DETERMINATION OF MINERAL AND PROXIMATE CONTENT OF BANANA MUSA SPP

By

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CERTIFICATION

This is to certify that this project was carried out by AHMED YAKUBU ABDULLAHI with matriculation number HND/23/SLT/FT/0771, submitted to the Department of Science Laboratory Technology, Chemistry Unit, Institute of Applied Science (IAS), Kwara State Polytechnic, Ilorin, in partial fulfillment for the requirement of the award of Higher National Diploma (HND) in Science Laboratory Technology (SLT).

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DEDICATION

This project is dedicated to Almighty God who alone grant wisdom, knowledge and inspiration and to my beloved parents.

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All praise is due to Almighty God the Lord of universe. I praise Him and thank Him for giving me the strength and knowledge to complete my HND programme and also for my continued existence on the earth.

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My special thanks goes to my parents, Alh. Ahmed Abdullahi Sabi and Hajia Rabi Barka and to my Uncle Alh. Bar. Umar Barka and also to my brothers, Comr. Iliyasu Ahmed Bala for their wonderfulness and support with love all through and to my champ Victor Daniel for his support and love God bless you all.

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ABSTRACT

This was designed to evaluate the phytochemical compositions of fruits of three Musa species at three stages of development. Spectrophotometric methods were used for the study. Results of the qualitative phytochemical assay of the pulp of the three Musa species harvested at different stages showed the presence of alkaloids, saponins, glycosides and flavonoids. Tannin was detected only in the ripe stage of plantain. Anthraquinones and phlobatannins were not detected in any of the samples at all the stages of development. The results of the quantitative phytochemical compositions of the three Musa species revealed that phenol content was highest in all the Musa species obtained at different levels of development followed by alkaloids. The quantity of tannins was observed to be high at the immature stages of development of the three Musa species. The quantity of each phytochemical in the different species was observed to have increased as fruit develops from immature to ripe stages. The results of the phytochemical compositions of the three Musa species at ripe stage show that the pulp of Musa species at the ripe stage contained phenols and saponins in abundance. Alkaloids and flavonoids were present in moderate quantities while tannin was absent in banana but present in plantain and saba banana, with a higher quantity in plantain. Phytochemical test is useful in the detection of bioactive principles and subsequently may lead to discoveries and development of the active ingredients so that they can be prevented from losing their potency. Most of these phytochemicals are present in the fruits at a concentration that may attract commercial exploitation.

CHAPTER ONE

1.1 INTRODUCTION

Banana is the common name for moncorapic flowering plants of the genus Musa, for the species Ensete Ventricoum, and for the fruit they produce. Bananas do not grown on trees the banana plant in classified as an arbores cent (tree-like) perennial herb and the banana itself is actually considered a berry. The correct name for bunch of banana is a hand of banana a single banana is a finger. One banana contains 467mg of potassium providing powerful protection to the cardiovascular system. Regular consumption of the potassium packed fruit helps gauds against high blood pressure, atherosclerosis and stroke. Although banana do not contain high amounts of calcium they do supply the body with an abundance of fructooligosucchairde a probiotic substance (one which encourages probioties, the friendly bacteria in the digestive system). As fructooligosaccharides a probiotic substance (one which encourages probiotics, the friendly bacteria in the digestive system).As fructooligosaccharidesferment in the digestive trace, they enhance the body's ability to abort calcium.

Banana fruit is one of the high calorie tropical fruits. 100g of fruit provides 90 calories. Besides, it contains goods amounts of health benefiting anti-oxidants, minerals and vitamins. Banana pulp is composed of soft, easily digestible flesh with simple sugars like fructose and sucrose that when eaten replenishes energy and banana are being used by athletes to get intend energy and as supplement food in the treatment plan for underweight children. The fruit contains a good amount of soluble dietary fiber (7% of

DRA per 100g) that helps normal bowel movements: there by reducing constipation problems.

It contains health promoting flavonoid poly-phenolic antioxidants such as lutein, in small amounts. These compound help act as protective scavengers against oxygen-drived free radicals and reactive oxygen species (Ros) that play a role in aging and various disease processes.

It is also a very good source of vitamin- B6 (phridoxine) provides about 28% of daily-recommended allowance. Phridoxine is an important B-complex vitamin that has a beneficial role for the treatment of neuritis and anemia. Banana is also a source of vitamin C. (about 8.7mg per 100g). consumption of foods rich in vitamin C. helps the body develop resistance against infections agents and scavenge harmful oxygen-free radicals. Fresh bananas provide adequate levels of minerals like copper, magnesium and manganese, are essential for bone strengthening and have a cardiac-protective role as well. Manganese is used by the body as a co-factor for the antioxidant enzyme, super oxide dismatase. Fortin, (2006)

1.2 STATEMENT OF PROBLEM

Despite the availability of banana in Nigeria and other parts of the world little information is available on the proximate, mineral, vitamin and photochemical composition of the banana pulp. This lead to the study the nutritional value of banana.

1.3 SIGNIFICANT OF THE STUDY

The study will make to know the nutritional value of banana. It will also help to know the healthy effect of the medicinal value of banana. Also to know if banana is toxic to the body at contain level of consumption.

1.4 AIM

The aim of this research work is to determine composition of fruits of three Musa Species at three stages of development

1.5 OBJECTIVE OF THE STUDY

The objective of the study are:

i. To collect and prepare the sample for analysis

ii. To determine the phytochemical content present

CHAPTER TWO

2.1 LITERATURE REVIEW

The use of traditional medicines in West Africa is probably as old as the duration of human settlement in the region (Abdul-aguye, 1997). A medicinal plant provides an important source of new chemical substances with potential therapeutic effects. These have been used in traditional medicine for the treatment of several diseases and aliments (Mukerjee et al., 1998). It is already important to the global economy with demand steadily increasing not only in developing countries but also in industrialized countries (Sofowara, 1993).

Herbalism or herbal medicine is the use of plants for medicinal purposes, and the study of such use (Briskin, 2000). Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care (Kamboj, 2000). Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today (Briskin, 2000). This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available (Gupta and Raina, 1998). Modern medicine recognizes herbalism as a form of alternative medicine as the practice of herbalism is not strictly based on evidence gathered using the scientific method (Talalay, 2001). According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (Evans, 1994). The use of plants for healing purposes predates human history and forms the origin

of much modern medicine. Modern medicine, does, however, make use of many plant-derived compounds as the basis for evidence-tested pharmaceutical drugs, and phytotherapy works to apply modern standards of effectiveness testing to herbs and medicines that are derived from natural sources (Talalay, 2001). Examples include aspirin (willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy) (Vickers and Zollman, 1999). Currently, a number of medicinal plants with antidiarrhoeal and antimicrobial properties are used in traditional herbal practice in many countries of the world. So it is important to identify and evaluate commonly available natural drugs that could be used against any type of diarrhoeal disease.

A number of herbs are thought to likely have adverse effects (Talay, 2001). Furthermore, "adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions have led to adverse reactions that are sometimes life threatening or lethal (Elvin-Lewis, 2001). Proper double-blind clinical trials are needed to determine the safety and efficacy of each plant before they can be recommended for medical use (Vickers, 2007). Although many consumers believe that herbal medicines are safe because they are "natural", herbal medicines and synthetic drugs may interact, causing toxicity to the patient. Herbal remedies can also be dangerously contaminated, and herbal medicines without established efficacy, may unknowingly be used to replace medicines that do have corroborated efficacy (Ernst, 2007). The World Health Organization (WHO), the specialized agency of the United Nations (UN) that is concerned with

international public health, published quality control methods for medicinal plant materials in 1998 in order to support WHO Member States in establishing quality standards and specifications for herbal materials, within the overall context of quality assurance and control of herbal medicines (WHO, 2010).

There are different methods of herbal preparations and the exact composition of an herbal product is influenced by the method of extraction. They are: Tisanes or herbal teas; are the resultant liquid of extracting herbs into water (Green, 2000). The methods used are, infusions (hot water extracts of herbs), decoctions (long term boiled extracts usually of harder substances like roots and bark) and maceration (old infusion of plants with high mucilage content) (Green, 2000). Tinctures; alcoholic extracts of herbs generally stronger than tisanes (Green, 2000). Syrups; extracts of herbs made with syrups or honey (Green, 2000).

In developing countries, diarrhoea continues to be one of the leading causes of mortality and morbidity in children less than 5 years old. According to World Health Report, diarrhoea is the cause of 3.3% of all deaths. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in children. The incidence of diarrhoeal disease still remains high despite the effort by many government and international organizations to reduce it. Nigeria, the fourth largest economy in Africa with an estimated per capita income of $350 has over half of its population living in poverty (WHO, 2007). This implies that very few people can afford orthodox medicine in curing diseases. Use of traditional medicines to combat the consequences of diarrhoea has been emphasized

by WHO in its Diarrhoea Control Programme. It is therefore important to identify and evaluate available natural drugs as alternatives to current antidiarrhoeal drugs, which are not always free from adverse effects. Several studies have shown the beneficial effects of a number of medicinal plants used traditionally in the treatment of diarrhoeal disease, one of such being Bombax buonopozense (Akudor et al., 2011), Vitex doniana (Ukwuani et al., 2012), Anacardium occidentale (Omoboyowa et al., 2013) etc.

Musa paradisiacae belongs to the Musaceae family and is cultivated in many tropics and subtropical countries of the world. It ranks third after yams and cassava for sustainability in Nigeria (Akomolafe and Aborisade, 2007). Musa paradisiacae is a rhizomatous perennial crop used as a source of starchy staple for millions of people in Nigeria (Adeniyi et al., 2006).Unripe Musa paradisiacae, which is the green plantain contains more starch than the ripe plantain in which the starch is converted to sugars (glucose, fructose and sucrose). It has been indicated to posses antidiabetic (Eleazu et al., 2013), antioxidant (Shodehinde and Oboh, 2012), antimicrobial (Hossain et al., 2011), and antiulcerogenic properties (Ralph et al., 1984). There have also been traditional claims that unripe Musa paradisiacae can be used in diarrhoeal treatments even though it has not been scientifically proven.

2.1.1 Musa paradisiacae

Fig. 1: Musa paradisiacae fruit (Gibert et al., 2009).

Fig. 2: Musa paradisiacae trees (Gibert et al., 2009).

Fig. 3: Musa paradisiacae flower (Gibert et al., 2009).

2.2 Taxonomy of Musa paradisiacae

Kingdom- Plantae

Division – Spermatophyta

Sub-division – Angiospermae

Phylum – Tracheophyta

Class – Liliopsida

Order – Zingiberales

Family – Musceae

Genus – Musa

Species – Paradisiacae

2.3 Common names of Musa paradisiacae

Musa paradisiacae is commonly known as plantain. Among the Igbos of Nigeria, it is known as “ogede or abrika”, in Yoruba as “ogede agbagba”, in “Igala as agbo”, and in Hausa as “agada or afutu”.

2.4 Origin of Musa paradisiacae

Bananas and plantains belong to the genus Musa. It was Linnaeus that first gave the scientific name Musa sapientum for all sweet bananas, and the scientific name Musa paradisiacae for plantains (Simmonds, 1962). However, Linnaeus did not know that the two species he had described were in fact hybrids and not two distinct species (Zeller, 2005). Therefore, those two names could not be relevant in modern taxonomy.

Genetic studies have then demonstrated that all edible bananas and plantains come from a common ancestor, Musa acuminata. Plantains also carry genes from another ancestor, Musa balbisiana (Lejju et al., 2005). The genome of each ancestor could be represented respectively by the letter A and B. Then, further studies showed that edible bananas are mostly triploids and their genome would be described as AAA. This means that they carry three sets of chromosomes derived from M. acuminate (Simmonds, 1962). Different hybrid combinations have been observed, such as AAB, BBB, and tetraploid groups (AAAA) were also described.

Therefore, an accurate classification for bananas seems to be a great challenge. However, one thing sure in that banana taxonomists seem to agree that there is no single scientific name that can be attributed to all edible bananas (Zeller, 2005; Solofo and Ellis, 2009).

Therefore, a new type of classification was proposed by Simmonds and that would abandon the Latin name to use instead a group indication like this: genus (Musa) + genome group (e.g. AAA) + subgroup name (e.g. Cavendish subgroup "Grand Nain"). In Panama, the sweet bananas come mostly from the Cavendish subgroup. The plantain subgroup is also triploid but has the genome group AAB (Simmonds, 1962).

2.5 Description of Musa paradisiacae plant

The common Musa paradisiacae has broad, irregular oval leaves, abruptly contracted at the base into a long broad, channelled footstalk. The fully grown blade is 1.3–2.4 meters long and about two third as broad, usually smooth, with several parallel veins. It is wind pollinated and propagates primarily by seeds which are held on the long narrow spikes which rise well above the foliage (Zeller, 2005).

Musa x paradisiaca (M. acuminata x M. balbisiana) is a sterile (without seeds or viable pollen) triploid (2n=3x=33 chromosomes) that is cultivated in warm climates for its tasty yellow-skinned fruit (Nelson et al., 2006). This is a large, fast-growing, suckering, herbaceous perennial that produces huge oblong to paddle-shaped leaves that grow to as much as 8' long with leaf sheaths overlapping to help form a trunk-like pseudo stem (false stem). The pseudostem can reach up to 2-9 m tall and with short underground stem (corm) with buds, from which short rhizomes grow to produce a clump of aerial shoots (suckers) close to the parent plant. The roots are adventitious, spreading 4-5 m laterally, descending to 75cm long, but mainly in the top of 15cm and form a dense mat. It develops from the underground rhizome (Gibert, 2009).

At maturity, the rhizome gives rise to flower (inflorescence) that is carried up along a smooth elongated unbranched stem piercing through the centre of the pseudo-stem, finally emerging out at the top in between the leaf cluster. Yellow flowers with purple-red bracts appear in summer on mature plants. The flower subsequently develops to plantain bunch consisting of 3 to 20 hands each with at least 5-10 fingers (fruits) (Zeller, 2005). The plant is also monocarpic, which means that a shoot can only flower once and will die after the fruit is produced. The leaf crown will be oriented downward due to gravity.

Raw green fruits are only eaten after cooking. Each fruit measures about 3 to 10 inches or more in length depending on the cultivar type. They tend to have coarse external features with prominent edges and flat surfaces. The flesh inside is starch rich with tiny edible black seeds concentrated at its core. Ripening process however enhances flavor and sweetness since the starch converts to sugar (glucose, fructose and sucrose) (Phebe et al., 2007). The genus honors Antonia Musa, Roman physician of the 1st century B.C.

No serious insect or disease problems. In some cases, insects like aphids, mealy bugs, moths, scale, thrips, fruit flies and spider mites may attack the plant. Susceptible to anthracnose, wilt and mosaic virus (Scott et al., 1970).

2.6 Distribution of Musa Paradisiacae

The plant is widely distributed throughout the tropical regions of Southeast Asia and western Pacific regions.

It is native to Southeast Asia, India and Burma through the Malay Archipelago to New Guinea, America, Australia, Samona, and tropical Africa (Ahmad et al., 2006). However, the cultivation is limited to Florida, the Canary Islands, Egypt, Southern Japan, and South Brazil. The top leaders exporting countries of plantain are Ecuador, Colombia, Costa Rica, Guatemala and Honduras. Panama occupies the 6th position. The large diversity that occurred in plantain has resulted in a variety of cultivars (Scott et al., 1970).

The number of Musa paradisiacae cultivated varieties (cultivars) has been reported to vary from one country to another. Swennen (1990) observed that at least 116 plantain cultivars exist in different parts of West and Central Africa. In Nigeria alone, more than 20 cultivars have been reported, although only a few are important commercially Swennen (1990). Musa paradisiacae is a major starch crop of importance in the human tropical zone of Africa, Asia, Central and South America. It is undoubtedly one of the oldest cultivated fruits in West and Central Africa. It is consumed as an energy yielding food and desert. It has been estimated that

Musa paradisiacae and other bananas provide nearly 60 million people in Africa with more than 200 calories (food energy) per day. Fruits such as Musa paradisiacae are an important contribution to the diets of many low and middle class people in many African settings (Stover and Simmonds, 1987). Bananas and plantains constitute the fourth most

important global food commodity (after rice, wheat and maize) grown in more than 100 countries over a harvested area of approximately 10 million hectares, with an annual production of 88 million tonnes (Frison and Sharrock, 1999). The all year round fruiting habit of Musa paradisiacae puts the crop in a superior position in bridging the 'hunger gap' between crop harvests. It therefore contributes significantly to food and income security of people engaged in its production and trade, particularly in developing countries. Musa paradisiacae is an important staple crop, supplying up to 25% of the carbohydrates for approximately 70 million people in the humid zone of subSaharan Africa. (IITA, 1998).

2.7 Cultivation and storage of Musa paradisiacae

Musa paradisiacae is grown in 52 countries with world production of 33 million metric tonnes (FAO, 2005). It grows more than any other plant in compacted soils, is abundant beside paths, roadside and other areas with frequent soil compaction. It is also common in grassland and as a weed among crops. Musa paradisiacae originated in the humid tropics and performs best under warm (27-30ºC) and very wet (200-220mm per month) conditions. The musa cultivars can stand warmer and drier climates (Gibert, 2009). The best soils are deep, friable loam with a good drainage and aeration. High soil fertility and organic matter content are desirable. The crop tolerates PH values of 4.5-7.5. It is sensitive to typhoons which cause blowdowns. A major problem of Musa paradisiacae is that the fruits are highly perishable (Scott et al., 1971). The most important physiological function affecting product quality during storage is respiration and transpiration. To

extend storage life, these functions should be reduced. This can be done by controlling temperature, humidity, ventilation, and atmospheric composition during storage (Scott and Gandanegara, 1974).

2.8 Historical uses of Musa paradisiacae

Every part of Musa paradisiacae including root system is used widely in various treatments. The fruit of unripe Musa paradisiacae is traditionally used in the treatment of diarrhoea, dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), in sprue, uraemia, nephritis, gout, hypertension, cardiac disease (Mwangi et al., 2007).

Unripe bananas and plantain fruits are astringent, and used to treat diarrhoea. The leaves are used for cough and bronchitis. The roots can arrest haemoptysis and posses strongly astringent, and antihelmintic properties. Plantain juice is used as an antidote for snakebite. Other uses are asthma, burns, diabetes, dysentery, excessive menstrual flow, fever, gangrene, gout, headache, haemorrhage, inflammation, insomnia, intestinal parasites, sores, syphilis, tuberculosis, ulcers, and warts (Coe and Anderson, 1999). In Suriname's traditional medicine, the red protecting leaves of the bud was used against heavy menstrual bleeding (menorrhagia).

Other therapeutic uses were against dysentery, migraine, hypertension, asthma and jaundice.

Indeed, they are very reliable sources of starch and energy ensuring food security for millions of households worldwide (Swennen, 1990).

It contains dietary fibre. Adequate amount of Dietary-fibre in the food helps normal bowel movements, thereby reducing constipation problems.

Musa paradisiacae is rich in vitamin C. Consumption of foods rich in vitamin-C helps the body develop resistance against infectious agents and scavenge harmful oxygen-free radicals.

Musa paradisiacae contains enough of vitamin A. In addition to being a powerful antioxidant, vitamin A plays a vital role in the visual cycle, maintaining healthy mucus membranes, and enhancing skin complexion.

As in bananas, they too are rich sources of B-complex vitamins, particularly high in vitamin-B6 (pyridoxine). Pyridoxine is an important B-complex vitamin that has a beneficial role in the treatment of neuritis, anaemia, and to decrease homocystine (one of the causative factors for coronary artery disease (CHD) and stroke episodes) levels in the body. In addition, the fruit contains moderate levels of folates, niacin, riboflavin and thiamine (Ogazi, 1996).

They also provide adequate levels of minerals such as iron, magnesium, and phosphorous. Magnesium is essential for bone strengthening and has a cardiac-protective role as well.

Musa paradisiacae are also rich in potassium. Potassium is an important component of cell and body fluids that helps control heart rate and blood pressure, countering negative effects of sodium (Ogazi, 1996).

CHAPTER THREE

3.1.1 Sources of Materials

Fresh plantain, banana and saba banana fruits used in this work were supplied through special arrangements with plantation farmers at Ilorin town in Kwara State Nigeria. The three *Musa* species used were *Musa paradisiaca* L, *Musa sapientum* L *and Musa saba* L. The species were identified and authenticated accordingly by a plant taxonomist of the Department of Botany. University Ilorin.

The fruits were collected fresh and used immediately in the analyses. The collection of the samples in these analyses was based on the rate of their development as recommended by [24]. Immature, green mature and ripe fruits were collected for the analyses (Plate 1^{a-c}, 2^{a-c} and 3^{a-c}). Fruits at each these stages of development were aged 30 – 45 days following fruit set for immature; 70 – 90 days of fruit set for green mature: while the ripe stage were those whose peels were showing 50% or more visible xanthophylls exposures or yellowing.

3.1.2 Sample Preparation

The samples were thoroughly washed under running water and the back removed exposing the pulp which was homogenized using a Kenwood warring blender and kept in the refrigerator until required for analysis.

3.2 Qualitative Phytochemical Analysis

The qualitative methods already established by [25 and 26], were used. The substances that were tested for included; alkaloids, Tannins, terpenoids, glycosides, flavonoids, Saponins and phenols.

3.2.1 Test for Alkaloids

The Mayer's, Dragendroff's, Wagner's, and Picric acid tests were used to test for alkaloids. One gram of the plant material was boiled for almost 2 minutes with 5ml of 2% hydrochloric acid in a steam bath and the material filtered. A volume 1ml portion of the filtrate was treated with 2 drops of the following reagents and for precipitate.

(a) Mayer's reagent (potassium mercuric iodide solution)

(b) Dragendroffs reagent (bismuth potassium iodide solution)

(c) Wagner's reagent (iodide in potassium iodide solution)

(d) 1% picric acid solution.

Turbidity or precipitate with either of the reagents was taken as evidence for the presence of alkaloids.

3.2.2 Test for Saponins

The Emulsion test and froth tests were used. The ability of saponins to produce emulsion with oil was used for the screening test. Twenty milligrams of sample was boiled in 20 ml of distilled water in a water bath for five minutes and filtered. Ten ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth

formation. Three drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development.

3.2.3 Test for Glycosides

Few drops of ferric chloride and concentrated sulphuric acid were added to a solution of the plant extract in glacial acetic acid. A reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer indicated the presence of glycosides [27].

3.2.4 Test for Anthraquinones

Two hundred milligrams of the samples each was boiled with 6 ml of 1% HCl and filtered. The filtrate was shaken with 5 ml of benzene, filtered and 2 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, violet or red colour in the ammoniacal phase indicated the presence of free hydroxyl anthraquinones.

3.2.5 Test for Phlobatannins

Eighty milligrams of the samples each was boiled in 1% aqueous hydrochloric acid; the deposition of a red precipitate indicated the presence of phlobatannins.

3.2.6 Test for Flavonoids

Fifty milligrams of the samples each was suspended in 100 ml of distilled water to get the filtrate. Five milliliters of dilute ammonia solution was added to 10 ml of filtrate followed by few drops of concentrated H_2SO_4. Presence of flavonoids was confirmed by yellow colouration.

3.2.7 Test for Tannins

The test for tannin was carried out using the method described by [23]. 50 mg of the samples each was boiled in 20 ml of distilled water and filtered. A few drops of 0.1% $FeCl_3$ was added in filtrate and observed for colour change; brownish green or a blue-black colouration was taken as evidence for the presence of tannins.

3.3 Quantitative Phytochemical Analysis

3.3.1 Saponin determination

Saponin was determined using the method as described by [28]. Twenty grams of the sample was put into a conical flask and 100ml of 20% aqueous ethanol was added. The Sample was heated over a hot water bath for 4hrs with continuous stirring at about55^{o}C. The mixture was filtered and residue was again extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^{0}C. The Concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer (upper layer) was discarded and the purification process repeated. 60ml of n- butanol was added (discard the bottom and recover the upper layer). The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in water bath. After evaporation, the samples were dried in the oven to dryness, a constant weight; the saponin content was calculated as percentage weight. The experiment was repeated on each of the samples.

% SAPONIN CALCULATION

%saponin = (W_2-W_1) / (weight of sample) X 100/1.

W_2 =weight of crucible + Sample after oven drying.

W_1 = weight of empty crucible

Weight of sample = 10g

3.3.2 Determination of Total Phenol

The phenolic content in the samples was estimated by Folin-Ciocalteu's colorimetric method described by [29], with some modifications. About 0.5 ml of each sample extract was mixed with 2.5 ml of distilled water. To this, 0.5 ml of Folin – Ciocalteu reagent (1:1) was added and incubated for 3 minutes.

To each tube, 2 ml of 20% Na_2CO_3 solution was added and the tubes were kept in boiling water bath for 1 minute. Tubes were cooled and the absorbance of reaction mixture was read at 650 nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/ml).

Total phenolic content was expressed w/w and calculated

using the formula Total phenolic content

(% w/w) = (GAE x V x D x 10^{-6} x 100) / W

GAE = Gallic Acid Equivalent (µg/ml)

V= Total Volume of

Sample (ml)

D = Dilution Factor

W = Weight of sample (g)

3.3.3 Tannin Determination

Tannin was determined using the method as described by [30].Half a gram (0.5) of the sample was weighed into 250ml conical flask and 50ml of distilled water added and then shaken on a rotating shaker (magnetic stirrer) for 1hr. The mixture was filtered into a 50ml volumetric flask. 5ml of the filtrate was pipetted into 50ml volumetric flask.(5ml of the filtrate was pipetted out into the test tube mixed with 2ml of 0.1M $Fecl_3$ in 0.1N HCl and 0.008M $K_4Fe(CN)_6$ (Potassuim ferrocyanide). The absorbance was measured at 120nm within 10min) For the standard solution, 0.1g of tannic acid was dissolved in 100ml of water to form tannic acid solution. From the tannic acid solution 5ml was pipetted into another 50ml volumetric flask. A blank sample was prepared using 5ml distilled water.

The three prepared solutions were put in an incubator for 1hr 30mins at 20-30^{o}C. After which the solutions were made up to the 50ml mark and absorbance of the solutions at 760nm were measured using Spectrophotometer.

Calculation:

Let absorbance of 5ml of extract be X

Let absorbance of tannic acid

solution be Y Let absorbance

of Blank be Z

Tannin in mg/100 = (X-Z)/(Y-Z).

3.3.4 Determination of Flavonoid Content

Total flavonoid content was determined following a method by [31]. In a 10 ml test tube, 0.3 ml of extract, 3.4 ml of 30% methanol, 0.15 ml of $NaNO_2$ (0.5 M) and 0.15 ml of $AlCl_3.6H_2O$ (0.3 M) were mixed.

After 5 min, 1 ml of NaOH (1 M) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made using rutin standard solution (0 to 100 mg/l) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of rutin equivalents per gram of dried fraction.

CALCULATION

Flavonoidcontent=(RExVxD10^{-}

^{6}x100)/W

RE = Rutin Equivalent (µg/ml)

V = Total volume of sample (ml)

D = Dilution factor

3.3.5 Determination of Alkaloids

Five grams of the plant sample was weighed into a 250ml beaker. 200ml of 10% HCL in ethanol was added and covered and allowed to stand for 4hrs.It was filtered and the extract concentrated on a water bath to one quarter of the original volume. Pigments and other unwanted materials were removed by shaking it with 100ml Chloroform in

separating funnel. Distilled water was added. The lower layer was collected and excess ammonia added to precipitate the free alkaloid.

The whole solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was later evaporated into dryness in an oven and weighed to a constant weight.

Calculation:

% Alkaloids = (W_2-W_1) / (weight of sample)

X 100/1. W_2 =weight of filter paper + Residue after oven drying. W_1 = weight of filter paper

Weight of sample = 5g.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results of Qualitative Phytochemical Compositions of Fruits of Three *Musa* Species at Three Stages of Development

Results of the qualitative phytochemical assay of the pulp of the three *Musa* species harvested at different stages showed the presence of alkaloids, saponins, glycosides and flavonoid. Tannin was detected only in the ripe stage of plantain. Anthraquinones and phlobatannins were not detected in any of the samples at all the stages of development (Table 1)

Table 1: Qualitative Phytochemical Compositions of Fruits of Three *Musa* Species at Three Stages of Development

	Banana			**Plantain**			**Saba Banana**		
	R	G M	I M	R	GM	I M	R	GM	I M
Tannin	-	-	-	+	-	-	-	-	-
Flavonoid	+	+	+	+	+	+	-	-	-
Saponin	+	+	+	+	+	+	+	+	+
Gylcoside	+	+	+	+	+	+	+	+	+
Anthroquinone	-	-	-	-	-	-	-	-	-
Phlobatanin	-	-	-	-	-	-	-	-	-
Alkaloid	+	+	+	+	+	+	+	+	+

+ =detected -= Not detected

R= Ripe stage GM= Green mature stage IM= Immature stage

4.2 Results of Quantitative Phytochemical Compositions of Fruits of Three *Musa* Species at Three Stages of Development

The results of the quantitative phytochemical compositions of the three *Musa* species showed that phenol content was highest in all the *Musa* species obtained at different levels of development followed by alkaloids. The quantity of tannins was observed to be high at the immature stages of development of the three *Musa* species. The quantity of each phytochemical in the different species was observed to have increased as fruit develops from immature to ripe stages (Table 4.5). Differences in Alkaloid content among the stages of development with respect to each fruit species were significant by Duncan's multiple range tests. Prominently, the saponin constituent of saba banana and banana at ripe stages were high, the difference between them were significant using Duncans multiple range tests.

Table 2: Quantitative Phytochemical Compositions of fruits of Three *Musa* Species at Three Stages of Development

Phytochemical components		**Phytochemical Compositions %**		
		Banana	Plantain	Saba Banana
Alkaloid	Immature	0.251 ± 0.003^{f}	0.643 ± 0.003^{e}	0.860 ± 0.005^{b}
	Green mature	0.770 ± 0.002^{cd}	0.187 ± 0.001^{f}	0.736 ± 0.004^{d}
	Ripe	0.778 ± 0.006^{bc}	1.027 ± 0.003^{a}	0.083 ±0.001g
Saponin	Immature	0.145 ± 0.005^{i}	0.773 ± 0.003^{f}	0.365 ± 0.000^{j}
	Green mature	1.175 ± 0.005^{c}	0.858 ± 0.003^{e}	0.179 ± 0.000^{h}
	Ripe	2.268 ± 0.003^{b}	0.973 ± 0.033^{d}	2.573 ± 0.003^{a}
Phenol	Immature	3.115 ± 0.005^{c}	2.498 ± 0.003^{f}	2.289 ± 0.001^{h}
	Green mature	3.310 ± 0.010^{b}	2.448 ± 0.003^{g}	3.392 ± 0.004^{a}
	Ripe	2.545 ± 0.005^{e}	2.697 ± 0.003^{d}	2.241 ± 0.016^{i}
Tannin	Immature	2.180 ± 0.462^{a}	ND	1.223 ± 0.049^{b}
	Green mature	1.138 ± 0.151^{c}	ND	ND
	Ripe	ND	2.360 ± 0.215^{a}	1.009 ± 0.151^{c}
Flavonoid	Immature	0.468 ± 0.002^{b}	0.114 ± 0.001^{g}	0.247 ± 0.004^{f}
	Green mature	0.444 ± 0.006^{c}	1.113 ± 0.001^{a}	0.330 ± 0.002^{d}
	Ripe	$0.071 \pm 0\ 000^{h}$	0.467 ± 0.001^{b}	0.292 ± 0.003^{e}

Results are in Means ± Standard Error. Means ± Standard Error followed by the same letter(s) in a column are not significant

4.3 Results of Phytochemical compositions of three *Musa* species at Ripe Stage of Development

The results of the phytochemical compositions of the three *Musa* species at ripe stage show that the pulp of *Musa* species at the ripe stage contained phenols and saponin in abundance. Alkaloids and flavonoids,were present in moderate quantities while tannin was absent in banana but present in plantain and saba banana, with a higher quantity in plantain (Fig 1).

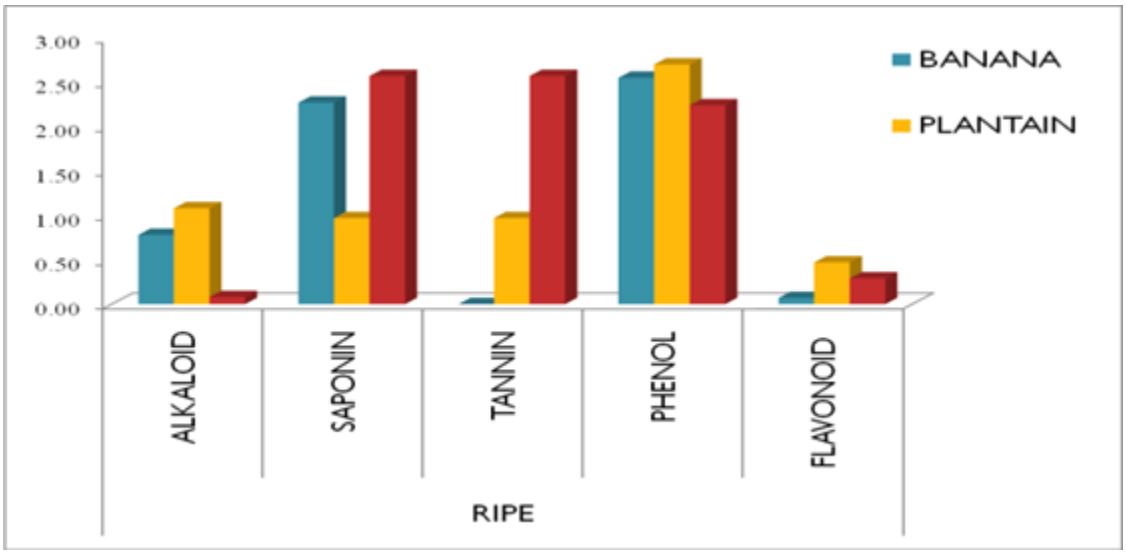

Fig 1: Phytochemical compositions of three *Musa* species at ripe stage of development

4.4 Results of Phytochemical compositions of three *Musa* species at green Mature Stage of Development

The results of the comparative phytochemical assay of the three *Musa* species showed that at the green mature stage, the phytochemicals present include phenols

which were present in abundance. Flavonoid content of plantain was higher than in banana and saba banana. Alkaloids and flavonoids were present in moderate quantities while Tannin contents of the samples increased in banana and saba banana but was absent in plantain (Fig 2).

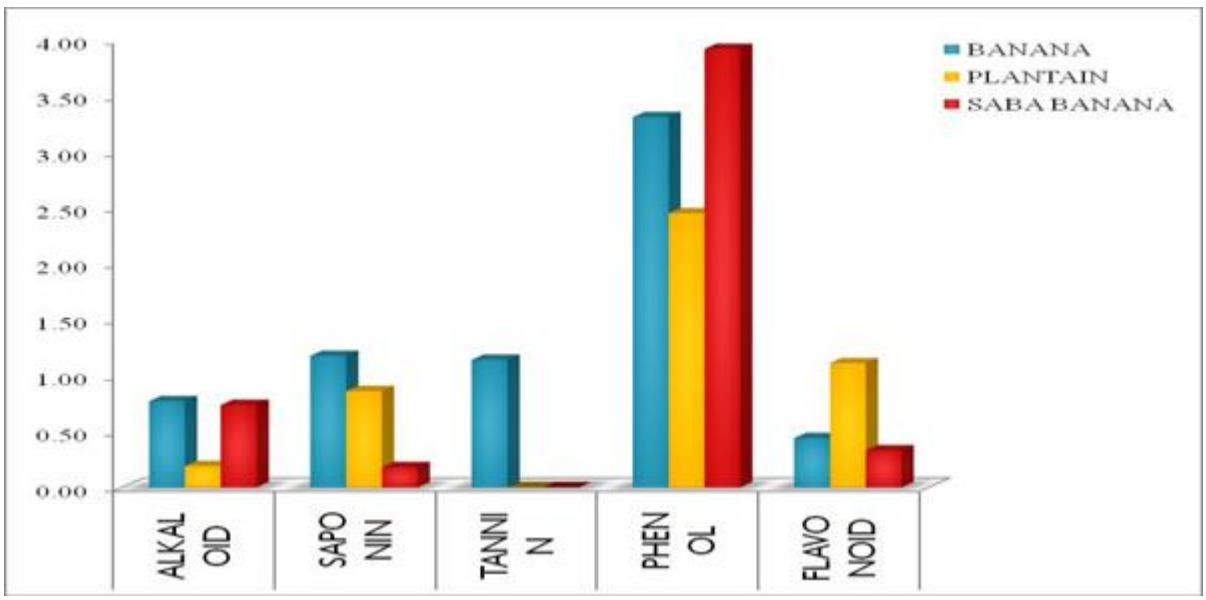

Fig 2 Phytochemical compositions of three *Musa* species at green Mature Stage of Development

Results of Phytochemical compositions of three *Musa* species at Immature Stage of Development

At immature stages, alkaloid, phenol and glycoside contents of saba banana were higher than as compared to banana and plantain (Fig 3). Tannin was not detected in saba banana and plantain. There was an increase in the tannin content of banana than as it was at the green mature stage (Table 2)

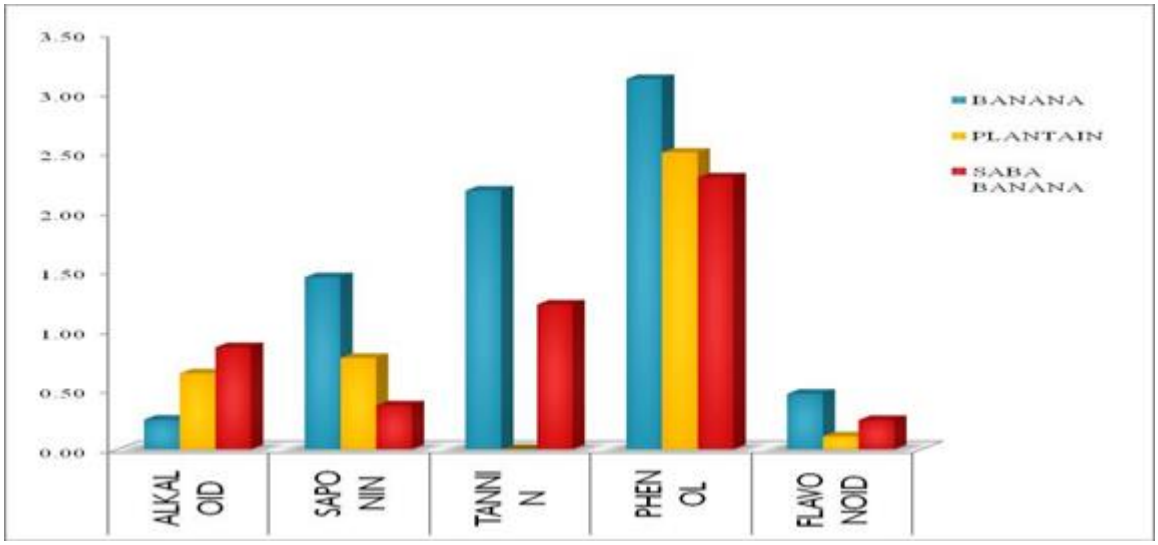

Fig 3: Phytochemical compositions of three *Musa* species at Immature Stage of Development

The Phytochemical assay revealed the presence of some phytochemicals like phenols, tannins, alkaloids, saponins, flavonoids and glycosides at varying concentrations. The presence of these phytochemicals confirms the three *Musa* species tested to be of medicinal value. This agrees with so many reports in the literature on their medicinal uses [32, 33 and 34]. Phytochemicals are non nutritive plant chemicals that have protective or disease preventive properties. Phenols in the three *Musa* species revealed no significant difference between them but rather between their stages of development. The phenol contents of the fruits were higher at the immature and green mature stages than at the ripe stages. This implies that the fruits at the immature and green mature stage are rich source of antioxidants because studies have shown that antioxidants capacity of plants is tightly correlated with phenol compounds [35 and 36]. Phenol is a major active ingredient in antiseptics and disinfectants due to their

antimicrobial activity. Phenols are also strong antioxidants that prevent oxidative diseases such as cancer and cardiovascular diseases. The plant phenols may interfere with all stages of the cancer processes, potentially resulting in a reduction of cancer risk [37]. The phenol compounds present in plants are also responsible for their contribution to colour, sensory and antioxidant properties of food [38 and 16]. There was no significant difference in flavonoids contents between the three different species but rather a slight difference was observed in the stages of development. Flavonoids are naturally occurring phenols. They are thought to have positive effects on human health. Studies have shown that flavonoids possess antibacterial, antiviral, anti-inflammatory, anticancer and anti allergic abilities [39 and 40]. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules. It has the ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals.. The decreasing trends in scavenging activity of plants with respect to crop maturity were attributed to phenols reduction at maturity.

Alkaloids content between the three species revealed no significant difference. Alkaloids are the most therapeutic plant substance. They are used as basic medicinal agents because of their analgesic, antiplasmodic and antibacterial properties

Alkaloids are nitrogen containing naturally occurring compounds found to have antimicrobial properties due to their ability to intercolate with DNA of the micro organisms . Significant difference was only observed at the stages of development. The alkaloid content of the fruits increased with ripening or development.

The quantitative analysis revealed the presence of tannin at some stages. Tannin was observed at the immature stages of banana (2.180 ± 0.462) and saba banana (1.223 ± 0.049). At the green mature stages tannin was observed in banana (1.138 ± 1.151) but was not detected in plantain and Saba banana respectively. Although it was observed in the ripe stages of plantain and Saba banana it wasn't observed at the ripe stage of banana.

The presence of tannin shows that the fruit has astringent properties, quicken the healing of wounds and inflamed mucous membrane. Tannins can also be effective in curbing hemorrhages as well as restrict swelling. They are also beneficial as mouthwashes, eyewashes, snuff and even as vaginal douches. Long term use of tannin containing plants is not recommended as tannin when applied internally, sour the mucus secretions, contract the membranes in such a manner that secretions from the cells are restricted. The quantity of tannins observed revealed no significant difference between the fruits of the three species of *Musa*.

CHAPTER FIVE

5.1 Conclusion

Phytochemical test is useful in the detection of bioactive principles and subsequently may lead to discoveries and development of the active ingredients so that they can be prevented from losing their potency and in general, most of these phytochemicals are present in the fruits at a concentration that may attract commercial exploitation.

REFERENCES

Abdon IC, del Rosario IF. Food composion tables recommended for use in the Philippines. Hand book1 (5th revision). Food and Nutrition Research Institute, National Science Development Board, Manila, Philippines, 1980.

Amorim EP, Vilarinhos AD, Cohen KO, Amorim VBO, Santos-Serejo JAD, Silva SO et al. Genetic diversity of carotenoid-rich bananas evaluated by Diversity Arrays Technology (DArT). Genetics and Molecular Biology. 2009; 32:96-103.

Aurore G, Parfaitb B, Fahrasmaneb L. Bananas, raw materials for making processed food products. Trends in Food Science & Technology. 2009; 20:78-91.

Coyne T, Ibiebele TI, Baade PD, Dobson A, McClintock C, Dunn S et al. Diabetes mellitus and serum carotenoids: findings of a population-based study in Queensland, Australia. American Journal of Clinical Nutrition. 2005; 82(3):685-693.

Doymaz I. Evaluation of mathematical models for prediction of thin-layer drying of banana slices.

Englberger L, Aalbersberg W, Ravi P, Bonnin E, Marks

Englberger L, Lyons G, Foley W, Daniells J, Aalbersberg B, Dolodolotawake U et al. Carotenoid and riboflavin content of banana cultivars from Makira, Solomon Islands. Journal of Food Composition and Analysis. 2010; 23:624-632.

Englberger L, Schierle J, Aalbersberg W, Hofmann P, Humphries J, Huang A et al. Carotenoid and vitamin content of Karat and other Micronesian banana cultivars. International Journal of Food Sciences and Nutrition, 2006; 57(5, 6):399-418.

Fungo R, Kikafunda JK, Pillay M. ß-carotene, iron and zinc content in Papua New Guinea and East African highland bananas. African Journal of Food Agriculture, Nutrition and Development. 2010; 10:2629-2644.

Fungo R, Kikafunda JK, Pillay M. Variation of ßcarotene, iron and zinc in bananas grown in east Africa. African Crop Science Conference Proceedings. 2007; 8:2117-2126.

Fungo R, Pillay M. ß-Carotene content of selected banana genotypes from Uganda. African Journal of Biotechnology. 2011; 10:5423-5430.

Ganapathi TR, Suprasanna PS, Bapat VA, Kulkarni VM, Rao PS. Somatic embryogenesis and plant regeneration from male flower buds of banana. Current Science. 1999; 76:1128-1231.

GC, Fitzgerald MH et al. Further analyses on

Gerard NN, Claudie DM, Juan RG, Kodjo T, Elie F, François XE. Carotenoid contents during ripening of banana hybrids and cultivars grown in Cameroon. Fruits. 2009; 64(4):197-206.

Holden JM, Eldridge AL, Beecher GR, Buzzard IM, Bhagwat S, Davis CS et al. Carotenoid content of U.S. foods: an update of the database. Journal of Food Composition and Analysis. 1999; 12:169-96.

International Journal of Food Properties. 2010; 13(3):486-497.

McLaren DS, Frigg M. Sight and Life Manual on Vitamin A Deficiency Disorders (VADD), 2nd ed. Task Force Sight and Life, Basel, Switzerland, 2001.

Micronesian banana, taro, breadfruit and other foods for provitamin A carotenoids and minerals. Journal of Food Composition and Analysis. 2003; 16:219-236.

Newilah GN, Lusty C, Van den Bergh I, Akyeampong E, Davey M, Tomekpe K. Evaluating bananas and plantains grown in Cameroon as a potential sources of carotenoids. Food. 2008; 2(2):135-138.

Offem JO, Njoku PC. Mineral distribution in the fruits of the plantain plant (Musa paradisiaca) in relation to mode and degree of maturation. Food Chemistry. 1993; 48(1):63-68.

Onwuka GI, Onwuka ND. The effects of ripening on the functional properties of plantain and plantain based cake. International Journal of Food Properties. 2005; 8(2):34735