**CHAPTER ONE**

**INTRODUCTION**

**1.1 Background of study**

Palm oil and kernel oil are edible plant oils derived from the fruits of palm trees. Palm oil is extracted from the pulp of the fruit of oil palm (*Elaeis guineensis);* palm kernel oil is derived from the kernel seed of the oil palm (Poku, 2002). Palm oil is naturally reddish in colour because it contains a high amount of beta-carotene. Palm oil has been used in food preparation for over 5,000 years. Palm oil is the most widely produced edible vegetable oil in the world and its nutritional and health attributes have been well documented (Chandrasekharan *et al.,* 2000). According to the Malaysian Oil Palm Statistics in 2005*,* it surpassed soybean oil as the most widely produced vegetable oil in the world. Palm oil is currently enjoying strong appeal worldwide as a cooking aid because it is free of Artery-clogging trans-fats. Besides being cleaner and more stable, it is consumed worldwide as cooking oil, in making of margarine and shortening, apart from being used as an ingredient in fat blends and a vast array of food products. In the United States, Palm oil’s principal edible use is as an ingredient in prepared foods (primarily baked foods). Food manufacturers choose palm oil because it has a distinct quality, requires little or no hydrogenation, and prolongs the shelf life of different products (Akintayo, 2002). Malaysia and Indonesia account for 83 percent of production and 89 percent of global exports. Palm oil is extracted from the ripened mesocarp of the fruits of oil palm tree (*Elaeis guineensis).* The oil palm fruit is a drupe formed in spiky tight bunches. The five leading producing countries are Indonesia, Malaysia, Thailand, Colombia and Nigeria. The oil palm tree gives the highest yield of oil per unit area of cultivated land, anestimated58.431million metric tons (MT) per year. Palm oil has a unique fatty acid (FA) and triacylglycerol (TAG) profile which makes it suitable for numerous food applications. It is the only vegetable oil with almost 50–50 composition of saturated and unsaturated fatty acids. CPO is used for cooking, frying, and as a source of vitamins. Fractionation of CPO yields mainly palm olein, the liquid fraction and palm stearin, the solid fraction. These fractions have distinct physical and chemical properties. CPO, palm olein and palm stearin are important constituents of several food and industrial products such as shortenings, ice cream, cosmetics, candles lubricants, toothpaste and biodiesel (Barriuso *et al.,*2013). Palm stearin is helpful in providing the solid fat functionality without the use of hydrogenation, thus, reducing trans-fat intake in the diets (Kellens *et al.,* 2007).

Inter-esterification of CPO also widens its scope of food applications. Inter-esterification can be used to incorporate essential poly- unsaturated fatty acids in order to obtain oil rich in essential fatty acids and enhanced antioxidant properties. Customized blends of CPO and fractions with other oils are used in different food products ranging from margarines to soup mixes and infant formulae (Manorama and Rukmini, 1992).

* 1. **Statement of the Problem**

Agricultural wastes are very important parts of the biotechnology industries as many of them are useful as preservatives, feed additives as well as raw materials in the production of useful products. The important of palm oil and other agricultural products in West African should never be underestimated as they have contributed greatly to the economic development in the country. Closely, allied to that is the fact that many health specialists have warned against the use of synthetic substances as feed additives or preservatives due to their health effects on humans.

In addition to the above, Musaceae species (plantain and banana) peels have been known to combat many problems in medical and biotechnology industries. Therefore, the importance of these related species of Musaceae are never to be overemphasized.

Thus, this project work seeks to compare the antifungal effects and the proximate composition of these two related species of Musa peels ash extract as oil palm preservatives.

* 1. **Justification of Study**

Musaceae specie peels plays major role as they are use in preservation of many dietary products. As such, this study focuses on comparing the antifungal effects as well as the proximate composition of these peels ash extract on oil palm.

**1.4. Significance of Study**

This study was carried out to compare the antifungal effect and proximate composition of the two related specie of Musaceae (plantain and banana) peels ash used as oil palm preservatives. This research also explores the importance of using agricultural wastes in prevention of lipids oxidation over synthetic compounds.

* 1. **Aim and Objectives of the Study**

The aim of the study is to compare the antifungal effect and proximate composition of the ash extracts of *Musa paradisiaca and Musa sapientum* peels on oil palm.

The aim was accomplished by the following objectives;

1. To compare the effect of antifungal properties of *Musa paradisiaca and Musa sapientum* peels-ash extract on palm oil.
2. To assess the effect of palm oil on the proximate composition of the two related species of Musaceae (*Musa paradisiaca and Musa sapientum)* peels ash extract
3. To explore the important of agricultural wastes as oil palm preservatives.

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.1 Palm oil**

Oil palm is one of the most profitable commercial high-tree crops, and has undergone one of the highest rates of expansion in comparison with other crops in the tropical world. Nevertheless, the conditions under which oil palm plantations expand as well as their social and environmental implications are ambiguous, which makes palm oil one of the most controversial globally traded commodities. On one hand, oil palm expansion has delivered important economic development for its host countries, including indirect benefits for local infrastructure development and rural poverty reduction. On the other hand, its development has often come at the expense of basic human rights and/or biodiverse, carbon-rich tropical forests, as local communities have been evicted from their lands and precious primary forest and peatland ecosystems have been destroyed by fire ( Sheil *et al*., 2009;Sayer *et al*., 2012).

Blended palm oil and palm kernel oil forms an important share of the global vegetable oil market, competing with other oils such as soybean. Its main use is as cooking oil and an ingredient in domestic products (e.g. processed foods, detergents, cosmetics), as well as biodiesel. As such, global demand for palm oil is growing rapidly. While the crop originated in West Africa, much of its industrial expansion under monocrop plantation systems has occurred in Southeast Asia (Malaysia and Indonesia). Governments in palm-oil-producing countries have seen its rural development potential and therefore supported its expansion. But consumer markets in developed economies are increasingly concerned about the associated social and environmental trade-offs. As a result, there have been intense debates around the pros and cons of oil palm agriculture, and numerous market-based and voluntary sustainability standards have emerged as a way to ameliorate some of these negative impacts.

The palm oil value chain has increased in complexity over time, and while the main producer countries are Malaysia and Indonesia, it fulfils markets all around the globe. The palm oil global value chain is made up of a wide range of stakeholders, from producers of all sizes, to processors, traders, consumer goods manufacturers (CGMs) and retailers. Despite being dominated by a handful of companies at the refining and international trading stages, production involves a wide range of suppliers from companies to smallholders, and manufacturing involves a wide range of CGMs in a market that is diversifying. This makes the palm oil value chain hard to govern for environmental outcomes, but given that the refinement and refined palm oil trade stages are concentrated in the hands of just a few corporate groups, these groups have often been the main target of international NGOs and environmental groups’ campaigns.



**Figure 1: Fruits of oil palm (*Elaeis guinensis*)**

**2.2 Ecological suitability for oil palm expansion**

The oil palm, *Elaeis guineensis* Jacq., is a monocotyledon which belongs to the Arecaceae family (also known as Palmaceae). The crop has an economic life-span of around 25–30 years, producing fruits throughout the year (Barcelos *et al.,* 2015). It produces roughly 3.8 tons per hectare (tons/ha) per year as a global average, 6 tons/ha in the best plantations in Southeast Asia and 10 tons/ha in genetic field trials (Rival and Levang, 2014). Oil palm has been labeled as a “natural oil machine” (Rival and Levang, 2014) due to its comparatively high productivity in relation to other oleaginous crops (e.g. soybean, sunflower and rapeseed) (Barcelos *et al.,* 2015). Oil palm has the lowest production cost of all vegetable oils in the global commodity market, and could meet growing global demand that is estimated to reach 240 million tons by 2050 (Corley 2009). Two types of vegetable oil are extracted from the palm fruit: crude palm oil (CPO) and palm kernel oil (PKO). These oils have different fatty acid profiles, which increases the crop’s versatility in several industrial applications (Barcelos *et al.,* 2015).

The oil palm requires warm and wet conditions to grow. Optimal temperatures are in the range of24–28 °C, and the average temperature during the coldest month of the year should not fall below15 °C (Corley and Tinker 2015). It is estimated that 2000–2500 mm of rainfall per year are required for optimal growth, with a minimum of 100 mm per month. The palm’s growth may be constrained by chemical (e.g. nutrient) or physical (e.g. water) soil deficiencies, but these can be overcome by irrigation and fertilizer application. In this regard, climatic conditions constitute the main factors determining land suitability for oil palm.

According to Pirker and Mosnier (2015) and Pirker *et al.,* (2016), only a small proportion of the total land that is biophysically appropriate for oil palm production can be classified as suitable to perfectly suitable, while significant tracts are marginally or moderately suitable. The most suitable lands are located in the Amazon region, although soil drainage and acidity may present limitations. In Central Africa, the Congo Basin and coastal region of Western Africa – mainly Sierra Leone and Liberia are most suitable, with limitations dictated by the local dry season and sand- and stone-rich soils.

In Southeast Asia, the most suitable lands are found in Indonesia and Malaysia, where extensive oil palm development is taking place. This expansion occurs in mineral soil and peatlands with diverse economic and environmental implications (Khasanah, *et al.,* 2015).

**2.3. Importance of palm oil**

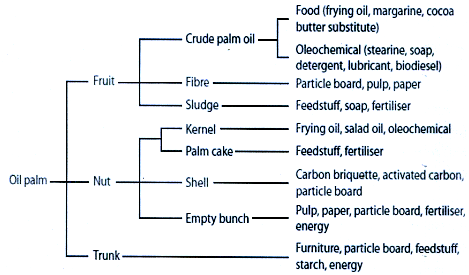
The palm oil tree gives the highest yield of oil per unit area of cultivated land and have contributed to the economic increase in many countries such as; Indonesia, Malaysia, Thailand, Colombia and Nigeria of an estimated 58.431million metric tons per year. One hectare of oil palm plantation is abletoproduceupto10timesmoreoilthanotherleading oilseed crops such as; coconut and soybean oil. Palm fruit produces two distinct types of oils: crude palm oil from the mesocarp and palm kernel oil (Gourichon, 2013; Robbelen, 1990). Both the crude palm oil and the palm kernel oil are important in world trade (Schroeder *et al.,* 2006). In 2012, the crude palm oil and the palm kernel oil were accounted for about 32% of global fats and oils production. Palm oil has overtaken soybean oil as the most important vegetable oil in the world (Oil World, 2013). Palm oil have contributed positively in both food and non-food industry as many of its products have been used as food additives, cosmetics, soap and in production of other important substances.

**2.3.1 Food Industry**

The food industry is responsible for 72% worldwide usage of palm oil (Oil World, 2012). Palm oil has been used either as additives or raw materials in the food industry in making of many dairy products such as; butter, bread, margarine, cracker, chocolate, doughnut, c and pastries. Due to it high melting point, palm oil has been used for frying and in food processing. Palm oil can be combined with harder fractions such as palm stearin to produce products with required consistency without hydrogenation. They are also added to frozen meals to prevent them from sticking and also function as natural preservative in processed meals (FAO, 2015). In making of ice cream, oil palm increases the melting point for ice cream, providing a suitable replacement for dairy fats which helps in giving them (ice creams) a thicker consistency while keeping them smooth and creamy.

**2.3.2. Non-Food Industry**

Palm oil is mostly use as personal care and cleaning products as well as providing foaming in soaps, shampoo and detergent. However, around 70% of personal care products (soap, shampoo, make and lotion) contained ingredients from palm oil (Tullis, 2019). Refined palm oil is use to create synthetic products or ingredient in the industry. In cosmetology, palm oil gives the smooth texture in lipstick and also increase the shelf life of the colour. Palm oil is also used as skin moisturizer due to it nutrient-dense profile and also beneficial to the skin. It gives the moisturizer anti-aging properties as well as softer (Oil World, 2015). Other non-food products produce from oil palm includes; toothpaste, deodorant, shaving cream and sunscreen.



**Figure 2: The products obtained from oil palm (Beveridge, 2009)**

**2.4. Plantain (*Musa paradisiaca Lin.*)**

Medicinal plants are frequently used in traditional medicine to treat different diseases. The World Health Organization (WHO) estimated that 80 % of the earth’s inhabitants depend on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts or their active components. This helped in exploration of different medicinal plants to find the scientific basis of their traditional uses (Jachak and Saklani, 2007). Ayurveda and other Indian literature mention the use of plants in treatment of various human diseases. Use of herbal remedies for prevention and cure of ailments is of increasing interest due to the superiority and efficiency of activity provided by phyto constituents in herbs and undesirable effects of modern medicine. Medicinal plants contain number of medicinal properties. One of such plant is *Musa paradisiaca.* It has been reported to have pharmacological activities such as antilithiatic, antioxidant, antibacterial, antidiabetic, antiulcer, antidiarrhoeal, hypocholesterolaemic, hepatoprotective, antisnake venom, wound healing, hair growth promoting, antifungal and antimenorrhagic activity.

**2.4.1 Taxonomy of Plantain *(Musa Parasidiaca L.)***

Kingdom: *Plantae*

Subkingdom: *Tracheobionta*

Super division: *Spermatophyta*

Division: *Magnoliophyta*

Class: *Liliopsida*

Subclass: *Zingiberidae*

Order: *Zingiberales*

Family: *Musaceae*

Genus: *Musa*

Species: *paradisiaca*

**2.4.2 Chemical Constituents of *Musa paradisiaca Lin.***

The whole plant as well as specific parts (Flowers, bracts, ripe, unripe fruits, leaves and stems) of plant extract and its active constituents have been used for the treatment of large number of human ailments. Flower consists of tannins, saponins, reducing and non-reducing sugars, sterols andtriterpenes. The structure of new tetracyclictriterpine isolated from the flowers of *Musa Paradisiaca Linn*. was determined as (24R)-4á-14á,24-trimethyl-5-cholesta-8, 25-dien-3â-ol **(**Adegboyega, 2006). The anthocyanins reported are 3-rutinoside derivatives of dephinidin, pelargonidin, peonidine andmalvidin**(**Jang, et al, 2012).The Fruit of the Musa species consists of carbohydrates, amino acids, sugar and starch. The skin of the fruit is rich in cellulose, hemicelluloses. The pulp protein is rich in arginine, aspartic acid, glutamic acid, methionine and tryptophan **(**Aravind*et al.,*2004). A new bicyclic Diarylheptanoid, 8-hydroxy-3-(4-hydroxyphenyl)-9- methoxy-4a,5,6,10b tetrahydro3Hnaphthopyran as well as four known compounds 1,2- Dihydro 1,2,3trihydroxy-9-(4-methoxy phenyl) phenalene 2-hydroxyanigorufone, 2-(4-hydroxy phenyl) naphthalic anhydride and 1,7 bis(4-hydroxy phenyl) hepta-4,6- diene-3-onewere isolated fromethyl acetate soluble fraction of the methanolic extract of fruits**(**Prasobh GR., *et al.,* 2016).Peeled fruits consist of two new acyl steryl glycosides Sitoindoside-III and Sitosterolmyo-inositylbeta-Dglucoside **(**Indera, *et al.,* 2011**)**. Fruit pulp consists of three forms of á-glucan phosphorylase **(**Adeolu *et al.,* 2013**)**.

**2.4.3 Distribution of *Musa paradisiaca* (Plantain)**

Edible plantains originated in the Indo-Malaysian region reaching to northern Australia. They were known in the Mediterranean region in the 3rd Century B.C and are believed to have been first carried to Europe in the 10th Century A.D. Early in the 16th Century, Portuguese mariners transported the plant from the West African coast to South America. It even spread into the Islands of the Pacific and to the West Coast of Africa as early as 200-300 BC (Rahman and Kabir 2003). In different countries about 300 varieties of bananas are grown, of which a vast majority have been growing in Asian, Indo-Malaysian and Australian tropics and are now widely found throughout the tropical and subtropical countries. India, Philippines, China, Brazil, Indonesia, Mexico, Colombia, Thailand are the top plantain and bananas producing countries. Plantains are today grown in every humid tropical region and constitute the fourth largest fruit crop of the world, following the grape, citrus fruits and apple **(**Aravind *et al.,* 2004).

**2.4.4 Morphology of *Musa paradisiaca***

The plantain plant, *Musa paradisiaca* often erroneously referred to as a "tree", is a large herb, with succulent, very juicy stem, which is a cylinder of leaf-petiole sheaths, reaching a height of 20 to 25 feet (6-7.5 m) and arising from a fleshy rhizome or corm. Leaves are tender, smooth, oblong or elliptic numbering 4 or 5 to 15, arranged spirally and they unfurl, as the plant grows, at the rate of one per week **(**Aravind *et al.,* 2004). The inflorescence, a transformed growing point, is a terminal spike shooting out from the heart in the tip of the stem. At first, it is a large, long-oval, tapering, and purple-clad bud. As it opens, it is seen that the slim, nectar-rich, tubular, toothed, white flowers are clustered in whorled double rows along the floral stalk, each cluster covered by a thick, waxy, hood like bract, purple outside, deep-red within. Female flowers occupy the lower 5 to 15 rows (Jachak and Saklani, 2007). Above them may be some rows of hermaphrodite or neuter flowers. Male flowers are borne in the upper rows **(**Aravind *et al.,* 2004). The bracts are soon shed and the fully grown fruits in each cluster become a "hand" of Bananas, and the stalk droops with the weight until the bunch is upside down. The fruit turns from deep-green to yellow or red, or, in some forms, green-and white-striped Jachak and Saklani, 2007).

**2.5 Banana (*Musa sapientum*)**

*Musa sapientum* (banana) is an elongated edible fruit-botanically a berry (Julia, 2009), produced by several kinds of large herbaceous flowering plants in the genus musa (Merriam-Webster, 2013). In some countries, bananas used for cooking maybe called plantain, which distinguished them from dessert bananas. Banana fruit is variable in colour, firmness and size, but is usually elongated and curved, with soft fresh rich in starch covered with a rind, which may be green, yellow, red, purple or brown when ripe. The fruits grow upwards in clusters near the top of the plant.

**2.5.1 Description of *Musa sapientum***

The banana plant is the largest herbaceous flowering plant (Picq *et al.,* 2013). The above ground part of a banana grows from a structure called “corm” (Stover and Simmonds, 1987). The plants are normally tall and a fairly sturdy with a tree like appearance, the trunk is usually a false stem or pseudostem. The plant, banana grows in a wide variety of soils which is as long as 60 centimetres deep and is not compacted when planted. However, the leaves of the banana plants are composed of a stalk known as the “petiole” with a blade “lamina”. The base of the petiole widens to form a sheath; the tightly packed sheaths make up the pseudostem, which is all that supports the plant. The edges of the sheath meet when it is first produced, making it tubular. As new growth occurs in the centre of the pseudostem the edges are forced apart (Stover and Simmonds, 1987). The leaves are spirally arranged and may grow about 2.7 metres long and 60 centimetres wide (Julia Morton, 2009).

When the banana plant is mature, the corm stops producing new leaves and begins to form a flower spike or inflorescence. A stem develops which grows up inside the pseudostem, carrying the immature inflorescence until eventually emerges at the top (Stover and Simmonds, 1987). Each of the pseudostem usually produces a single inflorescence which is also term as the “banana heart” (Angolo, 2009). Therefore, after fruiting, the pseudostem dies but offshoots will normally have developed from the base, so that the plant as a whole is perennial. In the plantation system of cultivation, only one of the offshoots will be allowed to develop in the order to maintain spacing. The female flowers appear in rows further up the stem from the rows of the male flowers. The ovary is inferior which means that the tiny petals and other flower parts appear at the tip of the ovary (GTR, 2008). The fruit of the banana develops from the banana heart in a large hanging cluster that made up of tiers which is up to 20 fruit to a tier. The hanging clusters is known as a bunch, comprising of 3-20 tiers or commercially as a banana stem and can weigh 30-50 kilometres. The fruit has been described as a leathery berry (Smith and James, 1977). There is a protective outer layer with numerous long, thin strings which runs lengthwise between the skin and the edible inner portion. The end of the fruit opposite the stem contains a small tip distinct in texture and often darker in colour (Tarantino, 2021).

**2.5.2 Cultivation of Banana**

The earliest domestication of bananas (Musa species) were initially from naturally occurring parthenocarpic individuals of Musa banksil in New Guinea. These were cultivated by Papuans before the arrival of Austronesian speakers. Numerous phytoliths of bananas have been recovered from the Kuk swamp archaeological site and dated to around 10,000 to 6,500 (Perrier *et al.,* 2009). From New Guinea, cultivated bananas spread westward into Island Southeast Asia through proximity. They hybridized with other subspecies of *Musa acuminata* and Musa balbisiana in the Philippines. These hybridization events produced the triploid cultivars of bananas commonly grown today. All widely cultivated bananas today descend from the two wild bananas *Musa acuminata* and *Musa balbisiana*. While the original wild bananas contained large seeds, diploid or polyploid cultivars with tiny seeds or triploid hybrids without seeds are preferred for human raw fruit consumption (Castle, 2015), as banana seeds are large and hard and spiky and liable to crack teeth. These are propagated asexually from offshoots. the plant is allowed to produce two shoots at a time; a larger one for immediate fruiting and a smaller “sucker” or “follower” to produce fruit in 6-8months and as non-seasonal crops, they are available fresh year round.

**2.5.3 Classification of *Musa sapientum***

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Liliopsida*

Order: *Zingiberales*

Family: *Musaceae*

Genus: *Musa*

Specie: *Musa sapientum*

**2.5.4 Morphology of *Musa sapientum (Banana)***

The banana (*Musa sapientum*) is a tree-like perennial plant. It can also be classified as an herb because it does not have woody tissues and the fruit-bearing stem dies down after the growing season. It is a perennial because suckers, shoots arising from lateral buds on the rhizome, take over and develop into fruit-bearing stems. The trunk is not a woody stem but a pseudostem, a compact assemblage of overlapping and spirally arranged leaf sheaths. The 'true' stem is made up of three parts: the underground rhizome, the aerial stem to which are attached the leaves, and the peduncle to which is attached the inflorescence (Robinson and Galàn, 2010). The stem starts on the rhizome's apical meristem, grows inside the pseudostem, and ends in the male bud.

Another important part is the "Mat" which is the banana-specific horticultural term for the clump formed by the rhizome, the fruit-bearing stem (or stems as more than one stem can be fruiting at the same time) and the suckers. Some people say stool. The botanical term is genet. The above-ground shoots are called ramets. Barring mutations in the lateral buds, the shoots on a genet are genetically identical to each other (i.e. clones). Wild species of bananas also form genets but, unlike cultivated bananas, they also reproduce sexually since their flowers are fertile. Pollination is required for the ovules to develop into seeds, which in turn stimulates pulp development in the fruit. Banana cultivars, which have flowers that are mostly sterile, produce fruits parthenocarpically, in the absence of pollination.

***2.5.4.1 Root System***

The root system of banana plants begins as a single rhizome that puts out suckers, which form new plants to replace the dying main plant after it fruits. The rhizome, suckers and their fibrous roots form a mass of roots known as the mat. In well-drained, loose soils, the roots are capable of reaching up to 5 feet deep and spreading up to 16 feet horizontally (Skutch, 2000). The root system is the means by which the plant takes up water and nutrients from the soil. The primary roots originate from the surface of the central cylinder whereas secondary and tertiary roots originate from the primary roots.

Additionally, the roots naturally produce many suckers that compete with the main stem for nutrients. Bananas are heavy feeders and require rich soil and plenty of water for healthy growth. If left unpruned, numerous pseudostems grow from suckers in the banana mat. Competition for nutrients weakens the plant and potentially leads to disease and problems with fruit production.

* + - 1. ***Rhizome***

The rhizome is commonly referred to as a corm, and occasionally as a bulb, but the botanically correct term is rhizome (Clonal, 2017). Rhizomes are characterized by horizontal underground growth; production of roots from multiple nodes; and production of clonal shoots. Corms, on the other hand, are vertical enlarged compact stems with a tunic of thin leaves and roots arising from a single node; features that do not describe well the banana's underground structure.

According to the Merriam-Webster dictionary (2010), a corm or rhizome is: “A rounded thick modified underground stem base bearing membranous or scaly leaves and buds and acting as a vegetative reproductive structure.” The corm is the site at which a banana plant produces “suckers,” or offshoots of young banana plants that grow in clusters around the “mother” plant. In the vegetative phase, the terminal growing point of the rhizome, the apical meristem, has the form of a flattened dome. At the transition from the vegetative to the floral stage, the meristem area becomes convex and rises above the surrounding leaf bases (Kirchoff, 1992). Flower bracts appear in place of leaves. Swellings, which differentiate into female flowers and then male flowers, appear at the base of the flower bracts.

* + - 1. ***Pseudostem***

The pseudostem is the part that looks like a trunk. This 'false stem' is formed by the tightly packed overlapping leaf sheaths. The pseudostem continues to grow in height as the leaves emerge one after the other and reaches its maximum height when the stem, which has been developing inside the pseudostem, emerges at the top of the plant (IPGRI-INIBAP and CIRAD). Even though the pseudostem is very fleshy and consists mostly of water, it is quite sturdy and can support a bunch that weighs 50 kg or more. Pseudo-stem have low glycemic index and have a high content of dietary fibre and antioxidant which is good for diabetes (Bhaskar *et al.,* 2011). Banana stem is rich in potassium and vitamin B6. Vitamin B6 helps in production of haemoglobin and insulin. This pseudostem can grow to be two to eight meters tall. Each pseudostem grows from a corm. A pseudostem is able to produce a single bunch of bananas. After fruiting, the pseudostem dies and is replaced. When most bananas are ripe, they turn yellow or, sometimes, red. Unripe bananas are green.

* + - 1. ***Stem***

Banana stems vary in size, averaging at least five centimeters in diameter when sold in markets, and are cylindrical to elongated in shape. The outer layer of the stem is a fibrous, green sheath that is inedible and tough to remove. Underneath this layer, the core is the edible portion of the stem and is white to pale green-yellow with a firm, dense consistency. Banana stems are crisp with a texture similar to jicama and have a mild, sweet-tart, vegetal flavor.

The 'true' stem provides support to the leaves and flowers, some of which will develop into fruits. The leaves and flowers are attached to a node, and the sections between nodes are internodes. The stem is subdivided in three parts: the underground rhizome, the aerial stem, and the peduncle (Robinson and Galàn, 2010). The aerial stem begins to develop after the formation of flowers on the rhizome's apical meristem. As it develops, it carries the inflorescence and the leaf bases upwards inside the pseudostem. When the aerial stem emerges at the top of the plant, it is called the peduncle.The aerial stem is often called the floral stem. But this is wrong because the flowers are attached to the peduncle. Only the leaves are attached to the aerial stem.

* + - 1. ***Leaf***

Banana leaves are large, wide, elongated, and slightly rounded, averaging two meters in length, a half a meter in width, and 8-12 leaves per tree. The surface of the leaves is waxy, flexible, and glossy, and range in color from lime, olive green, to dark green. There is a central midrib that runs the length of the leaf and two laminas, or leaf halves are found on either side of the midrib. The leaves do not have branching veins, and this makes them vulnerable to tearing easily. The leaf is the main photosynthetic organ. Each leaf emerges from the center of the pseudostem as a rolled cylinder. The distal end of the elongating leaf sheath contracts into a petiole, that is more or less open depending on the cultivar. The petiole becomes the midrib, which divides the blade into two lamina halves. The upper surface of the leaf is called adaxial while the lower one is called abaxial. The first rudimentary leaves produced by a growing sucker are called scale leaves. Mature leaves that consist of sheath, petiole, midrib and blade are called foliage leaves. Lamina veins run parallel to each other in a long S shape from midrib to margin. Veins do not branch, which results in leaves tearing easily. The cigar leaf is a recently emerged leaf still rolled as a cylinder. The lapse of time in which a leaf unfolds varies. Under favourable climatic conditions, it takes about seven days, but it can take up to 15 to 20 days under poor conditions. The new leaf is tightly coiled, whitish, and particularly fragile. The extension at the tip of the leaf is called the precursory appendage. After emergence, it withers and falls off.

***2.5.4.6 Sucker***

Sucker is a shoot that develops from a lateral bud on the rhizome and emerges from the soil usually near the parent plant. It is a form of asexual, or vegetative, reproduction, which makes the banana plant perennial. Suckers emerge and ensure a more or less continuous supply of shoots, each capable of producing an inflorescence. They have been used as planting material since the early days of domestication by severing them from the mat and transplanting them to a new location. Both wild species of bananas and cultivated bananas produce suckers. Wild species may produce few or many suckers (Turner, 2000).

Morphologically, there are two types of sucker: sword suckers, characterized by narrow leaves and a large rhizome, and water suckers; which have broad leaves and a small rhizome. Water suckers have a weak connection to the parent plant and as such will not develop into a strong plant. The number of suckers produced varies with the type of cultivar. The sucker selected to replace the parent plant after fruiting is called the follower or ratoon (Skutch, 2000).

* + - 1. ***Inflorescence***

The inflorescence of the banana is a complex structure that includes the flowers that will develop into fruits. The botanical term for the banana inflorescence is a thyrse (Kirchoff, 1992). The main types of flowers are the female flowers, which develop into fruits, and the male flowers. The female flowers which are known as "Pistillate" appear first. In cultivated bananas, the ovary develops into a seedless fruit by parthenocarpy. As it lifts, the bract (a modified leaf associated with a reproductive structure) exposes a cluster of female flowers that are normally arranged in two rows. These flowers will develop into a hand of fruit. The number of hands in the bunch depends on the number of female clusters in the inflorescence, and varies depending on the genotype and environmental conditions.

As the female flowers develop into fruit, the distal portion of the inflorescence elongates and produces clusters of male (staminate) flowers that produce pollen. In cultivated bananas, the amount of pollen is reduced or may be absent.

***2.5.4.8 Peduncle***

Botanically, peduncle is the stalk that supports the inflorescence. But in the Descriptors for bananas, the peduncle refers only to the stalk between the leaf crown and the first hand of fruit, whereas the stalk that actually supports the female and male flowers is called rachis (IPGRI and CIRAD, 1996). Jeff Daniells and David Turner have argued that in keeping with the botanical definition of the term, the peduncle extends to the meristem in the male bud and is composed of three sections: the transitional, female and male peduncles (Skutch, 2000).

i. Transitional peduncle

The transitional peduncle supports organs that are in transition from leaves to bracts: sterile nodes with a bract that abscises at bunch emergence. It corresponds that what is traditionally called the peduncle.

ii. Female peduncle

The female peduncle supports the female flowers that develop into fruits.

The bunch is the descriptive term that includes all the fruits. The fruits are arranged into hands, the former clusters of flowers that were each subtended by a bract. By analogy, the fruits in a hand are often called fingers.

iii. Male peduncle

The male peduncle supports the male flowers in the male bud. It corresponds that what is traditionally called the rachis, an ambiguous term that in botany has been used in relation to both vegetative and reproductive parts, whereas the term peduncle is only used for stems that support flowers. The part above the male bud can be bare or covered with persistent bracts. The scars (nodes) indicate where the bracts were attached. The male peduncle continues to grow as the fruits are maturing. Male bud

The male bud contains clusters of male flowers. Each cluster is subtended by a bract. The male bud is sometimes called the bell. In some cultivars, it ceases to grow after the fruits have set and can be more or less exhausted by the time the bunch reaches maturity. The presence or absence of the male bud is one of the traits used to distinguish cultivars.



**Figure 3: Diagram showing the morphological view of banana**



**Figure 4: Morphological view of *Musa paradisiaca***



**Figure 5: Diagram describing the root system of Banana**



**Figure 6: Diagram of banana inflorescences with flowers**

**2. 6. Nutritional Value of Musaceae Species**

**2.6.1. Carbohydrates**

Raw bananas with the exception of the peels contain of about 23% of carbohydrates. Carbohydrates perform numerous roles in living organisms. Polysaccharides serves as an energy store for starch and glycogen. Plantains are starchy and contain less sugar than bananas. Plantains provides a healthy dose of carbohydrates. One cup of boiled green plantains has 40 total grams of carbohydrates with nearly 4 grams of fiber and 3 grams of natural sugar (FCD, 2011). Plantains are high in resistant starch that gives them a low glycemic index of about 38.5 in raw and ripe plantains to 44.9 in boiled and unripe plantain (oladele and Williamson, 2016). However, banana is a rich source of carbohydrates which occur mainly as starch in unripe bananas. The carbohydrate composition of banana changes drastically during ripening. The main component of banana is starch. Green banana contains up to 80%of starch measured in dry weight. During ripening, the starch is converted into sugars and ends up being less than 1% when the banana is fully ripe (Gentile, *et al.,* 2016). The most common types of sugar in ripe bananas are sucrose, fructose and glucose. In ripe bananas, the total sugar content can reach more than 16% of the fresh weight (Pingyi *et al.,* 2010). Bananas have relatively low glycemic index of 42 -58, depending on their ripeness. The glycemic index is a measure of how quickly carbohydrate in food enter your bloodstream and raise blood sugar.

**2.6.2. Fibers**

Bananas are also a good source of other types of fiber, such as pectin. Some of the pectin in bananas are water-soluble. A high proportion of the starch in unripe bananas is resistant starch that passes through the gut. In the large intestine, the starch is fermented by bacteria to form butyrate, a short chain fatty acid that appears to have beneficial effect on human’s gut (Leonel and Alvarez, 2012). When bananas ripen, the proportion of water-soluble pectic increases whivh is one of the main reasons why bananas turn soft as they age. A 1-cup serving of sliced plantains contains 5grams of dietary fiber and are fiber rich, containing about 20% of the 25 grams of fiber per day which is the recommended fiber for men. Plantains and bananas contain both soluble and insoluble fiber with approximately 73% of insoluble fiber and 27% soluble fiber. The insoluble fiber helps add bulk to the human’s stool and lowers the risk for constipation while the soluble fibers help lower the blood cholesterol and blood glucose levels, lowering the risk for heart diseases and diabetes. The high fiber content also helps makes plantains more filling.

**2.6.3. Fats**

Plantains are naturally low in fat but easily absorb the oil they are cooked in. Fried plantains are very high in fat. A 1-cup serving fried plantains provides 14 grams of total fats, 4.4 grams of saturated fats, 5.1 grams of monounsaturated fats. Mono and polyunsaturated fats are good fats and may reduce the risk of heart disease. According to the American Heart Association (AHA, 2020), saturated fats should be limited in the diet because they increase the risk of heart disease. Adults should consume between 20 and 35 percent of their daily calories from fats. Banana are low in fat with less than ½ gram per medium sized banana.

**2.6.4. Proteins**

Bananas are pretty low in protein as well with less than 1.5 grams per medium banana. Plantains contain a minuscule of 1.3 grams of protein. Plantains are not a significant source of protein. A medium plantain has less than 2 grams.

**2.6.5 Vitamins and Minerals**

Plantains contain iron, vitamin, vitamin B6, folate, potassium, magnesium, copper and vitamin A (Famakin *et al.,* 2016). According to the USDA, a cup of plantains provides 12.5 milligrams of vitamin c, which is about 15% of daily recommended intake for humans. Plantains contain folate which is a vital nutrient for women trying to conceive. Banana contains several vitamins, including vitamin A, B, and C. Bananas are high in vitamin B6; one medium sized banana can provide up to 33% of the daily value of vitamin B6. Banana is also rich in potassium of around 400mg/100g. A diet high in potassium can lower blood pressure in people with elevated levels and benefits heart health. It also contains dopamine which is an important neurotransmitter of the brain. Several flavonoids are also present in both plantains and bananas such as; catchins, and low in sodium.

**2.7. Functional Properties of Musa Species**

**2.7.1 Traditional Uses**

All parts of the Banana plant have medicinal uses. The flowers are used in treating bronchitis, dysentery, menorrhagia and ulcers (Ghani, 2003). Cooked flowers are used to treat diabetes. The astringent plant sap is given in cases of hysteria, epilepsy, leprosy, fevers, hemorrhages, dysentery and diarrhea, and it is applied on hemorrhoids, insect and other stings and bites. Young leaves are placed as poultices on burns and other skin afflictions. The astringent ashes of the unripe peel and of the leaves are taken in dysentery and diarrhea and are also used for treating malignant ulcers. The roots are administered in digestive disorders, dysentery and other ailments. It also has anthelmintic property (Khare, 2007). Banana seed mucilage is given in cases of catarrh and diarrhea in India. Antifungal and antibiotic properties are found in the peel and pulp of fully ripe Bananas. The plant is also used in inflammation, pain and snakebite.

**2.7.2 Pharmacological Uses**

Several effects of *Musa. paradisiaca and Musa sapientum* are documented in traditional as well as scientific literature. However, the main pharmacologial effects of this plant (*Musaceae species)* are -diuretic, analgesic, antiulcer, wound healing, antioxidant, hypoglycemic activities mutagenic effects in which few are reported below:

* + - 1. ***Antiulcerogenic property***

In a study conducted to evaluate the effect of orally administered banana pulp powder (0.5mg/kg orally twice daily for 3 days) in ulcers induced by aspirin, indomethacin, phenylbutazone, prednisolone and cysteamine in albino rats and histamine in guinea pigs suggest that banana powder treatment not only strengthens mucosal resistance against the ulcerogens but also promotes healing by cellular proliferation (Goel *et al.,* 1986). Banana is used in the herbal medicine to treat peptic ulcer disease. The use of *Musa. sapientum* in peptic ulcer as a component of herbal medicine has been evaluated and found effective (Goel and Sairam, 2002). Dunjić et al. (1993) reported that pectin and phosphatidylcholine in green banana strengthens the mucous phospholipid layer that protects the gastric mucosa. The active ingredient of banana responsible for antiulcerogenic effect, identified to beleucocyanidin, a flavonoid. The leucocyanidin at 5mg/kg orally demonstrated a significant (p <0.05) protective effect against aspirin induced erosions (Shaw *et al.,* 1999). Pannangpetch *et al.* (2001) reported that the antiulcerative effect of banana may vary depending on different varieties of banana. They showed that the ethanolic extract of both *Musa. sapientum* and *Musa paradisiaca* have significant gastro protective effect but only *Musa paradisiaca promotes* ulcer healing by a similar mechanism like prostaglandins.

* + - 1. ***Antidiarrhoeal activity***

The antidiarrhoeal activity of banana in rats was observed as early as in 1930s. This effect in the intestinal diseases was attributed to the pectin content of banana. Later banana diet was reported to be effective and advantageous in bacillary dysentery in a proctoscopic study on 127 patients of age nine month to forty-eight years (Block, 1941). Banana flakes has also been tested and found effective in the treatment for diarrhoea in critically ill patients receiving enteral feedings (Emery *et al.,* 1997). The antidiarrhoeal activity of green banana diet was found very effective in children with diarrhoea (Rabbani *et al.,* 1999; 2001). According to a report by Bernal, *et al.,* in 1997, where a clinical trial was conducted to evaluate the efficacy of a solution of 50 gm/L of plantain flour and 3.5 gm/L of sodium chloride for rehydration of children with acute diarrhoeal disease. The plantain flour based solution proved effective for the treatment of dehydration due to acute diarrhoeal diseases (Bernal *et al.,* 1997).

* + - 1. ***Antioxidant activity***

Antioxidant activity was reported with aqueous acetone extract of banana peel by β-carotene bleaching method, DPPH free radical scavenging and linoleic acid emulsion method. Plasma oxidative stress is significantly reduced only after a single banana meal in healthy human due to the presence of dopamine, ascorbic acid and other antioxidants present in banana (Yin *et al.,* 2008). Glycosides and monosaccharide components are mainly responsible for the antioxidant activity (Mokbel and Hashinaga, 2005). Vijayakumar *et al.,* (2008) reported the antioxidant activity of the extracted flavonoids from *M. paradisiaca* in rats. They found that the flavonoids from banana stimulated the activities of superoxide dismutase (SOD) and catalase which might be responsible for the reduced level of peroxidation products such as malondialdehyde, hydroperoxides and conjugated dienes. The antioxidant effects of crude extracts from green banana and yellow peel were investigated and the results indicated that the extract of green peel recorded more significant activities than that of yellow peel at other solvents extracts **(**Sanjeev *et al.,* 2012).

* + - 1. **Wound healing activity**

The wound healing activity of both methanolic and aqueous extract of plantain and banana in rats was studied and both extracts were found to increase hydroxyproline, hexuronic acid, hexosamine and superoxide dismutase as well the wound breaking strength and reduced glutathione level. They also decreased the wound area, scar area and lipid peroxidation. The effects were attributed to the antioxidant property of the plantain and banana **(**Agarwal *et al.,* 2009).

* + - 1. **Antimalarial activity**

Kaou *et al.,* (2008) reported that the decoction of the leaves of *Musa paradisiaca* added to *Ocimum americanum* and *Ocimum gratissimum* is used as to treat malarial in Comores, Ngazidja. But *in vitro* study using *Plasmodium falciparum* chloroquine-resistant strain proves this plant ineffective in malaria.

* + - 1. **Diuretic activity**

Ash of the peel of *Musa Sapientum (*banana*)* showed an increase in urine volume and potassium (K+) as well as other electrolyte excretion than normal saline in a study in rats. Successive ethanolic extract also give this diuretic effect (Jain *et al.,* 2007). Phytochemicals such as saponin, flavonoids and terpenoids are known to be responsible for this effect (Rizvi *et al.,* 1980; Sood *et al.,* 1985; Chodera *et al.,* 1999).

* + - 1. **Anti-allergic activity**

The water extract of pulp of ripe *Musa sapientum* has been reported to have significant anti-allergic activity on antigen induced degranulation in RBL-2H3 cells with an IC50 value of 13.5±2.4 (Tewtrakul *et al.,* 2008).

* + - 1. **Anti-snake venom activity**

Borges *et al.,* (2005) reported the *in vitro* neutralizing capacity of *Bothrops jararacussu* and *Bothrops neuwiedi* snake venoms by the stem juice of *Musa. paradisiaca*. The phospholypase A2 (PLA2) and hemorrhagic activities induced by the venom was inhibited by the extract as it forms unspecific complex with the venom protein. However, the *in vivo* activity of the extract in mice was not significant to protect against the venom (Borges *et al.,* 2005).

***2.7.2.9 Hypocholesterolaemic activity***

Hemicellulose and other neutral detergent fibers (NDF) from the unripe *Musa. paradisiaca* fruit showed low absorption of glucose and cholesterol and low serum and tissue levels of cholesterol and triglycerides (Usha *et al.,* 1984). Flavonoids isolated from unripe fruits showed hypolipidemic activity evidenced by decrease in cholesterol, triglycerides (TG), free fatty acids and phospholipids levels in serum, liver, kidney and brain of rats. The cholesterol lowering effect was attributed to a higher degradation rate of cholesterol than synthesis (Vijayakumar *et al.,*2009). Methanolic root extract of *M. paradisiaca* showed totalcholesterol (TC), triglyceride (TG), LDLc and VLDLc lowering effect in diabetic rats (Mallick *et al.,* 2006). The pectin content in the juice of the inflorescence stalk of *Musa. sapientum* has also been reported to possess cholesterol and triglyceride lowering activity inrats (Gomathy *et al.,* 1989).

* + 1. ***Mutagenic effect***

Mutagenic effect of the *Musa paradisiaca* fruit peel extract was assessed by the Single-cell Gel Electrophoresis and micronucleus assays. Peripheral blood cells of Swiss mice were collected 24 h after treatment for the SCGE assay and 48 and 72 h for the micronucleus test after feeding the extract in three different concentrations (1000, 1500 and 2000 mg/kg Body Weight). It was concluded that the two higher doses of the extract of *Musa. paradisiaca* induced significant increases in the average numbers of DNA damage in peripheral blood leukocytes for the two higher doses and a significant increase in the mean of micronucleated polychromatic Erythrocytesin the three doses tested (Sanjeev, 2012).

* + 1. ***Phytochemicals property***

Carbohydrates have been isolated from *M. sapientum* (Anhwange, 2008). Catecholamines such as norepinephrine, serotonin, dopamine (Waalkes *et al.,* 1958; Vettorazz, 1974). Tryptophan, indole compounds (Shanmugavelu and Rangaswami, 1962), pectin have been found in the pulp. Several flavonoids and related compounds (Leucocyanidin, quercetin and its 3-O-galactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside) were isolated from the unripe pulp of plantain (Lewis *et al.,* 1999; Lewis and Shaw, 2001; Ragasa *et al.,* 2007). Serotonin, nor-epinephrine, tryptophan, indole compounds, tannin, starch, iron, crystallisable and non-crystallisable sugars, vitamin C, B-vitamins, albuminoids, fats, mineral salts have been found in the fruit pulp of *Musa paradisiaca* and *M. sapientum* (Ghani, 2003). Acyl steryl glycosides such as sitoindoside-I,sitoindoside-II, sitoindoside-III, sitoindoside-IV and sterylglycosides such as sitosterol gentiobioside, sitosterol *myo*-inosityl-β-D-glucoside have been isolated from fruits of *M. paradisiacal* (Ghoshal, 1985)*.* Jang et al. (2002) isolated a bicyclicdiaryl heptanoid, *rel*(3*S*,4a*R*,10b*R*)-8-hydroxy-3-(4-hydroxyphenyl)-9-methoxy-4a,5,6,10b-tetrahydro-3*H*s naphtho[2,1-*b*] pyrin, and1,2-dihydro-1,2,3-trihydroxy-9-(4-methoxyphenyl) phenalene, hydroxyanigorufone, 2-(4-hydroxyphenyl) naphthalic anhydride,1,7-bis(4-hydroxyphenyl) hepta-4(*E*),6(*E*)-dien-3-one.

**CHAPTER THREE**

**MATERIALS AND METHODS**

**3.1 STUDY AREA**

This research work was conducted at Microbiology laboratory, Akwa Ibom State University and Biochemistry laboratory, University of Uyo.

**3.2 MATERIALS**

Refrigerator, palm oil, extracts from Musa species, autoclave, microscope, Peptone Detrose Agar (PDA), incubator, Petri dishes and reagents.

**3.3 SAMPLE COLLECTION AND PROCESSING**

The unripe plantain and banana were purchased from Ukam market and the peels were sun dried for 4weeks and burnt to ashes. The palm oil was also obtained from Ukam market in Akwa Ibom state. All the samples were taken to Microbiology and Biochemistry laboratory for analysis

**3.4 ANTIFUNGAL ASSAY**

***3.4.1 Fungi isolation***

A serial dilution of 10-5 was carried out for the oil sample. After the dilution, 1ml from dilution factor 103, 104 and 105 were plated into petri-dishes and appropriate medium (potato dextrose agar) was poured into the petri-dishes and swirled gently to mix. The plates were allowed to solidify. Then, the plate was incubated at room temperature for 2-5 days and appropriate growth were observed after 2-5 days (Kirk and Farell, 1987).

***3.4.2 Purification of fungi isolates***

After seventy-two (72) hours of isolation, plates were observed for obvious growth and plate that displayed growth were marked and sub-culture to obtain pure colonies. After obtaining the pure colonies, they were preserved and grown in sterile McCartney’s bottle for further use. The stock bottle was kept in a refrigerator at 4oC (*Bragulat et al., 2001)*

***3.4.3 Identification of fungi isolates***

Microscopic and macroscopic approach was used for the identification of the fungi. The colony characteristics of the fungi isolates were compared with guide provided by Benson, 2002, while the microscopic morphology was determined using lactophenol cotton blue stain as described by Pepper and Gerba, 2005. After the microscopic and macroscopic approach was successfully used, the fungi isolates identified were; *Aspergillus niger, Rhizopus stolonifer, Aspergillus fumigates and Rhizopus oryzae*

***3.4.4 Fungal susceptibility testing***

After each of the fungi isolates were successfully identified, fungal susceptibility test was carried out by agar well diffusion method. Pentose Dextrose Agar (PDA) was prepared and the ash extract of the two samples were added to the petri plates. 3mm in diameter of the PDA were cut with a cork borer and placed in the centre of the petri plates containing different concentration of the ash extracts. Each replicates were incubated at 280C for 4days. Radial growth was monitored for the two extracts and aliquot of the ash extract were diluted with distilled water to give a concentration of 1.0mg/ml, 0.5mg/ml, 0.25mg/ml and 0.125mg/ml respectively and were incubated at 280C for 4days and observed for fungal growth. The lowest concentration of the ash extract that inhibited the growth of the fungi was recorded as growth inhibition and was expressed in percentage.

**3.5 PROXIMATE ANALYSIS**

**3.5.1 Moisture Content**

Weighing bottle was washed and dried in an oven at 800C for some minutes, cooled and weighted (a), 2g of the burnt banana and plantain peels was weighed into different weighing bottle and the weight of the bottle and its content was taken as (b). Then, the weighing bottle and its content were then dried in an oven at the temperature of 1050C for 24 hours. The sample was then removed from the oven and allowed to cool in desiccators at room temperature, weighed with a minimum exposure to atmosphere **(**Pearson, 2003). This was repeated till a constant weight was obtained (c)

Calculatation:

Moisture (% wet weight) =

b-c x 100

b-a

Where:

a = weight of beaker only

b = weighed beaker + sample before oven drying

c = weighed beaker + sample after oven drying

**3.5.2 Preparation of sample for subsequent analysis**

After taking part of the fresh sample for moisture content determination, the remaining sample was dried to a constant weight before subsequent analysis (Gregory, 2005). The low temperature (50- 600c) was employed to reduce any possible effect of high temperature on the protein (and probably other nutrients) in the sample, such effect includes

1. Protein denaturation
2. Loss of vitamins
3. Decomposition of anions

However, the oven dried material was ground in a mortar into a powdered form, often necessary to pass through a sieve of a particular mesh size and then stored at a temperature in dry-air-tight container specifically having a plastic cover.

The following parameters were determined from the grounded sampled; Ash, Fibre, Lipid, Protein, Etc.

**3.5.3 Ash and Organic Matter**

Crucible with lid was ignited in a muffle furnace at the temperature of 1050C for an hour. It was transferred to desiccators to cool and weighed (a). Few grams (1-5g) finely ground dry sample was put into the pre-weighed crucible. Then, the weight of the crucible and its content (sample) was taken (b). the crucible was charred and its content on a Bunsen flame in a fume cupboard, to drive off most of the smoke (until smoking ceases), then was transferred to a muffle furnace heated at (500- 6000c) to burn off all the organic matter; then was allowed at this temperature for 2 hours. The crucible was allowed to cooled, cover and place in a desiccators and weighed (c).

Calculation:

Ash % = weight of ash x 100

Weight of sample 1

c-a x 100

b-a 1

Where

a = weight of empty crucible

b = weight of crucible + sample before ashing

c = weight of crucible + ash

The portion of sample which burnt off is organic matters

Organic matter (%) = 100 – (%) ash

* + 1. **Estimation of Crude Fibre**

2g of material with petroleum was defatted for 2hours. Then, was boiled under reflux for minute with 200ml of a solution containing 1.25g of H2SO4 per 100ml solution. The solution was filtered through cotton cloth on a fluted funnel. The sample was washed with boiling water until the washing was no longer acidic. The residue was transferred into a beaker and was boiled for another 30 minutes with 200ml of a solution containing 1.25g of NaOH per 100ml. then, the final residue was filtered and was washed with a boiling water several times until it was base (NaOH) free. The residue was finally washed twice with ethanol and qualitatively transferred into a pre-weight crucible, oven dried at 1500C (Io) and was incinerated in a furnace at 5500C, allowed to stand at this temperature for 2 hours and was then cooled in a desiccators and weigh la (loss in weight).

Calculation:

Ia- Io × 100

Weight of original sample taken

*Where;*

Ia = weight of empty crucible

Io = weight of crucible and its content after incineration (ash fibre)

* + 1. **Determination of Crude Fat**

2.0g of the sample was weighed into an extractor thimble, which was already been washed dried in an oven and was then plug lightly with cotton wool. 150ml of petroleum ether (Boiling point 60 - 80 0c) was poured into 250ml capacity round bottom flask. The soxhlet extractor was fitted into round bottom flask which was seated on a heating mantle. Then the soxhlet apparatus was assembled and allowed to reflux for 4 hours. The extract was poured into a dried pre- weighted beaker (W1) and the thimble was rinsed with a little quantity of the ether back to the beaker and the beaker was heated on a steam bath to dried off the excess solvent and was cooled in desiccators and weigh(W2).

Calculation:

Weight gain in flask × 100

Weight of sample 1

W2 - W1 × 100

Weight of sample 1

Where:

W2  = weight of beaker + fat

W1  = weight of empty beaker only

**3.5.6 Crude Protein Determination**

The crude protein of the sample was determined using kjeldahl method;

1g of the sample was accurately weighed into a standard 250ml kjeldahl flask containing 1.5g CuSo4 and 1.5g Na2S04 as catalyst and 5ml concentrated H2S04. The kjeldahl flask (digestion) was placed on a heating mantle and was heated gently to prevent frothing for some hours until a clear bluish solution was obtained. The digested solution was allowed to cool and this was quantitatively transferred to 100ml standard flask and make up to the mark with distilled water. 20ml portion of the digest was pipette into a semi micro kjeldahl distillation apparatus and treated with equal volume of 40% NaOH solution. The ammonia evolved was steam distilled into a 100ml conical flask containing 10ml solution of saturate boric acid to which 2 drops Tashirus indicator (double indicator) has been added. The tip of the condenser was immersed into the boric acid double indicator solution and then the distillation continued until about 2/3 of the original volume obtained and the tip of the condenser was rinsed with a few millimeters of distilled water in the distillate which was then titrated with 0.1M HCL until a purple-pink end point was observed. The blank determination was also carried out in the similar manner as described above except for the omission of the sample. The crude protein was obtained by multiplying the % Nitrogen content by a factor (6.25).

2N (food) + 4H2SO (NH4)2 SO4

(NH4)2 SO4 + 2NaOH Na2SO4 + 2H20 + 2NH3

2NH3 + 2H3BO3 2NH4H2BO3

Ammonium borate

2NH4H2BO3 NH4Cl + HBO3

I mole HCl = 1 mole of N = 14gN

Crude protein = Nitrogen × factor

Calculation:

(Sample titre - Blank titre × 0.1 × 0.014 × 20 × 100 × 6.25)

Weight of sample 10 1

Note:

Most protein contain about 16% Nitrogen, so that 16mg N2 = 100mg

1 mg N2 =100 = 6.25mg of protein

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The Nitrogen value was therefore multiplied by 6.25 to get the weight protein.

**3.5.7 Determination of carbohydrate**

This was determined as the different obtained after subtracting total organic nitrogen (protein), lipid, ash and fibre from the total dry matter.

**3.5.8 Estimation of caloric value (energy)**

The caloric values of the sample were obtained by multiplying the value of the crude protein, lipid and carbohydrate by 4, 9, 4kcal respectively and taking the sum of the product.

**3.6 STATISTICAL ANALYSIS**

All the data obtain was expressed as mean ± standard deviation and analyzed using One Way Analysis of Variance (ANOVA). Significant means was separated by applying Duncan multiple range post hoc test as outlined by Duncan (1955).

**CHAPTER FOUR**

**RESULT AND DISCUSSION**

**4.1 Antifungal Effect of *Musa sapientum* and *Musa paradisiaca* Peels -Ash extract on Oil Palm**

The fungi isolated from palm oil in this study demonstrated susceptibility to both the ash extract of *Musa paradisiaca* and *Musa sapientum* peels respectively. At 1.00mg/ml concentration of ash extract of *Musa paradisiaca* peels, the growth of *Aspergillus fumigatus, Aspergillus niger, Rhizopus stolonifer and Rhizopus oryzae* were inhibited 75%, 100%, 56.67% and 76.67%. At the same concentration, ash extract of *Musa sapientum* peels inhibited the growth of these organisms 60%, 85%, 43% and 50.20% respectively as shown in Table 4.1 and Table 4.2 respectively. At 0.50mg/ml concentration of ash extract of Musa paradisiaca peels, inhibition was 60%, 70%, 26.67% and 56.67% while at the same concentration, the ash extract of *Musa sapientum* peels gave 45.67%, 54.67%, 23.54% and 42.40% (Table 4.1 and Table 4.2). At 0.025mg/ml concentration of *Musa paradisiaca* peels, the fungi were inhibited 50%, 53.33%, 10% and 23.67% while the ash extract of *Musa sapientum* recorded 43.40%, 40%,5% and 20.58% respectively. At 0.125mg/ml concentration, the ash extract of *Musa paradisiaca* peels inhibited the growth of fungi by 40%, 26.67%, 5% and 20% while the ash extract of *Musa sapientum* at the same concentration gave 35.32%, 22%, 3.67% and 14% as shown in Table 4.1 and Table 4.2 respectively.

It was observed that the ash extract of *Musa paradisiaca* demonstrated high antifungal property as compared to the ash extract of *Musa sapeintum* as shown in table 4.1.1 and 4.1.2 respectively and was also observed that the growth inhibition of test fungi reduces as the concentration of the ash extract reduces. This may due to the level of the antifungal concentration in the two samples or the geographical region or origin of the Musaceae species as supported by a research work carried out by Janovsky *et al.,* (2003) who attributed the antifungal properties of plant origin to it origin and geographical region. Also the difference in the two samples base on their antifungal property can be attributed to the level of antioxidants presence in the ash extract of the two samples such as; alkaloids, flavonoid and tannins (Harborne, 1973; Tsuchiya *et al.,* 1994; Hutchings *et al.,* 2003; Okorondu *et al.,* 2010).

**Table 4.1 Growth inhibition (%) of fungi by ash extract of *Musa paradisiaca* on oil palm isolates**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Isolates** | **Extract Concentration** | | | |
|  | **1.0mg/ml 0.5mg/ml 0.025mg/ml 0.125mg/ml** | | | |
| 1. ***Fumigates*** | **75.00** | **60.00** | **50.00** | **40.00** |
| 1. ***niger*** | **100.00** | **70.00** | **53.33** | **26.67** |
| ***R. stolonifera*** | **56.67** | **26.67** | **10.00** | **5.000** |
| ***R oryzae*** | **76.67** | **56.67** | **26.67** | **20.00** |

**\*Data represents mean of triplicate determinations**

**\*Reduction in radial fungal growth as compared with control and expressed as percentage**

**\**Aspergillus fumigates \*Aspergillus niger \*Rhizopus stolonifera \*Rhizopus oryzae***

**Table 4.2 Growth inhibition (%) of fungi by ash extract of *Musa sapientum* on oil palm isolates**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Isolates** | **Extract concentration**  **1.0mg/ml 0.50mg/ml 0.025mg/ml 0.125mg/ml** | | | |
| ***A. fumigates*** | **60.00** | **45.67** | **43.40** | **35.32** |
| 1. ***niger*** | **85.50** | **54.67** | **40.00** | **22.00** |
| ***R. stolonifera*** | **43.00** | **23.54** | **5.000** | **3.670** |
| ***R. oryzae*** | **50.20** | **42.40** | **20.58** | **14.00** |

**\*Data represents mean of triplicate determinations**

**\*Reduction in radial fungal growth as compared with control and expressed as percentage**

**\**Aspergillus fumigates \*Aspergillus niger \*Rhizopus stolonifera \*Rhizopus oryzae***

**4.2 Effect of oil palm on the proximate composition of ash extract of *Musa paradisiaca* and *Musa sapietum* peels**

**4.2.1 Moisture content**

The results in Table 4.3 showed that the total moisture content of ash extract of both *Musa paradisaica* peels and *Musa sapientum* peels was influenced by palm oil. These result revealed that there was a significant increase (p>0.05) in the moisture content of both the two samples due to the addition of the palm oil. The value of total moisture in the ash extract of the *Musa sapientum* peels alone recorded 1.34**.** These levels were increased by the palm oil which recorded 1.68 ± 0.001% while the ash extract of the plantain peels alone recorded 1.17±0.003% of moisture and 1.29 ± 0.002% when blended with palm oil. This might be due to the addition of palm oil to the samples which act as liquid. This is similar to the work reported by Almeida *et al.,* (2011) who recorded different moisture content in the same species of Musa peels.

**4.2.2 Ash content**

Table 4.3 reported that there is significant decrease (p < 0.05) in the ash extract of the two samples. The ash content of the *Musa sapientum* peels alone was observed to contained 24.30 ± 0.004% and the values were decreased after addition of palm oil to the sample which recorded 23.64 ± 0.001 % while the ash content of the plantain peels contained 26. 11 ± 0.002% and the values were reduced to 24.50 ± 0.006 after addition of the palm oil. It was observed that plantain peels extract contained the highest value of ash compare to banana peels extract. The variation in the ash content relies upon the plant species, geographical origin, method of mineralization, and the organic content of the oil palm. The results are in agreement with those reported by Emaga *et al.,* (2008) who revealed the ash content of banana peels to be 22.2%.

**4.2.3 Crude fibre**

The crude fibre content of ash extract of *Musa paradisiaca* and *Musa sapientum* peels are shown in Table 4.3. The results indicated that the crude fibre of both the ash extract of *Musa paradisiaca* and *Musa sapientum* peels significantly decreases (p <0.05) by addition of palm oil. It was observed that the ash extract of *Musa sapientum* alone recorded 22.18 ± 0.004% and 21.72 ± 0.004% when mixed with palm oil. It was also observed that the ash extract of *Musa paradisiaca* peels alone recorded 23.64 ± 0.006 and 22.31 ± 0.002 after addition of palm oil. The highest levels of fibre content were observed in the ash extract of *Musa paradisiaca* peels compared to that of *Musa sapientum* peels. This is due to the differences in varieties of cultivars which have shown in previous research that the fiber content of 14.83 % in *Musa sapientum* peel compare to other cultivars. The presence of oil palm can also reduce the fibre content of those two samples, as well as the increase in the temperature during the processing of determining the fibre content (Wang *et al.,* 2008).

**4.2.4 Protein content**

The protein content obtained in Table 4.3, demonstrated a significant decrease (p < 0.05) in the ash extract of *Musa paradisiaca* and *Musa sapientum* peels respectively when oil palm was introduced. It was observed that the protein content in the ash extract of *Musa sapientum* peels alone recorded 4.23 ± 0.003% and 2.45 ± 0.003% when oil palm was added. The protein content of the ash extract of the *Musa paradisiaca* peels alone recorded 5.25 ± 0.004% and 3.26 ±0.004% after addition of palm oil. The decrease in protein content of the two ash extract of the *Musa paradisiaca* and *Musa sapientum* peels may be as a result of the sample content as the ash extract contain low level of protein and oil palm also contain no or little amount of protein. This is with the agreement of Ladeji *et al*., (2004) and Ferreira *et al.,* (2013) that attributed the increase and decrease of protein content in *Musa paradisiaca* and *Musa sapientum* peels to chemical transformation of starch present in the sample and the instrument used for measurement.

**4.2.5 Lipid content**

From Table 4.3, the highest level of lipid content was detected in ash extract of the *Musa sapientum* peels which recorded 2.60 ± 0.003% and the lipid content was increase after the addition of palm oil to 3.66 ± 0.003% while the ash extract of *Musa paradisiaca* peels contained 1.81 ± 0.004% and increases to 2.34 ± 0.002% after addition of oil palm. This could be as a result of high content of lipid in palm oil as shown in table 4.2 where oil palm recorded the highest value of lipid content (Adeniyi *et al.,* 2009).

**4.2.6 Carbohydrate content**

The carbohydrate content as shown in table 4.3 indicated that the ash extract of *Musa sapientum* peels contained the highest level of carbohydrates which recorded 47.26 ± 0.25% and 48.53 ± 0.012% when mixed with palm oil. The carbohydrate content of the ash extract of *Musa paradisiaca* peels recorded 37.94 ± 0.015% and 46.99 ±0.0011%n when mixed with palm oil. It was observed that palm oil influenced the carbohydrates levels of the two samples. This might due to the different cultivars of Musaceae or the geographical origin of the two samples. These results are comparable to the result which suggested that the banana peels are good sources of nutrients particularly carbohydrates and fiber (Kala and Mohen.2012).

**4.2.7 Caloric content**

From Table 4.3, the caloric values of both the ash extract of *Musa paradisiaca* and *Musa sapientum*peels were influenced by oil palm. It was observed that the ash extract of *Musa sapientum*peels contained the highest level of calories which contained 224.38 ± 0.542Kcal and 227.46 ± 0.245Kcal when oil palm was added while the ash extract of *Musa paradisiaca* peels contained 189.05± 1.31Kcal and 236.86 ±4.56Kcal respectively. This could be as a result of the palm oil, as the increase in lipid content of palm oil can influenced the caloric values of the samples when mixed.

**Table 4.3 proximate composition of plantain (*Musa paradisiaca)* and banana (*Musa sapientum*) peels ash soak in oil palm**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Samples** | **Moisture**  **(%)** | **Ash(%)** | **Fibre(%)** | **Protein(%)** | **Lipid(%)** | **CHO(%)** | **Energy (kcal)** |
| **Control**  **(oil)** | **0.19bc±0.001** | **0.23c± 0.001** | **0.00± 0.00** | **0.046d± 0.002** | **90.49a±0.01** | **0.82d±0.07** | **891.53a ±1.005** |
| **B-Ash** | **1.34b±0.002** | **24.30ab± 0.004** | **22.18ab± 0.004** | **4.23b± 0.003** | **2.60c±0.025** | **47.26b±0.25** | **224.38bc±0.542** |
| **P-Ash** | **1.17c±0.003** | **26.11a±**  **0.002** | **23.64a ±0.006** | **5.25a± 0.004** | **1.81e±0.004** | **37.94c±0.015** | **189.05c±1.310** |
| **B-Ash + oil** | **1.68a±0.001** | **23.64b± 0.003** | **21.72b± 0.004** | **2.45c± 0.03** | **3.66b±0.003** | **48.53a ±0.012** | **236.86 b±4.56** |
| **P-Ash + oil** | **1.29ab±0.002** | **24.50ab± 0.006** | **22.31ab±0.002** | **3.26c ±0.004** | **2.34d±0.002** | **46.99ab± 0.033** | **227.46bc±0.245** |

**\*Mean with different superscripts in a column are significantly different at p<0.05**.

**\*B-Ash – Banana ash extract \*P-Ash – plantain ash extract \*B-Ash + Oil – Banana ash extract with Oil palm \*P-Ash + Oil – plantain ash with Oil palm**

**CHAPTER FIVE**

**CONCLUSION AND RECOMMENDATION**

**5.1 Conclusion**

The two ash extract of the Musa species peels used in this research work demonstrated antifungal properties and justify that *Musa paradisiaca* peels ash extract possess the highest antifungal property compare to the ash extract obtained from *Musa sapientum* peels. The result obtained in this work further justify that there is a significant difference (p<0.05) in the proximate composition of the two samples as when mixed with palm oil as preservative.

**5.2 Recommendation**

It could be recommended that the waste obtained from *Musa paradisiaca* and *Musa sapientum* can be useful as organic or natural antifungal compare to the synthetic ones and that oil palm has the potential of influencing the nutritional content present in the Musa species peels. As such, the wastes from these two species of Musaceae should be a preferable alternative for natural antifungal.

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