materials. Prasad and Tyagi (2015) and Koudou et al., (2007) reported that some spices such as ginger, turmeric, *Monodora myristica, Tetrapluera tetraptera,* curry, garlic, etc., contain several numbers of phytochemicals and antioxidants, as observed also in the results obtained from this research work. Therefore, the predominance of the phenolic compounds in the crude extracts could be attributed to the presence of secondary and tertiary essences in all the samples analyzed. Thus, the implications of the presence of these phytochemicals is that, they will be useful in many beverage applications, pharmaceutical and other allied industries.

4.2.3. Quantitative Analysis/Evaluation of the Phytochemicals/Bioactive Compounds in the Crude Extracts

The mean scores for the quantitative screening/evaluation of the phytochemicals/bioactive compounds in the crude extracts were stated in Table 4.3, while the graphical representation is shown in appendix 2 of this work. Thus, from the results obtained, the saponin contents of samples AGX, ATX and AMX were 2.71, 2.53 and 2.48 mg/g, respectively. The presence of these phytochemicals in the spices (*Z. officinale, T. tetrapleura,* and *M. myristica*) is an indication of their potency, and owing to the facts that most of the phytochemicals and bioactive compounds are important antioxidants which are valuable both in foods and other allied/ pharmaceutical industries. The statistical analysis indicated that sample AGX is significantly different ($P \leq 0.05$) from all the samples, while samples ATX and AMX are not significantly

different (P≥0.05). The flavonoid contents for the samples ranged from 14.33 to 10.68 mg/g, with sample AMX having the highest mean score of 14.33, followed by sample AGX and ATX with mean scores of 11.84 and 10.68, respectively. Thus, they are statistically different from each other. Also, there were significant differences (P≤0.05) in the terpenoids contents of all the samples, with sample AMX recording the highest mean score (7.30), followed by sample AGX with a mean value of 5.87 while sample ATX had the least score of 5.36. The results of the tannins indicated that sample AMX recorded 5.46, followed by sample ATX and AGX with mean values of 5.29 and 4.84, respectively. However, sample AMX was not statistically higher (P≥0.05) than sample ATX. The alkaloids showed significant differences (P≤0.05) among all the samples, with ATX, AGX and AMX recording mean values of 10.17, 7.47 and 6.84 mg/g, respectively. The total phenolic contents of the sample AMX was 26.06, the highest, while sample ATX recorded the least value of 20.93 mg/g, while sample AGX scored 23.26. Thus, the presence of phenolic compounds in the crude extracts is also tantamount to their potentials in foods and allied industries, as well as in pharmaceuticals. Phenols have also been seen as a strong ingredients in foods and pharmaceutical preparations and other allied chemicals (Takrama *et al.* 2012). The statistical analysis revealed that all samples differed significantly (P≤0.05). The mean scores for the steroids showed that sample ATX recorded 4.31, the highest, followed by sample AGX with a value of 3.36 mg/g, whereas sample AMX had the least mean score of 2.02. Statistically, all the samples differ significantly (P≤0.05). Overall, Appendix 2 clearly showed that phenolic compounds are predominantly present in all the samples of the crude extracts as it was found to have the highest peaks. The results of this work are, thus, in consonant with the values reported by Alara *et al.,* (2019), which reported that ginger has more phenolic compounds as well as steroids. Furthermore, the higher contents of these phytochemicals (terpenes, gingerols, terpenoids, etc) as reported in this could be used as an indicator and justification of their health importance in both foods and allied industries/pharmaceuticals. Terpenes, terpenoids,

as well as saponins have also been reported to very strong anti-stress depressant and the samples of the plant materials evaluated in this work have confirmed to possess reasonable amounts of these phytochemicals/bioactive compounds as also reported in ginseng plant (Christensen, 2009). As a result, the crude extracts from these spices (*Zingiber officinale, Tetrapluera tetraptera* and *Monodora myristica*), having been confirmed to contain some of the aforementioned compounds, could be utilized in the formulation or development of nutraceutical beverages for stress managements and other health related issues.

4.2.4. Antioxidant Assays

4.2.4.1 DPPH (2,2-diphenyl-1-picrylhydrazyl)Radical Inhibitory Activity of the crude Extracts.

The mean scores of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical inhibitory activities for all the samples at different concentrations were presented in Table 4.4.

However, at the concentration of 50 (µg/ml), sample AMX recorded the highest mean score (DPPH) of 56.61 µg/ml, followed by sample AGX with a mean score of 49.26 µg/ml, whereas ATX and Control samples scored 44.64 and 41.07 % respectively. There were significant differences ($P \leq 0.05$) among all the samples, and the control recorded the least PPH value (41.07%) at the concentration of 50 µg/ml.

Also, at the concentration of 100 µg/ml, sample AMX (*Monodora myristica* crude extracts) gave the highest DPPH value of 71.27 %, while sample ATX (*Tetrapluera tetraptera* crude extracts) scored the least with a value of 60.06 %; thus, differs significantly ($P \leq 0.05$) among each other. In addition, sample AGX and the control (Ascorbic acid) recorded mean scores of 66.38 and 67.24 % respectively. Statistically, the control sample (Ascorbic acid) is not significantly ($P \geq 0.05$) higher than sample AGX. A study conducted by Arala *et al.* (2020) suggested that tropical spices such as ginger and *M. myristica* contain high amounts of antioxidants up to the range of 60 to 80 %; thus, the values obtained in this work fell within the range.

At 200 µg/ml, the antioxidant (DPPH radicals activity) analyzed showed that all the samples were significantly different from each other. Thus, the sample with the highest mean score was AMX (*Monodora myristica* crude extracts), with a value of 85.54%, while the control had the least mean score of 75.35. By implications, it revealed that the *Monodora myristica* has higher antioxidant potentials, compared to other samples, as such, could be very useful in foods and beverage formulations due to its high antioxidants. More so, samples AGX (*Zingiber officinale* crude extracts) and ATX (*Tetrapluera tetraptera* crude extracts) recorded a mean score of 80.79 and 77.60 %, respectively. At the 400 µg/ml concentration, the results showed that sample AMX had the highest mean score (88.02), followed by sample AGX with a mean score of 87.14. Sample ATX recorded a mean score of 84.65%, while the control sample gave the least mean value of 82.33 %. Therefore, the results revealed that increase in the concentration, invariably amount to increase in the antioxidant activity of the samples. However, as clearly observed, the higher DPPH activity also translates the high bioactive compounds in the different samples and the values obtained here is similar to the values reported by Tapsell *et al.* (2006) with DPPH activity ranging from 43% to 84%. The statistical analysis, however, revealed that the samples are significantly different ($P<0.05$). Also, the graph (appendix 2) showed that the increase was progressive; thus, the antioxidant in the form of DPPH is directly proportional to the concentration. That is to say, the DPPH (2,2-diphenyl-1-picrylhydrazyl) increases with concentration. Suekawa *et al.*, (2014) also reported higher DPPH activity in ginger, which is in line with the scores obtained in this work, and further stated that the higher DPPH activity is an indication that ginger (*Zingiber officinale)* is a good source of antioxidant and could be applied in foods and allied industries, as well as in the formulation of nutraceuticals. Vaibhav *et al.* (2019) opined that food materials with high DPPH activity is an evident of being a strong antioxidant compounds which protection against damage caused by free radicals played important roles in the devolopment of many chronic disease including cardiovasculer diseases,

aging, heart disease, anaemia, inflamation and cancer. Therefore, the implication of the high antioxidant activities as obtained in this work is that the crude extracts would be useful ingredients in beverage and pharmaceutical formulations, as well as other allied industries.

4.2.4.2. Ferric Reducing Antioxidant Power (FRAP) Activity of the Crude Extracts at Different Concentrations.

The FRAP assay is high-throughput, adaptable and can detect antioxidant capacities as low as 0.2 mM Fe^{2+} equivalents. The assay measures the antioxidant potential in samples through the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by antioxidants present in the samples. The mean scores of the ferric-reducing antioxidant power (FRAP) activity of the crude extracts at different concentrations are clearly shown in Table 4.5. The FRAP as determined at different levels using 50, 100, 200 and 400 µg/ml, respectively showed distinct variations in their activity. The results revealed that, at 50 µg/ml, the control sample had the lowest mean score (18.20) while sample AGX had the highest mean score (73.39%). In addition, samples AMX and ATX recorded mean values of 40.84 and 34.42 µg/ml, respectively. Sequel to the statistical analysis conducted, the results suggested that there were significant differences ($P \leq 0.05$) among all the samples of the crude extracts evaluated (Table 4.5). Therefore, the results which placed ginger (*Zingiberoffficinale*) extracts as the sample with the highest FRAP activity implies that ginger extracts has higher potentials in reducing iron (iii) ion to iron(ii) ion (ferric iron to ferrous iron) in any system/mixture where it is incorporated. In addition, sample ATX (*Tetraplueratetraptera* extracts) has shown a lower ferric reducing ability; which implies that the potency of the *T.tetraptera* extract as an antioxidant and iron reductant is much lower than that of samples AMX and AGX.

All the samples showed significant differences ($P \leq 0.05$) at 100 µg/ml, including the control, with the lowest mean value of 29.64%. However, AGX, ATX and AMX recorded 82.42, 61.72 and 50.18 µg/ml, respectively. The means obtained suggested that sample AGX had the highest

value, preceded by sample ATX. At 200 µg/ml, sample AGX had the highest antioxidant (FRAP) value with 88.51 µg/ml, preceded by sample ATX with 77.22 (Table 4.5). However, sample AMX recorded a mean value of 58.30 µg/ml, and the control sample gave a mean value of 38.80. Therefore, it was revealed that all the samples differ significantly (P≤0.05).

Furthermore, at 400 µg/ml, all the samples replicated similar trends, with samples AGX and Control having the highest and lowest mean scores of 89.93 and 62.69 µg/ml, respectively, while samples ATX and AMX recorded mean values of 79.53% and 76.80% respectively. Thus, statistically, all the samples of the crude extracts were significantly different (P≤0.05) from each other. It can also be observed from the graph (appendix 5) that the increase in the concentration consequently results in to increase in the ferric-reducing antioxidant powers (FRAPs) of both the control samples and the crude extracts. Therefore, the increase in the antioxidant potential which is evident through FRAP is a strong indication of the ability of the crude extracts to reduce iron (Fe) present in any compound, as such, very suitable for use in both foods and beverage formulations (including nutraceuticals).

4.2.4.3. Lipid Peroxidation Inhibition Activities of the Extracts at Different Concentrations.

Lipid peroxidation is a process generated naturally in small amounts in the body, mainly by the effect of several reactive oxygen species (hydroxyl radical, hydrogen peroxide etc.). It can also be generated by the action of several phagocytes (Nwozo, *et al*. 2011).The results of the lipid peroxidation inhibition activities of the extracts at different concentrations where shown in Table 4.6 and Appendix 6. At 50 µg/ml, the control gave the highest mean value (2.86 µg/ml), followed by sample AMX (*Monodoramyristica* crude extracts) with a mean score of 2.23 µg/ml. Thus, they are significantly different (P≤0.05). Also, samples AGX (*Zingiberofficinale)* and ATX (*Tetrapleura tetraptera)* scored 2.01 and 1.73, respectively, and statistical analysis showed that the two samples also differ significantly (P≤0.05) from each other. On the contrary, sample AMX had the highest mean value of 3.26 µg/ml, at 100 µg/ml, while the control sample had the

least mean value of 2.88 µg/ml. In addition, samples AGX and ATX scored 2.63 µg/ml and 1.97 µg/ml, respectively. The values obtained indicated that sample ATX had the least lipid peroxidation inhibition at 100 µg/ml. Thus, the statistical analysis showed that all the samples of the crude extracts were significantly different ($P \leq 0.05$) from each other, including the control, as shown in Table 4.6.

Furthermore, the lipid peroxidation activities at 200 µg/ml tend to increase for all the samples. Sample AMX gave a mean score with a value of 3.50 µg/ml. Sample AGX scored 2.88, while sample ATX had the least mean score of 2.29 µg/ml. Statistical analysis showed that there were also significant differences ($P \leq 0.05$) among all the samples analyzed, including the control. At 400 µg/ml, the same trends were replicated, with sample AMX having the highest mean score of 4.55, whereas samples AGX and ATX recorded mean scores of 3.48 and 2.91 µg/ml, respectively while the control sample gave the least mean score (2.88 µg/ml). Thus, it showed that sample ATX had the lowest Lipid peroxidation inhibition activities while the control had the highest activity at 400 µg/ml. Thus, all the samples were significantly different ($P \leq 0.05$). Figure 4.1(e) also showed that Lipid peroxidation is directly proportional to concentration. The control sample, however, showed the highest peak at the 400 (µg/ml) concentration. Chan *et al.* (2011) reported that lipid peroxidation of plant extracts increases with increased concentration. Therefore, it can be deduced that the differences among the samples could be attributed to the variations in the intrinsic/extrinsic factors and nature of the crude extracts such as the moisture contents, chemical compositions, free radicals, temperature and light intensity. However, it was observed that the values of the lipid peroxidation inhibition increase with increasing concentrations, and by implications, it could be deduced that the different samples (*Zingiber officinale, Tetrpluera tetraptera,* and *Monodora myristica)* have the potentials as antioxidants to inhibit the peroxidation of lipids in foods and other compounds which are formulated with the

aforementioned extracts. This however, made it suitable for use in some foods, especially beverage formulations, as well as in pharmaceutical industries.

4.2.5. The Bioactive Compounds of the Extracts: (Tocopherols, Carotenoids, Gingerols, Terpenes, Vitamins C and B_6 and B_{12}) (Mg/100g)

The means for the tocopherols, carotenoids, gingerols, terpenes, Vitamin C, Vitamin B_6 and B_{12} for the crude extracts were stipulated in Table 4.7. Appendix 6 also revealed the different peaks for the bioactive.

The mean scores for the tocopherol analysis indicated that sample ATX had the highest mean value of 0.041(mg/100g), followed by AGX and AMX, with mean values of 0.029 and 0.024(mg/100g), respectively. Thus, the statistical analysis showed that sample AGX and ATX were not significantly different ($P<0.05$). Similarly, samples AGX (*Zingiberofficinale* crude extracts) were not significantly higher ($P\geq0.05$) than sample AMX.

As analyzed, the carotenoid contents of the crude extracts indicated that the values for the samples AMX (*Monodoramyristica* crudeextracts) had the highest mean score of 17.66(mg/100g), followed by sample AGX with a mean score of mg/g, whereas sample ATX recorded the lowest carotenoid values of 7.55(mg/100g). The statistical analysis, however, suggested that all the samples differed significantly ($P\leq0.05$).

The gingerols were analyzed for all the samples of the crude extracts. It was found that AGX had the highest mean score of 18.79(mg/100g), followed by sample ATX (13.26), while AMX gave the lowest mean score. Statistically, all the samples were significantly different ($P\leq0.05$). However, it could be observed from the literature that gingerols (6-gingerols and 8-gingerols) are predominant in sample AGX, a crude extract from *Zingiber officinale* (Yeh *et al.*, 2013).

The terpenes content of the samples of the crude extracts was also determined. The assay results were similar to gingerols, with sample AGX having the highest mean value (9.46 µg/ml), followed by the ATX with a value of 8.64(mg/100g), while sample AMX had the least value of

5.49. Statistical analysis revealed that significant differences ($P \leq 0.05$) existed among all the samples of the crude extracts.

Furthermore, the results of the vitamin C content of the crude extracts suggested that sample AMX scored the highest mean (9.33 (mg/100g)), followed by sample AGX which recorded a mean value of 9.04(mg/100g). However, the two samples statistically revealed no significant differences ($P \geq 0.05$). That implies that sample AMX is not significantly higher than sample AGX in vitamin C content. On the contrary, sample ATX had the lowest mean score of 6.21(mg/100g); as such, it differs significantly ($P \leq 0.05$) from the rest of the samples (Table 4.7). This is also of great value in bevarge formulations as Vitamin C is regarded as a vital food nutrient and antioxidant.

The vitamin B contents of the crude extracts were determined. Thus, among the vitamin B complexes determined are vitamin B_6 and B_{12}. Sample AGX showed an increase in vitamin B_6 with a mean value of 8.56, followed by sample AMX with a mean value of 7.88(mg/100g), whereas sample ATX recorded the least mean value of 7.26(mg/100g). Statistically, all the samples showed significant differences ($P \leq 0.05$). The result suggested that the ginger extracts had more vitamin B_6 when compared to *Tetrapluera tetraptera* extracts which is higher compared to results obtained by Manju and Nalini, (2005).In addition, the vitamin B_{12} contents of the crude extracts from the different tropical spices were highly significant ($P \leq 0.05$); thus, the samples followed similar trends as in the case of vitamin B_6. Hence, sample AGX recorded the highest mean value (15.00 mg/100g), preceded by sample AMX which scored 13.89(mg/100g), while sample ATX had the least mean score of 12.09 mg/g. The results also indicated that the samples of the crude extracts contained varying amounts of vitamin B_{12} and, as a result, has revealed that it could be used in nutraceutical. Also, tocopherols were less significant and had the lowest peak in all the samples of the crude extracts analyzed. Therefore, the presence of these vitamin B complexes, phytochemicals and bioactive compounds in the crude extracts analyses has revealed their potential use in the areas of nutraceuticals/functional beverages development as well as pharmaceutical preparation/other allied industries.

4.2.6. The Antioxidant Activities of the Different Beverage Samples at Different Concentrations

4.2.6.1 Percentage DPPH radical inhibitory activities of the Beverage Samples.

The mean scores for the percentage DPPH for the beverage Samples at different concentrations were expressed in Table 4.8(a). At 1.00 ml, sample GTM had the highest mean score of 68.20 %, followed by sample AGM with 66.78, whereas sample ATY recorded the lowest value of 54.85, which differs significantly ($P \leq 0.05$) from each other. However, samples ATM, AGT and the control recorded mean values of 61.30, 61.90, and 61.07%, respectively. Thus, statistical analysis revealed that they are not significantly higher ($P \geq 0.05$) than each other. At 2.00 ml concentration, all the samples are significantly higher than each other, with sample AGY scoring 77.96 % while sample ATY recorded 64.39%. In addition, when the beverage concentration was increased to 5.00ml, sample GTM recorded 92.15. Sample AGM and AMY had mean values of 88.87 and 88.55 %, respectively; thus, they are statistically not higher ($P \geq 0.05$) than each other, while all other samples differ significantly from each other ($P \leq 0.05$). Also, sample AGT and AMY are not significantly different ($P \geq 0.05$). At 10ml concentration, sample GTM gave a mean score of 91.88 %, the highest, while the control sample recorded the lowest value. Thus they are significantly different ($P \leq 0.05$) from every sample. Samples ATM and AGM recorded 90.37 and 89.70, respectively, and statistical analysis revealed that they are not significantly different from each other, whereas samples AGY, AMY and AGM had a mean score of 88.90 %, 89.55 and 89.70 %, respectively, with no significant differences ($P \geq 0.05$) among them. Also, the control sample recorded the least value of 82.33 % at 10ml concentration. Thus statistical analysis showed that it is significantly different ($P \leq 0.05$) from all other samples. From the results, it could be observed that the DPPH value increases progressively with an increase in the concentrations of the samples. This, however, could be attributed to the inherent factors within the samples. The values obtained in this research are slightly higher than that obtained by (Prasad and Tyagi, 2015). The high DPPH radical activities obtained in this research at different concentrations shows that the extracts could be very useful in nutraceuticals and pharmaceutical industries.

4.2.6.2 Percentage Nitric Oxide Scavenging Activities in the Beverage Samples at Different Concentrations.

The mean scores of the nitric oxide radicals in the beverage samples were expressed in Table 4.8(b). Sample GTM had the highest mean score of 40.54%, followed by the control sample with a value of 39.61 %, while sample AGY recorded the lowest mean value of 33.72; thus, they showed significant differences (P≥0.05) between each other and across all other samples. Samples ATM and AGM scored 38.59 and 38.19, respectively, such are not significantly higher (P≥0.05) than each other. AGT and AMY also show no significant differences (P≥0.05), as they recorded mean scores of 36.49 and 36.03 µg/ml, respectively. At 2 ml concentration, sample GTM gave the highest mean score of 64.87 %, followed by sample ATM with a total mean value of 62.86 with significant differences (P≤0.05), whereas the values were recorded by samples AGY and the control (44.89 and 44.70 % respectively) and it was revealed that there were no significant differences (P≥0.05) between the two samples. However, samples AGT, AMY and ATY are related and are not significantly different (P≥0.05). At 5ml concentration, the control recorded 39.61 %, the least, while sample GTM scored 77.61 %, placing it as the highest value. The two samples are significantly different from each other and also differ significantly (P≤0.05) from other samples. On the contrary, samples AGT and AGY recorded mean scores of 65.73 and 65.25 %, respectively, and statistically, they are not significantly higher (P≥0.05) than each other. When the concentration is elevated to 10 ml, the values tend to increase, with sample GTM recording a mean value of 95.20, while the control scored the lowest mean (67.39). All the samples, including the control, are significantly different (P≤0.05) except for AGT and AGM, which recorded mean scores of 90.75 and 90.63%, respectively, as they are not statistically higher than each other. The means recorded in this work are similar to the ones reported by (Yeh *et al.,* 2013) when the antioxidant properties of ginger (*Zingiber officinale)* were analyzed. It was also noticed that the Nitric Oxide Scavenging activity values tend to increase as the

concentration of the extracts increases at different levels of formulations. This could result from endogenous factors in the beverage samples, such as antioxidants, phenols and other phytochemicals.

4.2.6.3. Percentage Ferric Reducing Antioxidant Power (FRAP) activities of the Beverage Samples at different Concentrations.

Table 4.8(c) outlined the different mean scores for the beverage samples' ferric-reducing antioxidant power (FRAP) activities at different concentrations. The FRAP activities for the beverage samples at 1 ml revealed that sample AGT had the highest mean score of 76.00 %, followed by sample AGY and AGM, whereas the control recorded the lowest mean value of 38.09 %. Statistically, all the samples showed significant differences ($P \leq 0.05$). At 2 ml, samples AGM recorded mean values of 71.14 and 71.72, respectively. Hence, they are not significantly different. Moreover, all other samples differ greatly ($P \leq 0.05$). Notwithstanding, sample AGT gave the highest mean score (88.57), while the control recorded the least mean value of 38.09 %. At an increased concentration of 5 ml, the values tend to increase, with sample AGT recording the highest FRAP activities value (90.49 %), followed by sample AGY with a mean score of 89.50 %. However, the control sample also recorded the least mean value of 38.09 %. Statistical analysis showed that all the values are significantly different ($P \leq 0.05$) from each other. Furthermore, the mean values for the samples at 10 ml revealed that samples AGM and AMY were 86.22 and 86.80 %, respectively, which showed that they are not significantly higher than each other. The control (Eviron Health Drink) sample also recorded a mean value of 38.09 %, while the sample GTM was 68.94, the least. Thus, sample AGT had the highest mean score and differed significantly ($P \leq 0.05$) from all other samples. In general, it could be seen that the FRAP

activities of all the samples are directly proportional to their increased concentration. The results obtained here, however, were in line with the report made by Barbosa *et al.* (2014).

4.2.6.4. Lipid Peroxidation Inhibition Activities of the Beverage Samples at Different Concentrations (µg/ml).

The mean scores of the lipid peroxidation inhibition activities of the beverage samples at different concentrations were stated in Table 4.8(d). From the table, eight samples were analyzed (with control inclusive). The control has the highest mean score of 2.86 at 1 ml while sample ATY recorded 1.81, which is the least; thus, it differs significantly ($P \leq 0.05$) from all other samples. However, samples AGY, AMY, AGT, AGM, ATM and GTM recorded 2.37, 2.33, 2.32, 2.34, 2.07 and 2.20 µg/ml, respectively. However, from statistical analysis, they are not significantly different ($P \geq 0.05$) from each other. At 2 ml concentration, sample AMY recorded the highest mean value of 3.36 µg/ml, followed by sample AGM with a value of 3.12 µg/ml, while sample ATY recorded the least (2.03 µg/ml), and they were all significantly different ($P \leq 0.05$) from each other, and likewise, sample AGM which is not closely related to any other samples. However, AGT and AGY are not significantly different concerning lipid Peroxidation Inhibition activities. At 5 ml concentration, no significant differences ($P \leq 0.05$) among samples GTM, ATM, ATY and AGY. Sample AMY recorded a mean score of 3.75 µg/ml which is the highest, while the lowest mean value was recorded by sample AGT and the control (2.68µg/ml respectively), thus not significantly different from each other. Amaravathi *et al.* (2014), however, reported that gingers are a major source of antioxidants and lipid peroxidation inhibition activities were also found to be high as reported in this work. High lipid peroxidation inhibition activities is of great importance, especially with food materials containing fat.

More so, when the concentration was raised to 10 ml, it was statistically observed that the control had the least mean score (2.86 µg/ml). Sample AMY with a mean score of 4.63 was the highest, while ATY scored 3.02 and as a result, these samples are significantly different, ($P \leq 0.05$).

Sample GTM and AGM also showed similarities in lipid peroxidation inhibition activities; likewise, samples AGY and ATM are not significantly higher (P≥0.05) than each other. However, (Yeh *et al.,* 2013) reported that increased analyte concentration results in increased antioxidant activities of the samples. The results obtained in this work were in consonant with the work done by Ekeledo *et al.* (2013) when they blended pineapple juice, ginger and turmeric. Therefore, the high lipid peroxidation inhibitions obtained in this research has proven the suitability of of the extracts for use in the development of nutaceutical, pharmaceuticals and allied industries.

Therefore, it could be deduced that the lipoid peroxidation values tend to increase with the increase in the concentration of the samples. In addition, the variations and the similarities observed among the samples could be attributed to the intrinsic factors within the sample materials.

4.2.7. Sensory Properties of the Beverage Samples

The mean scores of the sensory evaluation for the formulated beverage produced (appendix 7) and the control samples (Eviron health drink) are described in Table 4.9. The sensorial parameters checked include colour, taste, aftertaste (mouth feel), flavour and general acceptability.

From the results obtained based on colour, sample CTY which is the control (commercial drink containing ginseng extracts) has the highest mean score of 8.40, followed by sample AGY with a mean score of 7.80, whereas sample AGT recorded the least mean score of 6.50. However, the results of statistical analysis revealed that sample CTY (control) is significantly different (P≤0.05) from all other samples. Also, sample AGY, ATY and ATM are not significantly different. The results also revealed that sample AGY, AGT and ATM are not significantly higher than (P≥0.05) each other. Samples GTM and AMY are also not significantly different (P≥0.05).

However, the mean scores for the taste revealed that the control sample is not significantly higher than sample AGY, which recorded 8.20 and 8.00, respectively. Samples AGT and ATY recorded 7.80 and 7.50, respectively. Thus are not significantly different (P≥0.05) from each other. More so, sample AMY recorded 6.55 while GTM recorded 6.50, respectively and are significantly different (P≤0.05) from each other, while AGM and ATM scored 6.0 and 6.20, respectively. Thus, statistical analysis revealed that AGM is not significantly lower than (P≥0.05) sample ATM. The results obtained here is in line with the research conducted by Pee and Bloom (2009).

The mean score for the aftertaste (mouth feel) for the control sample and ATY were 8.50 and 8.00, respectively, and the control sample is not significantly higher than ATY. Sample AGY also recorded 7.50 as opposed to sample AGT 7.00. The samples, however, are not significantly different from (P≥0.05). Sample ATM and AGM are not also significantly different (P≥0.05) from each other. However, samples AMY, AGM and GTM recorded 5.50, 5.20 and 5.00, respectively. Statistical analysis revealed that all three samples mentioned above are not significantly different (P≥0.05) from each other, though sample GTM had the least mean score, not significantly lower than AGM and AMY.

For the flavour, sample AGM recorded the lowest mean score, while the control had the highest mean score of 5.50 and 8.00, respectively. Thus, the AGM differs significantly from all the samples. Statistically, samples CTY (control), AGY, and ATY were closely related in terms of flavour as they did not show any significant differences (P≥0.05). The three samples, however, recorded mean scores of 8.00, 7.58 and 7.80, respectively. In the same vein, samples AGT and GTM recorded 7.10 and 7.40, respectively, and statistically, sample AGT is not significantly lower than (P≥0.05) GTM. Furthermore, samples AGT and ATM are not statistically different (P≥0.05). Nevertheless, samples AGM recorded the least mean score of 5.50, thus, differs significantly from every other sample.

The control and AGY scored 8.00 and 7.90 for general acceptability, respectively. Sample AGY is not statistically higher than (P≥0.05) the control. The statistical analysis also revealed that AGM is the least acceptable sample, with a mean value 5.80. Also, samples AGY, AMY and AGT are not significantly higher than each other (P≥0.05), while sample ATM revealed statistical differences. In furtherance to that, sample GTM and AMY recorded a mean score of 6.50; thus, no statistical difference between them. A study conducted by Ekeledo *et al.(2013)* also reported that ginger-based pineapple drinks were generally accepted by the panelists who were presented with the drinks. Finally, the statistical analysis revealed that the control and sample AGY (beverage with ginger extracts) are generally acceptable, while the samples not generally accepted were the beverage with *Monodora myristica* extracts and the one containing the combinations of all three extracts. The results obtained in this work were also similar with the report made by Ekeledo *et al.* (2013), with ginger drinks being the most preferred. However, the preference for the sample containing ginger and and *Tetrapleura tetraptera* extracts implies that they would be very useful in beverage formulations. The samples of the formulated beverages were shown in Appendix 6.

4.2.8. Physicochemical Properties of the Beverage Samples Developed

The mean scores of the physicochemical of the beverages formulated were shown in Table 4.10. The total titrable acid (TTA), pH, Viscosity, Total suspended solids and total sugars (Brix) values were stated in the table. From the results obtained, sample AGM recorded the highest mean score but was not statistically higher than (p≥0.05) samples ATY, AMY and AGT, which recorded mean values of 3.21, 2.90 and 2.92, respectively. Similarly, sample AGY recorded a mean score of 2.60 for TTA and is not significantly higher than (p≥0.05) sample ATM with a mean score of 2.56. However, the latter shows no statistical difference (p≥0.05) between samples AMY and AGT. That implies that they are closely related. Notwithstanding, the control (CTY) had the least mean score of 1.92 for total acidity and was preceded by sample GTM with a value

of 2.08; Thus, statistical analysis suggested that the control sample is not significantly lower than sample GTM. Omobuwajo, *et al.,* (2003) reported a lower total titrable acidity in a research conducted using *Monodora myristica.*

The pH of the formulated beverages clearly showed that the samples were acidic. Scientifically, pH is the measure the acidity or alkalinity of a solution or substance. Sample CTY (control) recorded the least pH value of 3.66 and differed significantly ($p \leq 0.05$) from all other samples. Sample AGM recorded the highest mean pH score and differed significantly from all other samples analyzed. More so, sample AGY had a mean score of 4.50, significantly different from other samples. Samples ATM, AMY, ATY, AGT and GTM scored 4.25, 4.28, 4.23, 4.16 and 4.18, respectively; thus, statistical analysis suggested they were not significantly higher than each other ($p \geq 0.05$). Therefore, the results showed that the control was more acidic than all the samples. The high acidity of the beverage samples could be attributed to the nature of the constituents of the carrier upon which the extracts were incorporated. The implications of the pH levels obtained in this work is that, the formulated beverage is moderately acidic and can be suitable for consumption as there might not be feelings of astringency or unpleasant aftertaste. It could also have some preservative effects on the beverage samples. However, the values obtained in this work suggested high acidity, as reported in the work already established by Ekeledo *et al.* (2013).

The viscosity of the beverage samples was also recorded, and from Table 4.10, it is observed that sample AGT had the highest mean score of 90.41 while ATY had the least score of 60.71. As mentioned earlier, the two samples are significantly different and differ greatly from all other samples ($p \leq 0.05$). Sample ATM with a value of 85.62 varies significantly from all other samples ($p \leq 0.05$), likewise sample GTM with a mean score of 81.45. Samples AMY and AGM scored 77.47 and 77.71, respectively. Thus, sample AMY is not significantly lower ($p \geq 0.05$) than

sample AGM. In the same manner, sample AGY and CTY (Control) are not significantly different ($p \geq 0.05$) from each other, with each scoring 70.29 and 69.45, respectively.

The total soluble solids can be regarded as the total suspended solids. It is also the measure of the clarity of a solution in the sense that it confers clarity to a beverage mixture or any liquid sample solution. Thus, only samples ATM and AGT with respective means scores of 4.33 and 4.38 mg/l showed similarities in that they are not significantly different ($p \leq 0.05$). Samples AMY with a value of 2.94 mg/l was not significantly higher than the control, which recorded a mean score of 2.93 mg/l and the lowest value. Nevertheless, sample ATY had the highest mean score of 5.38, followed by ATY with a value of 4.60 mg/l. However, sample AGM and GTM scored 4.48 and 3.46^{o}Brix respectively. As such, they differ significantly from each other ($p \leq 0.05$). The means recorded here compared favourably with the work reported by (Prasad and Tyagi, 2015). The total sugar measures the Brix level of any liquid beverage. The means scores recorded showed that the brix levels for the different samples vary from each other. The control sample gave the highest mean value of 7.17 ^{o}Brix, significantly different from all other samples. Statistical analysis also revealed that sample AGY differs significantly from every sample with a brix value 6.22^{o}Brix. Sample ATY and AMY are related to sample AGT in that they are not significantly different ($p \geq 0.05$), scoring 4.20, 4.10 and 3.94^{o}Brix respectively.

Moreover, sample AGM and GTM recorded mean values of 3.40 and 3.46. Statistically, the former is not significantly higher than ($p \geq 0.05$) the latter. The total brix (^{o}Brix) measures the sugar level in a beverage; and it is already known that sugars are one of the major constituents of beverages. Thus the study recorded mean values for brix between the ranges of 3.50 to 10.63 ^{o}Brix.

4.2.9. Shelf life Studies on the Beverages Developed.

The formulated beverage samples were subjected to shelf life studies by storing them on shelves at room temperature and at refrigeration condition for an interval of 3 months. However, the pH were determined at intervals. The pH of the samples ranged from 4.25 to 3.60 during the period of storage. Therefore, the changes in the pH could be due to the chemical changes within the beverage samples. Thus, there were stability in the pH of the samples between 0 to 30 days of storage with pH range of 4.25 to 4.00.

In addition, there were no colour changes, precipitate formation and off-flavour development observed during the initial day (0-7th day) of production to 21 days of storage. There were noticeable colour changes and slight off-flavour developments on samples ATY, AMY and AMT within 42 days of storage while the changes became intensified within 60 to 90 days of storage. Some of the beverages tend to be clearer due to settling under gravity leading to phase seperations while the ones under refrigeration conditions remained unchanged. There were no phase separations and precipitates noticed within a one month storage duration, while the changes were observed from 42 days to 90 days of storage periods. On the 80th day, the samples stored on the shelf showed more precipitates and phase separations, while for the samples under refrigeration, the colour change and layer formation were slightly noticed. Therefore, the changes could be attributed to the different compositioons as well as the chemical components of the different samples of the beverage. Furthermore, the sedimentation values for the samples ranged from 7.01% to 8.06%. However, between 0 to 30 days storage, the sedimentation rate ranged between 7.01% to 7.02%. Thus, it implies that there were stability in the sedimentation rates until the value increased from 42 days storage periods with a value ranging from 7.52% to 8.60%. At 90days storage periods, there were increased colour changes, off-flavour formation and phase separations of the beverages samples. Similar research conducted by Chukwu *et al.* (2017) also revealed that most beverages stored under shelves instead of refrigeration temperature tend to form gas bubbles within 7 to 8 weeks of storage due to microbial spoilage

due to some circumstantial and intrinsic factors such as the nature and the compositions of the samples of the beverage materials. The implications of this is that the sample could not be stored under shelf over a prolong period of time.Notwithstanding, under refrigeration conditions, some of the samples showed no distinct changeswhile some of the formed separation in a few samples and the formation of sediments. This implied that, the samples when stored over a prolonged period of time, can settle under gravity (sedimentation), as such, would need clarification and suitable stabilizers and emulsifiers to hold both the solid and liquid/oil phases together.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION.

5.1 Conclusion.

The **Ehuru** (*Monodora myristica)* yielded more crude extracts (essential oils) than other spice samples (*Zingiber officinale and Tetrapluera tetraptera*) using microwave-assisted extraction.

The phytochemicals such as saponins, flavonoids, terpenoids, gingerols, alkaloids, phenolic compounds, steroids, tannins and cardiac glycosides are present in ginger (*Zingiber officinale*), *Monodora myristica* and *Tetrapluera tetraptera*. Phenolics were present in all the samples of the extracts. Terpenes, carotenes, tocopherols, Vitamin C, and gingerols, which are the major target anti-stress components, were also found in the crude extracts.

The beverage containing single-strength of ginger extracts has higher antioxidant activity (DPPH, FRAP, and Lipid peroxidation inhibition activities). The samples' antioxidant activity values increased as the concentration increases.

The physicochemical properties of the samples showed they are moderately acidic, falling within the pH range of 4.16 to 4.83, in relation to the control sample (Eviron health drink) with higher acidity of 3.66. The samples also showed to contain less total sugars than the control.

The sensory evaluation results showed that the control samples (Eviron health) and the developed beverage with a blend of ginger crude extracts were the most preferred, followed by the sample with *Tetrapluera tetraptera* extracts. The single strength of *M. myristica* was the least preferred.

The shelf life studies of the formulated beverages revealed that the product containing no preservatives (citric acid), can stay on a shelf at room temperature for about four weeks while it will last for more than eight weeks in refrigeration condition.

5.2. Recommendations

- Due to higher antioxidant activity as well as essential phytochemicals (terpenes, terpenoids, gingerols, phenolics, etc) of the beverages containing *Tetraplueratetraptera* and *Zingiberofficinale*crude extracts, it is recommended that, the extracts from the plant material (pods and rhizomes) be used in beverage/nutraceutical formulations.
- Formulation optimization: Further research is needed to optimize the formulation of the nutraceutical beverage, including the selection of ingredients, the ratio of ingredients, use of emulsifiers and the addition of other active ingredients to enhance the antioxidant properties of the beverage. However, the darker colour and the stronger flavour of the crude extracts (essential oils), especially that of *Monodora myristica,* can be reduced by purification to obtain a purer form of the target extracts.
- Comprehensive chemical characterization of the crude extracts is recommended for further studies to ascertain/identify the quantities of fundamental constituents of the extracts in ginger, *Tetrapluera tetraptera, and Monodora myristica.*

- It is recommended that further studies be carried out on ginger, *Tetrapluera tetraptera, Monodora myristica,* in areas of nutraceuticals, involving animal studies to ascertain the efficacy of the beverages formulated from them, including the need for further optimization of the extraction conditions and formulation of the nutraceutical beverage and the need for larger-scale clinical trials to validate the results.

5.3. Contributions to Knowledge

- Microwave Assisted Extraction can be used for extraction of phytochemicals (gingerols, tannins, steroids, flavonoids, terpenes, phenolics, etc) from tropical spices (eg. ginger, *T. tetraptera,* and *M. myristica).*
- This study has introduced a new frontier towards the utilization of tropical spices such as ginger (*Zingiber officinale*), *Tetrapluera tetraptera,* and *Monodora myristica* for the production of functional beverage. However, a triple mix of extracts from the spices (Ginger, *M. myristica, T. tetrapluera*) gave highest DPPH (91.88%) at 10 ml inclusion in the beverage.
- This study advances our understanding of the potentials of tropical spices as sources of bioactive compounds for the development of natural products with potential health benefits, thus, contributing to the field of nutraceutical/functional beverages
- Ginger (*Zingiberofficinale*) was found to contain the highest amounts of gingerols (18.79 mg/100g) and terpenes (9.46 mg/100g) which are the major components of the spices.

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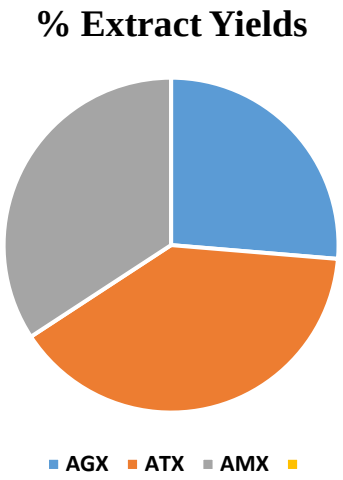
APPENDICES

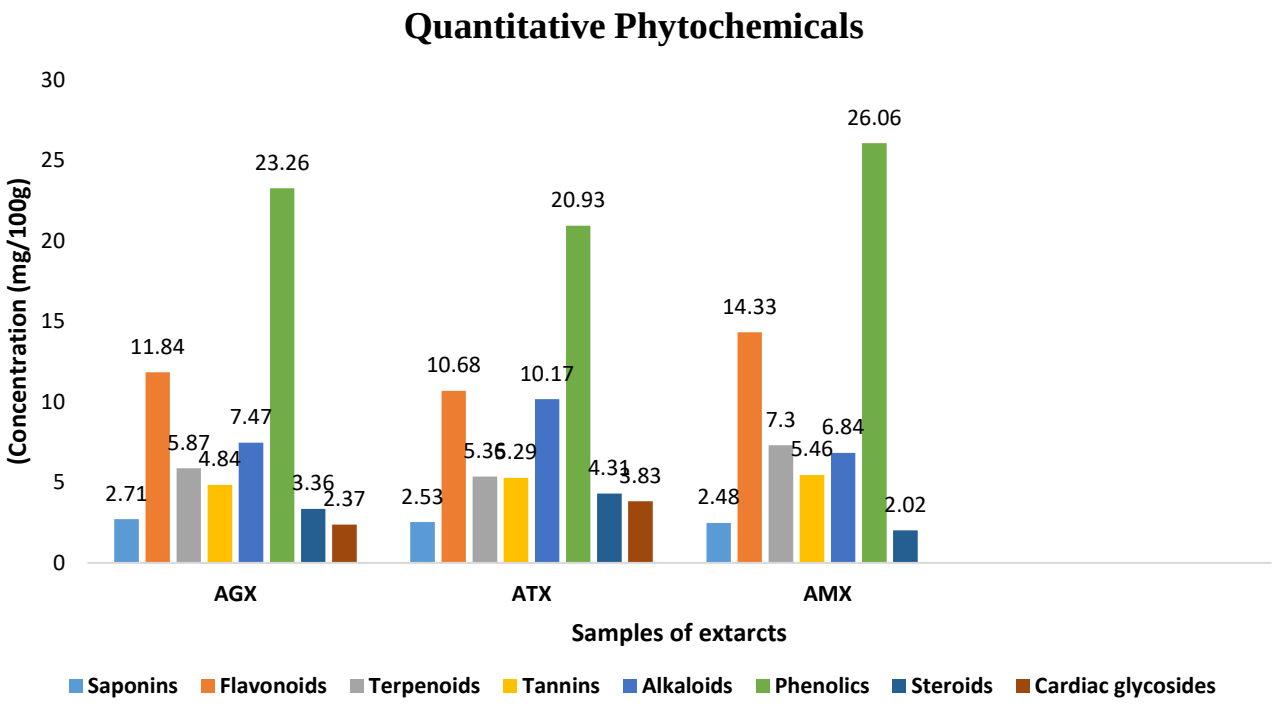
Appendix 1 (a). The percentage Extract Yields for the different samples of Crude extracts.



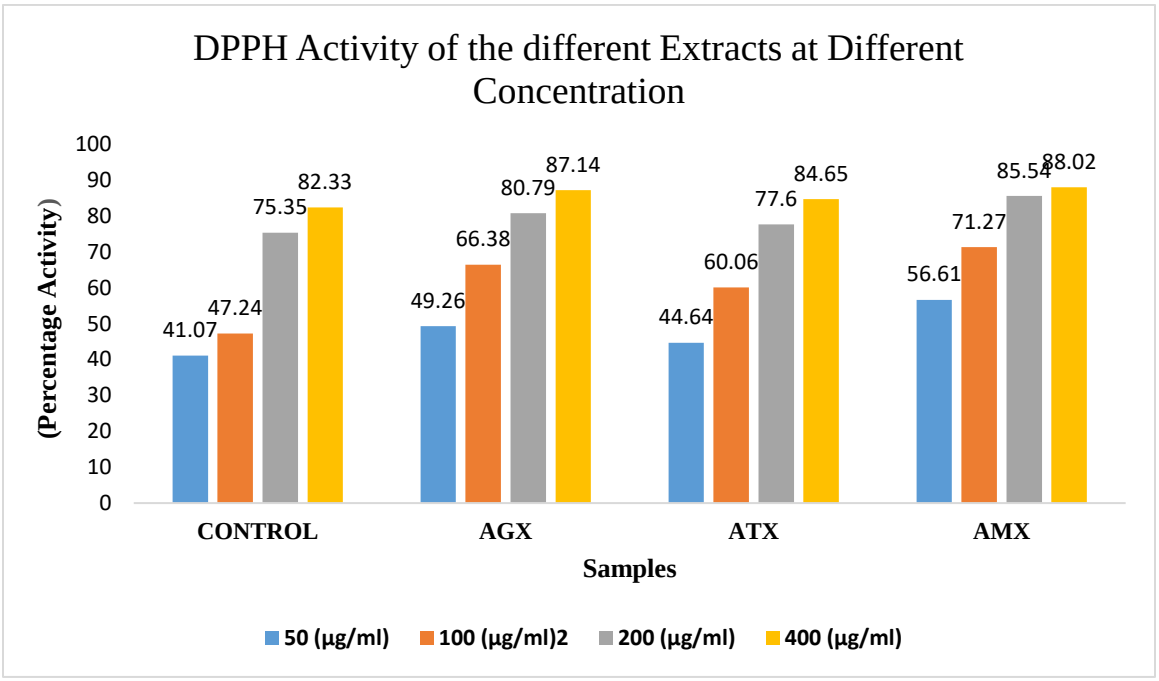
Appendix 1 (b). The sample containers containing the crude extracts from Ginger (*Zingiber officinale*), *Tetrapluera tetraptera and Monodora myristica.*.

Note: In all cases, the following acronyms stands for:
AGX = Ginger Extracts
ATX = *Tetrapleura tetraptera* crude extracts
AMX = *Monodora myristica* crude extracts

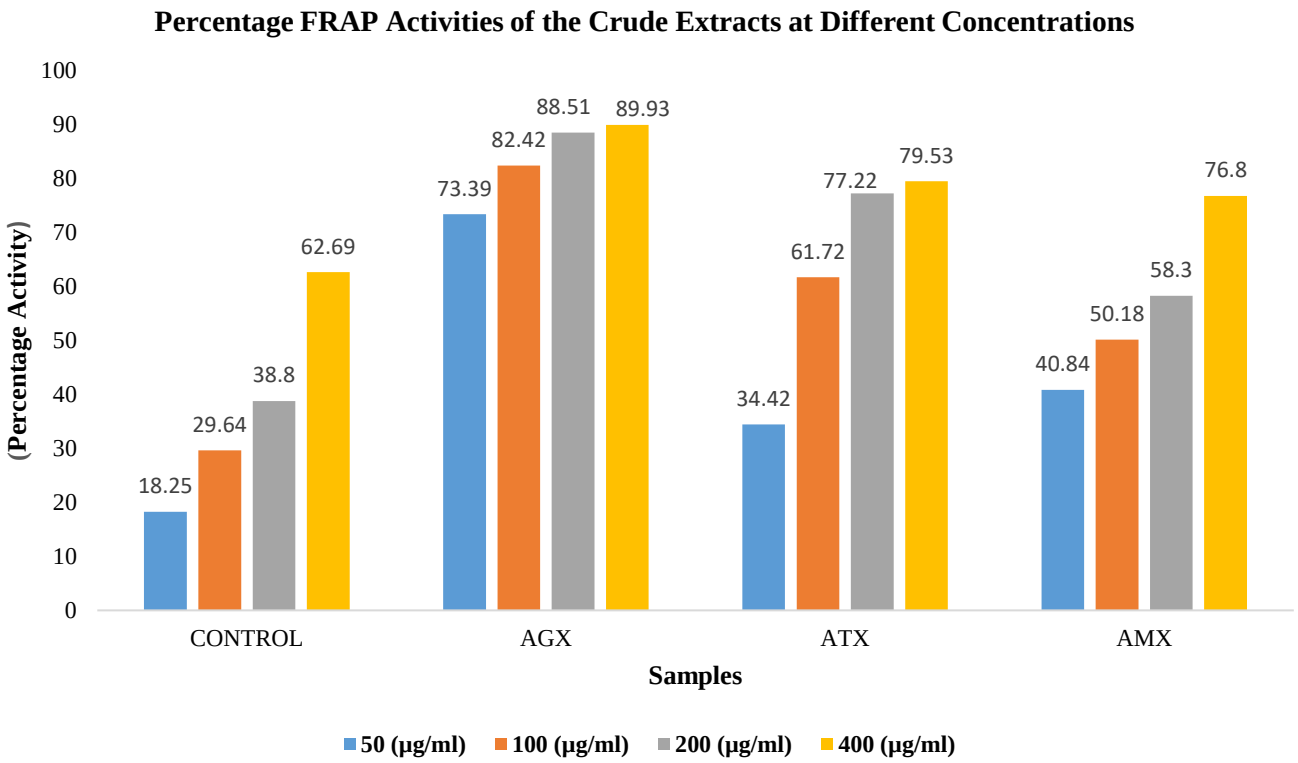
Appendix 2. Quantitative Phytochemicals



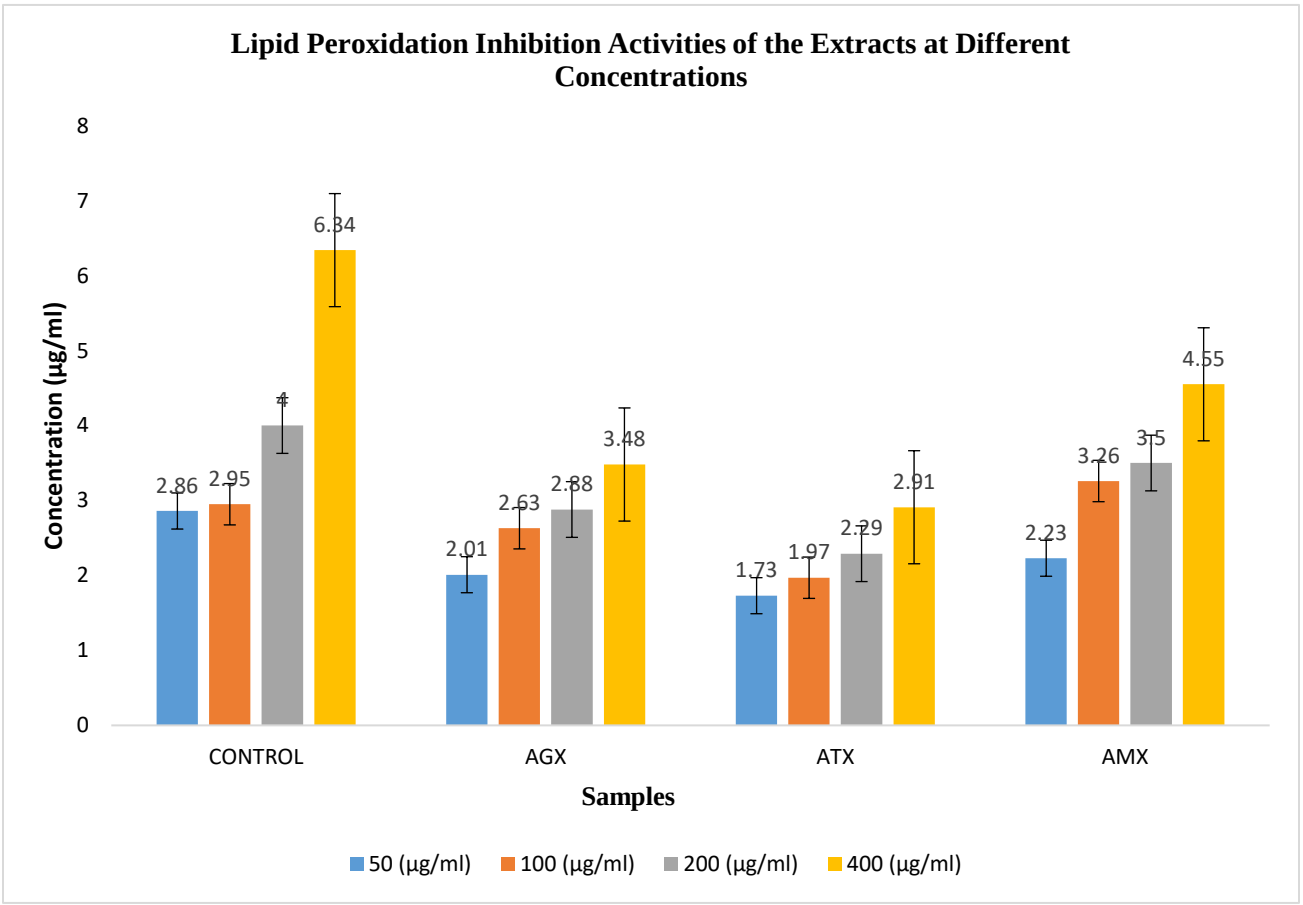
Appendix 3. DPPH Assays of the Crude Extracts at Different Concentrations.



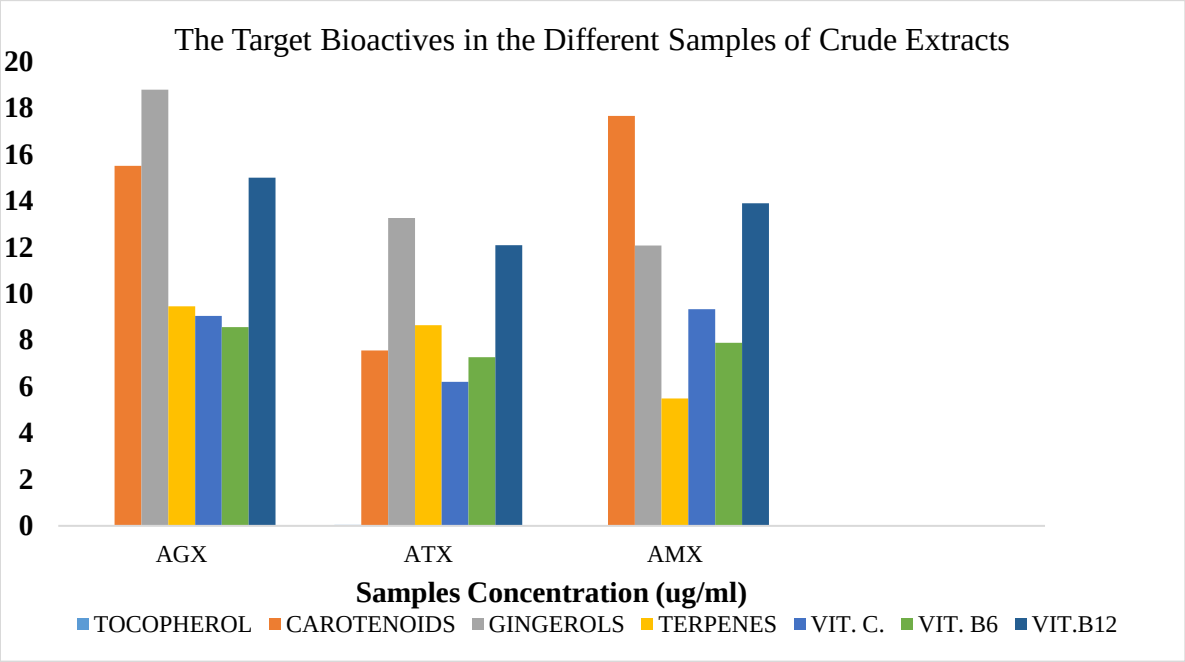
Appendix 4. The FRAP activity of the Crude Extracts at Different Concentrations



Appendix 5. Lipid Peroxidation Inhibition Activities of the Extracts at Different Concentrations



Appendix 6. The different bioactive compounds in the Extracts at Different Concentrations



Appendix 7 (a). Samples of the formulated Beverages

Appendix 7 (b). The bottled samples of the formulated Nutraceutical Beverages.

Appendix 8. Sensory Evaluation of the Beverage Samples

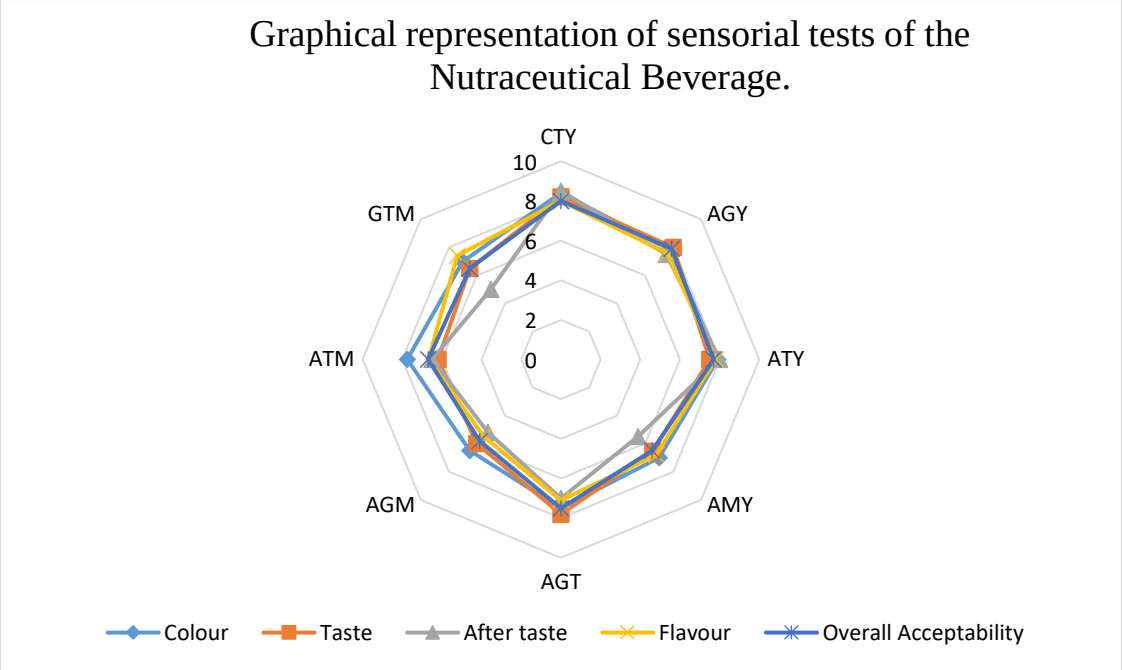


Figure 2. Graphical representation of sensorial tests of the Nutraceutical Beverage