# Evaluation of Starch Properties, Phytochemical Composition and Antimicrobial Activities of Yam Bean (*Pachyrhizus erosus* L. Urban)

By

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## DECLARATION

STUDENT

I hereby declare this thesis is the outcome of my own original research and that it is neither in part or whole been presented for another certificate in this university or elsewhere.

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## ABSTRACT

Yam bean (*Pachyrhizus erosus* L. Urban) is an underutilized tropical legume with limited industrial uses. Hence the starch properties, phytoconstituents and antimicrobial activities of yam bean were investigated to identify potential applications for the crop. Physicochemical and functional properties of yam bean starch were assessed. Phytochemical composition of yam bean leaf, seed and root extracts were conducted qualitatively. Total phenolics of the extracts were quantified by the Prussian Blue assay. Antimicrobial activities of the extracts were tested against a panel of microorganisms using the broth macrodilution method. Starch extraction yield, moisture, ash, phosphorous, protein and lipid contents of the starch were 23.63 % (db), 12.47 %, 0.50 %, 0.06 %, 0.41 % and 0.69 % respectively. Amylose content was 23.25% and starch pH was 4.95. The starch had swelling power and solubility 15.23 g/g and 7.57 % respectively, paste clarity of 2.48 % transmittance and water binding capacity of 96.81 %. The starch paste had pasting temperature of 71.45 °C, peak viscosity of 563.50 BU, final viscosity of 550.50 BU, breakdown viscosity of 235.5 BU and setback viscosity of 146 BU. Phytochemical screening of yam bean leaf, seed and root extracts showed the presence of tannins, saponins, glycosides, steroids, terpenoids and flavonoids. The total phenolic content was higher in the root (52.30 mg GAE / g) than in seed (34.77 mg GAE / g) and leaf (17.49 mg GAE / g). All the extracts inhibited growth of all the test organisms. There were significant differences ($p \leq 0.05$) in the antimicrobial activity of the yam bean extracts with the leaf extract as the most active (MIC $\leq$ 5 mg/mL) of all. Based on its starch properties, yam bean may be used as thickener in food products requiring short processing time, reduced heat and in opaque

foods like mayonnaise, sauces, gravies and dressings. Unique phytoconsttituents and antimicrobial activity makes yam bean suitable as additive in functional foods for prevention and treatment of diseases and as natural food preservative.

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## DEDICATION

This work is dedicated to my family for their unflinching support, love and investment into my education.

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# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 Background

In Ghana, only a few crops are being exploited as source of food with even fewer as source of income (Aboagye *et al.,* 2007). Overreliance on a shrinking food basket poses a great risk of increase in intensity and frequency of pest and disease outbreak, extreme drought and flood (Jaenicke and Pasiecznik, 2009). To achieve food stability in Ghana, there is the need to reduce dependency on just a few crops through diversification with local sources. The issue of underutilized crops has thus become a prominent subject for discussion within international, national and academic circles. Underutilized crops provide a better buffer to reduce nutritional, environmental and financial vulnerability (Aboagye *et al.,* 2007) especially in developing countries. However, very few of them have been given the necessary attention in terms of research, hence, hindering their effective use.

One of the most underutilized tuber producing legume is the yam bean (*Pachyrhizus sp.*) which is native to South and Central America and usually eaten as a vegetable crop (Zanklan *et al.,* 2007). Yam bean has high adaptability to drought-stress conditions such as found in tropical regions like West Africa and nitrogen-fixation ability which makes it highly suited to the needs of small-scale farmers and has a potential of becoming an integral part of sustainable land-use systems (Grum and Sorensen, 1998). Literature provided abundant information about the chemical composition of yam bean which

suggests that the crop can be of great industrial value. For instance the seeds and tubers contain about 25 – 50 % (db) of starch (Lopez *et al.,* 2010; Zanklan *et al.* 2007; Leidi *et al.,* 2003) which can be extracted for applications in the food and pharmaceutical industries. The crop also has rich nutraceuticals value indicated by the presence of phytochemicals in the root (Heredia and Cisneros-Zevallos, 2009; Aquino-Bolanos and Mercado-Silva, 2004), seed (Abid *et al.,* 2006; Phrutivorapongkul *et al.,* 2002) and leaf (Al-Razimi and Alkhathlan, 2000). Despite all these unique features of yam bean, little is known about the production of the crop for industrial use (Lopez *et al.,* 2010). There is therefore the need to expand utilization of yam bean in the food and health industries. Its potential to be processed into value-added products, however, depends on the physical and chemical properties of its constituents.

## 1.2 Problem statement

Yam bean roots, like many root and tuber crops are highly perishable being susceptible to mechanical injury during transport after harvest and deterioration by pest during storage (Aquino-Bolanos and Mercado-Silva, 2004). Therefore it is necessary to process yam bean roots into value added products to minimizing postharvest losses. The portfolio of commercial starch source does not cover the entire spectrum of potential starch functionality (Piyachomkwan *et al.,* 2002) hence the need to find other non-conventional starch sources with unique properties. Yam bean has potential for starch production (Lopez *et al.,* 2010) and in recent times, the starch properties of yam bean have received substantial attention in terms of agronomics (yield performance). However, few studies exist on the physicochemical and functional properties of native

yam bean starch. Moreover, available data on the physicochemical and functional properties of yam bean remain disparate and unequal with no published reports on the properties of native yam bean starch extracted from materials grown in Africa. This limits information on potential food applications of the starch as these properties are important to determine suitability of starch for specific food applications.

Additionally, despite the increase in awareness of bioactive compounds in yam bean during the last decade, investigations on specific industrial applications for these compounds are sparse. Current demands for natural plant products could create new opportunities for adding value to yam bean and minimizing postharvest loss by using yam bean as food preservative, food sanitizer or sterilizing microbial infections and nutraceuticals products. Assessment of phytochemical composition of yam bean and its pharmacological activities is thus necessary to exploit yam bean for such applications.

### 1.3 Justification

The only starch factory in Ghana, Ayensu Starch factory (ASCo) has been defunct since 2011 and did not reach its objective of starch import substitution for the years it was operational (Angelucci, 2013). As a result, Ghana largely relies on importation to meet local demand for starch and its derivatives. There is a potential for higher production volumes to meet domestic demand for starch and for export if underutilized crops like yam bean with potential for starch production are studied and their unique properties classified. Consequently farmers' incomes would increase ensuring sustainable starch production in Ghana.

In recent years due to health problems and better life expectancy, natural sources of bioactive substances have gained wide interest. Literature indicates that consumption of naturally occurring bioactive compounds may lower the risk of serious health disorders because of the protective effects of such compounds and some of these phytochemicals may also act as natural food preservatives (Michel *et al.,* 2012; Paradiso *et al.,* 2009). Therefore using yam bean as a natural source of nutraceuticals and biofortification of food products would be beneficial for consumer health and foster complete utilization of the yam bean. Confirmation of the nutraceutical potential of yam bean demands systematic investigation of different parts of the plant.

Knowledge on the physicochemical and functional properties of yam bean starch would lead to a better understanding of its characteristics thereby providing information on its end use quality while knowledge on the phytochemical composition and antimicrobial capacities of yam bean would serve as basis for developing it into an exploitable nutraceuticals product

## 1.4 Objectives

The overall aim of this study was to evaluate starch functionality, phytochemical constituents and antimicrobial properties of yam bean grown in Ghana.

### 1.4.1 Specific objectives

1. To determine the physicochemical and functional properties of yam bean starch.
2. To assess the phytochemical composition of yam bean leaf, seed and root
3. To assess the antimicrobial activities of yam bean leaf, seed and root against food-borne and clinical microorganisms.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Description of yam bean (*Pachyrhizus* sp.)

Yam bean is a tuber producing legume belonging to the genus *Pachyrhizus* and has been defined to be close relatives of soybeans and the phaseolus bean. It consists 3 cultivated species (*P. ahipa*, from Bolivia and northern Argentina; *P. erosus,* from the semiarid tropics of Central America, Philippines, West Africa; and *P. tuberosus*, from the tropical lowlands of Andean mountain range (Bhat and Karim, 2009). The leaf, seed and root (tuber) of *P. erosus* are shown in Plate 1.

Unlike its close relative the soybean (*Glycine max*), the yam bean is grown exclusively for its storage roots. This is because the seeds of the yam bean possess high rotenone content (nearly 1 % of seed weight), thus making it toxic for human consumption (Bhat and Karim, 2009). Usually, it is grown for use as a root vegetable, the exception being the *P. tuberosus* Chuin type from the Ucayali River of Peru, which has a high dry matter content and is processed to produce flour (Zanklan *et al.,* 2007). Yam bean grows well in tropical and sub-tropical regions, in both acidic and basic soils, and has a high nitrogen fixation potential (Stevenson *et al.,* 2007). Mycorrhizae are also associated with the crop, thus facilitating phosphorus uptake. For these reasons, yam bean is highly suited to the needs of small farmers, and from both an ecological and a socioeconomic perspective, it has the potential to become an integral part of sustainable land-use systems (Zanklan *et al.,* 2007).

Seed

Leaf

Root

Plate 1 Yam bean (*P. erosus*) parts

Despite its attractive agronomic characteristics, the crop currently has little or no commercial use (Lopez *et al.*, 2010). Some of the alternatives to add value to this crop and to expand its applications for commercial products like starch and nutraceuticals have been reported and will be reviewed in this chapter.

## 2.2 Starch properties and prospects in Ghana

Starch derived from cereals (wheat, rice, corn, and barley), tubers and roots (potato, cassava, sweet potato, etc) play important role in human diet as the main source of carbohydrates. The application of starch in the food industry is determined by their physicochemical and functional properties (Hoover, 2001). Therefore, knowledge about their properties is important. Starch has been the subject of intensive research over many decades, resulting in a vast body of published literature on preparative and analytical methods, molecular structure, physical, chemical and biochemical properties, functionality and uses (Copeland *et al.,* 2009). It is not intended in this section to review the extensive literature on starch. Rather, the aim is to provide a reasonably concise discussion of current knowledge of some starch properties in relation to yam bean (*Pachyrhizus erosus*) that is relevant to its functionality in foods and justify exploration of non-conventional sources of starch.

### 2.2.1 Chemical composition

#### *2.2.1.1 Amylose*

Starch granules are composed of a mixture of two polymers: an essentially linear polysaccharide called amylose and a highly branched polysaccharide called amylopectin, primarily forming the crystalline regions. The large size and highly branched structure of amylopectin are responsible for the high viscosity of amylopectin dispersion (Copeland *et al.,* 2009). Amylopectin constitutes about 75 % of most common starches. Most starches contain about 25 % amylose (BeMiller and Huber,

2008). An amylose content of 23.6 % for yam bean was reported by Stevenson *et al.* (2007).

### *2.2.1.2 Minor components*

Proteins, phosphorus, lipids, ash and moisture are present in small amounts within the starch granules. Protein content within starches varies depending on the botanical sources. The presence of protein can cause unwanted colour in starch and starch hydrolytic products via reaction between amino acid groups and reducing sugars (Mailard reaction). Moreover, proteins may also affect surface charge and the rate of hydration (Cui, 2005). Amaya-Llano *et al.* (2011) reported a protein content of 0.09 % for yam bean starch which is within range (>1 %) generally reported for legume, root and tuber starches.

In various starches, phosphorus is present as phosphate monoesters (esterified phosphorus), phospholipids, or inorganic phosphate. Compared to cereal starches, root and tuber starches contain higher amount of phosphorus (Taggart and Eliasson, 2004). In root and tuber starches, phosphorus is present as phosphate monoester. Phosphate monoesters could contribute to the high viscosity, high transparency, water binding capacity, and freeze thaw stability of the starches. Repulsion between phosphate groups on adjacent amylopectin chains may increase the hydration by weakening the extent of bonding between the crystalline domains (Hoover, 2001). On the contrary, cereal starches contain only small amount of phosphorus, which is present as phospholipids. These phospholipids tend to form complexes with amylose and long branched chain of amylopectin which result in limited swelling and lower transmittance of cereal paste

(Taggart and Eliasson, 2004). According to Mélo *et al.* (2003) yam bean has a low phosphate content of 0.009 %.

Lipids occupy the same site within amylose helices, their presence may interfere with the determination of amylose content, measured using iodine-binding method. Therefore, removal of lipid is necessary before measurement of amylose content using this method (Hoover, 2001). The effects of surface lipids on starch properties have been explained by Copeland *et al.* (2009). According to the authors, surface lipids affect the diffusion of water into starch granules. As a consequence, it may alter starch properties by reducing water binding capacity, swelling and solubility of starches. In addition, starch-lipid complexes alter the rheological properties of pastes by preventing amylose from contributing to the thickening power of gelatinized starch. Moreover, lipids increase gelatinization temperature, reduce gel rigidity, retard retrogradation and reduce the susceptibility to enzymic hydrolysis. The formation of starch-lipid or starch-surfactant complex could improve the textural properties of some foods (Moorthy, 2002). A lipid content of 0.7 % was found in yam bean starch (Amaya-Llano *et al.*, 2011). The ash and moisture contents of yam bean starch as reported by Amaya-Llano *et al.* (2011) were 0.27 % and 5.9 % respectively. An ash content of 0.5 % limit is recommended for grade A commercial starches while a 10 – 20 % range for moisture is also recommended for commercial starches as higher moisture contents can lead to microbial damage and subsequent deterioration in quality (Mufumbo *et al.*, 2011).

### 2.2.2 Functional properties

#### *2.2.2.1 Water binding capacity*

Water binding capacity (WBC) is important in determining the quality and texture of some food products because it stabilizes them against effects such as syneresis which sometimes occur during retorting and freezing. High water binding capacities are desirable as they increase the unit yield of products (Zakpaa *et al.,* 2010; Ellis *et al.,* 2003; Oduro *et al.,* 2001). WBC depends on the type of cultivar used, and environmental growing conditions; ultra-structural composition of starch molecules such as degree of association of amylose and amylopectin, degree of available water binding sites (OH groups and glucose oxygen atoms) (Zakpaa *et al.,* 2010). In general, tuberous starches have higher water-binding capacities than those of cereal origin, and the majority of workers have demonstrated that sweet potato starch has a higher WBC (66.3 to 211.6 %) than potato (93 %) and cassava starches (72–92 %). There is no available report on WBC of yam bean starch.

#### *2.2.2.2 Swelling power and solubility*

When starch is heated in excess water, the hydrogen bonds are broken disrupting the crystalline structure. Water molecules then become exposed to the hydroxyl groups on the amylose and amylopectin causing an increase in granular swelling and solubility. Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline regions. Differences among swelling power of various starches can be attributed to different intensities of molecular association forces inside the granules (Mélo *et al.,* 2003). These forces are governed by

many factors, including amylose / amylopectin ratio and by the characteristics of the amylose and amylopectin in terms of molecular weight / distribution, degree and length of branching, and conformation. Complexation of amylose with lipids reduces the solubility of starch in water and decreases swelling (Hoover, 2001). The rate and extent of swelling and breakdown in starch paste can be affected by the presence of proteins (Cui, 2005; Copeland *et al.,* 2009). In a previous study, the swelling power and solubility of yam bean at 95 °C were 56.4 g/ g and 27.2 % respectively (Mélo *et al.,* 2003). These authors also reported that the swelling power and solubility of yam bean were higher than those of cassava (48.0 g/g and 26 % respectively) and corn (24.0 g/g and 25.0 % respectively) but lower than that of potatoes (>100.0 g/g and 82.0 % respectively).

***2.2.2.3 Paste clarity***

Clarity is an important characteristic of starch paste quality that determines their application in the food industry because it gives shine and opacity to products. Paste clarity or the lack of it, can be advantageous or in some instances a serious problem. Products containing short chain amylose or fractured short chains of amylopectin, such as cereal starches, generally contribute opacity in finished food products. For some foods this may not be a significant issue, however, for some this change from clear is found unacceptable. Gravies, sauces, dressings, puddings and other savory foods usually are non-clear or opaque. The foods requiring more clarity such as fruit fillings and jellies require the use of the predominately amylopectin starches (waxy variety) (Eliasson, 2004) According to Mélo *et al.* (2003), the gel from yam bean starch paste was intermediate in clarity (11.4 %) when compared to cassava (21.84 %) and

maize (5.60 %). In another study a lower paste clarity value (7.26 %) was reported for another yam bean (*P. ahipa*) starch (Lopez *et al.,* 2010). These authors attributed the lower paste clarity in yam bean paste compared to cassava to the presence of molecules less susceptible to retrogradation (Lopez *et al.,* 2010; Mélo *et al.,* 2003).

### *2.2.2.4 Pasting properties*

A paste is defined as a viscous mass consisting of a continuous phase of solubilized amylose and/or amylopectin and a discontinuous phase of granule ghosts and fragments. Pasting refers specifically to changes in the starch upon further heating after gelatinization has occurred, including further swelling and leaching of polysaccharides from the starch granule, and increased viscosity which occurs with the application of shear forces. The Brabender viscoamylograph and rotational viscometers are used to examine the rheological properties of starches (Hoover *et al.,* 2010). The use of starch in industry as a thickener in many food systems or as a binder is based on its paste characteristics, hence, measurement of pasting characteristics of extracted starches is important. Swelling of starch granules and the resistance of starch granules to disruption by high heat and high shearing force primarily governs the amylogram patterns of starch (Tulyathan *et al.,* 2005).

Table 2.1 Pasting characteristics of native yam bean root starch

| Method | Moisture (%) | Starch concentration (%) | Pasting Properties | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | Pasting temp (°C) | Peak viscosity | Trough | Final | Break-down | Setback | |
| **RVA (RVU)** | nd | 8 | 72.3 | 282.2 | 145.5 | 214.0 | 138.4 | 75.6 | Stevenson *et al.*(2007) |
| **RVA (RVU)** | nd | nd | 68.79 | 419.82 | 130.69 | 263.38 | nd | 132.77 | Martinez-Bustos *et al.*(2007) |
| **RVA (RVU)** | 5.9 | nd | 71.5 | 363.9 | 317.0 | 561.1 | nd | 244.1 | Amaya-Llano *et al.*(2011) |
| **BVA (BVU)** | 10 | 5 | 64.5 | 580 | 480 | 780 | nd | nd | Méloet *al.*(2003) |

nd – not determined

Few studies have investigated the pasting properties of native yam bean starch (Table 2.1). The rapid viscoanalyzer (RVA) and Brabender viscoamylograph (BVA) have been widely used to simulate food processing in order to examine the pasting characteristics of yam bean starch. These two instruments (RVA and BVA) have been shown to differ in rotational speed, spindle geometry, extent of shear, temperature-time programs and viscosity units (Suh and Jane, 2003; Walter *et al.,* 2000). The pasting curves of these starches have been determined at different or unknown concentrations and the pH at which the measurements were carried out has not been reported. Thus, it is difficult to make a meaningful comparison of the data reported. In their study, Mélo *et al.* (2003) reported that yam bean starch paste has high viscosity profile, high retrogradation tendency and low stability on cooking and concluded that the functional properties of yam bean starch are very similar to most starchy roots. Another study by Stevenson *et al.* (2007) also concluded that yam bean starch paste has high paste viscosity and low

gelatinization temperature which makes it suitable as a food thickener in applications where heat is minimized to prevent adverse colour or off-flavour reactions.

### 2.2.2 Environmental effects on starch properties

While there is some understanding of the influence of genotype on starch structure and morphology, our knowledge of how these are affected by environmental factors during crop growth is still limited. Environmental conditions (especially temperature) have a very significant effect on the synthesis and properties of starch (Copeland *et al.,* 2009; Tester and Karkalas, 2001). The effects of environmental factors on starch properties have been explained by Tester and Karkalas (2001). According to the authors, environmental factors are thought to affect starch properties by influencing amylose and amylopectin structure (by regulating the activities of key biosynthetic enzymes), starch composition, granule dimensions, crystallinity, functional properties, starch hydrolysis and digestibility.

In many cases, the influence of environmental factors is greater than varietal differences or even between species. Although it is difficult to control this variable in view of unpredictable climatic conditions, environmental factors affect raw material and product quality. The issue of importance is to understand what effects environmental factors may have had on product quality (Tester and Karkalas, 2001). According to Burrell (2003), an increase in temperature of 5 °C can lead to 10 - 15 % decrease in yield of some wheat varieties. In a study on potato starch, Protserov *et al.* (2002) established a positive correlation between growth temperatures and yield. Noda *et al.* (2001) also confirmed that an increase in soil temperature corresponded to increase in amylose content, average

granule size, gelatinization temperature, enthalpy of gelatinization and lower values of peak viscosity and setback viscosity in a sweet potato cultivar. In investigating the effect of geographical factors on starch characteristics of a yam bean cultivar, Stevenson *et al.* (2007) observed some differences in pasting properties, amylopectin weight and gyration radius but concluded that there were sufficient similarities to suggest that yam bean starch cultivated over a wide geographical area would have similar industrial applications. Since starch is usually heated in aqueous medium at temperatures approaching that of boiling water or more in food processing, Tester and Karkalas (2001) were of the opinion that minor variations in gelatinization temperature and paste viscosity is unlikely to be critical for most processes and that the significance of environmental factors on starch synthesis and properties is perhaps of particular importance to farmers where yields may be reduced if sub-optimum growth conditions are experienced.

### 2.2.3 Starch sources, demand and constraints in Ghana

Corn, wheat, rice, potato and cassava dominate the world as primary raw material sources for starch in food applications (Eliasson, 2004) with mung bean being a unique and important legume source of starch (Tan *et al.,* 2007). Corn is cultivated in warmer climates, with half of the world's production grown in the U.S.A., its biggest crop. China, the second largest producer in the world, grows about 10 %. Wheat, requiring a more temperate climate, is primarily grown in the former USSR, North America and Europe. Approximately 90 % of world rice production comes from South and Southeast Asia. Approximately 70 % of the world's potato supply is grown in the cool, moist

climate of Europe and Russia, while cassava is cultivated in the narrow tropical band about the equator (Eliasson, 2004).

Although starch from these crops has been extensively studied, the interest to explore new starch sources exists (Abo-El-Fetoh *et al.,* 2010). This is because the available commercial starch sources do not possess the entire spectrum of starch functionality desired in food products (Piyachomkwan *et al.,* 2002). As such, the native properties of commercial starches sometimes require some form of chemical modification to yield the desired functionality. With increasing consumer demand for more "natural foods" (Copeland *et al.,* 2009), starches from non-conventional sources that have desirable functional properties could play a significant role in improving the quality of different food products and could replace chemically modified starches that are currently being used in a number of products.

In Ghana, starch has found its application in the food, textiles, pharmaceutical, paper and plywood industries. The main sources of starch are maize, cassava and potato starch of which most was imported. Therefore as part of the goal of the government to move Ghana into the club of nations producing industrial starch for the global market the Ayensu Starch Company Ltd. (ASCo) was established in 2001 (Addy *et al.,* 2004) at Bawjiase in the Central Region under the President's Special Initiative. It was established as a commercial company to boost cassava cultivation for starch production. However, presently, the company is defunct (Angelucci, 2013). According to data extracted from FAOSTAT and UN Comtrade on starch tradeflows by Angelucci (2013), presented in Table 2.2, Ghana was a net exporter of starch only in 2005 and the cassava

starch producing plant which at least for the years in which it was operational, 2004 -2008, did not reach its objective of starch import substitution.

Table 2.2 Ghana cassava starch import and export flows from 2005 to 2010

| **FAOSTAT** | **2005** | **2006** | **2007** | **2008** | **2009** | **2010** |
|---|---|---|---|---|---|---|
| **Import Quantity (tonne)** | 15 | 329 | 233 | 224 | 434 | N/A |
| **Import Value (USD)** | 14, 000 | 177, 000 | 86, 000 | 218, 000 | 283, 000 | N/A |
| **Export Quantity (tonne)** | 302 | 115 | 28 | 1 | 1 | N/A |
| **Export Value (USD)** | 84, 000 | 51, 000 | 33, 000 | 1, 000 | 1, 000 | N/A |
| **UNCOMTRADE** | **2005** | **2006** | **2007** | **2008** | **2009** | **2010** |
| **Import quantity tonne** | 40 | 171 | 233 | 224 | 353 | 350 |
| **Import value (USD)** | 14, 252 | 69, 405 | 86, 284 | 216, 413 | 154, 190 | 424, 826 |
| **Export quantity** | 350 | 7 | 28 | 1 | N/A | N/A |
| **Export value (USD)** | 88, 152 | 2, 546 | 33, 472 | 640 | N/A | N/A |

Source: Angelucci (2013); NA – not available

UNCTAD provided a succinct account on the rise and collapse of ASCo (UNCTAD, 2011). According to their report ASCo was a success initially, recording $300,000 profits in its first year of operation. It boasted sales agreements for further production with customers including Unilever, Nestle, Dera of the Czech Republic, and ELSA Foods. The 7,000 contract farmers supplying cassava were guaranteed year-round purchase of their cassava crop. However, things quickly turned for the worse after 2002. In fact, from 2006 to 2008 the factory operated at only 20 % of its installed capacity. ASCo blamed this on an inadequate supply of roots and low export prices obtained from the European market. Many reasons, including low cassava yields, falling international

starch prices, high perishability, and a rising local demand for cassava for gari were the major constraints that bedeviled the company. The European market, which is a major consumer of starch, proved hard to infiltrate. Dominated by potato and corn starch and highly protected by high tariffs ASCo managed to sell only about 2,000 tons of cassava starch to the European market at $ 200 per ton. In Asia too, Ghana's starch was non-competitive because Thailand, a commercial and low cost producer of cassava starch, has captured the market and is said to have the advantage of closeness and high operational efficiency. Though ASCo's starch had comparative advantage in the ECOWAS sub-region, Nigeria, which has the largest market demand for starch in the region, has also banned imports of starch from Ghana (UNCTAD, 2011).

Considering the above account on the demise of ASCo, the implication is that in order to revive the starch factory and make it more competitive in both the local and international starch markets, it is expedient that ASCo makes a paradigm shifts from relying solely on cassava as raw material but to expand its raw material base to include new and cheaper alternative starch sources. Yam bean could therefore be considered as a new and low cost raw material source to compliment cassava in the starch industry due its attractive agronomic characteristics. Starch is a versatile polymer able to position itself as an important raw material not only in the food industry but also in the pharmaceutical and textile industries. Similarly, aside starch, yam bean also has other properties that could broaden its industrial application beyond starch production and transform it into a nutritious crop with therapeutic value due to the presence of phytochemicals.

## 2.3 Potential of phytochemicals as nutraceuticals

### 2.3.1 Nutraceuticals

Food can be both nutritious and therapeutic. The term "nutraceuticals" can be explained "as a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease." Nutraceuticals may range from isolated nutrients, dietary supplements, and diets to genetically engineered "designer" food, herbal products, and processed products such as cereals, soups, and beverages. A nutraceutical is also any nontoxic food extract supplement that has scientifically proven health benefits for both the treatment and prevention of disease. The increasing interest in nutraceuticals reflects the fact that consumers hear about epidemiological studies indicating that a specific diet or component of the diet is associated with a lower risk for a certain disease (Tapas *et al.*, 2008).

Phytochemicals, as plant components with discrete bioactivities are being widely examined for their ability to provide health benefits. It is important to establish the scientific rationale to defend their use in foods, as potential nutritionally active ingredients. Research supporting beneficial roles for phytochemicals against cancers, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, psychotic diseases, spasmodic conditions, ulcers and other bioactivities is based on chemical mechanisms using *in vitro* and cell culture systems, various disease states in animals and epidemiology of humans (Dillard and German, 2000).Public health authorities consider prevention and treatment with nutraceuticals as a powerful instrument in maintaining health and to act against nutritionally induced acute and chronic diseases, thereby promoting optimal health, longevity and quality of

life. Thus ongoing research on bioactivities of phytochemicals from diverse plant sources will lead to a new generation of foods, which will certainly cause the interface between food and drug to become increasingly permeable (Andlauer and Fürst, 2002).

### 2.3.2 Ethnomedicinal use and phytochemical composition of yam bean

There is extensive literature on the ethnomedicinal uses of yam bean seed and tuber. According to a report by Sørensen (1996) the seeds have been used to treat various skin diseases while the root has also been used to cure fevers, treat skin diseases, gastrointestinal ailments and respiratory tract diseases. The beneficial medicinal effects of plant materials typically result from the secondary products (phytochemicals) present in the plant although it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Parekh *et al.,* 2005). They also vary between tissues, among species from plant to plant and from season to season (Das *et al.,* 2010).

Available information on phytochemical composition of yam bean lends some credence to its ethnomedicinal uses. The seeds contain saponins (Sørensen 1996), rotenoids (Sørensen 1996; Phrutivorapongkul *et al.,* 2002), isoflavonoids (Abid *et al.,* 2006; Phrutivorapongkul *et al.,* 2002), phenylcoumarin (Phrutivorapongkul *et al.,* 2002), pterocarpanoids (Al-Razimi and Alkhathlan, 2000) and an antifungal protein known as SPE-10 (Wu *et al.,* 2002; Song *et al.,* 2005). The tuberous root contains phenolics (Aquino-Bolanos *et al.,* 2000; Heredia and Cisneros-Zevallos, 2009), isoflavonoids,

pterocarpanoid and furans (Lukitaningsih, 2009); and the leaf also contains pterocarpanoids (Al-hazimi and Alkhathlan 2000) though there is no available reference on ethnomedicinal use of the leaf. These compounds elicit a broad spectrum of activities including antibiotic, antihelmintic, antidiarrhoeal, anti-inflammatory, spasmolytic, antifungal, antioxidant, antisecretory, antiviral and insecticidal (Tiwari *et al.,* 2011; Bejar *et al.,* 2000).

### 2.3.2 Importance of herbal extracts to food quality and safety

The appearance of food is one of the major determinants of its appeal to consumers and consequently, sales of the product. Microbial contamination and lipid oxidation are the main factors that determine food quality loss and shelf life reduction (Loizzo *et al.,* 2010). Therefore preventing microbial contamination is highly relevant to food processors. Microbial contamination is of serious concern to both the food industry and the general public. This is due to food spoilage and food-borne disease outbreaks associated with microbial contamination. Popular among food-borne bacteria isolated in foods are *Campylobacter jejuni*, *Salmonella*, *E. coli*, *Listeria monocytogens* and *Staphylococcus aureus* (Park *et al.,* 2011). Their effects could be infection or intoxication. These food-borne pathogens have been isolated from fresh fruits and vegetables such as apples (Park *et al.,* 2011), lettuce (Park *et al.,* 2011; Cooley *et al.,* 2007; DeWaal *et al.,* 2002), mixed fruits, prepared salad (Feglo and Sakyi, 2012; Sivapalasingam *et al.,* 2004), tomatoes (Cummings *et al.,* 2001) and fresh cut melon (Del Rosario and Beuchat, 1995). Also included are ready to eat street vendored foods like ice-kenkey, cocoa drink, fufu, fresh ground red pepper (normally eaten with kenkey) and pasta (Feglo and Sakyi 2012), beef (Adzitey 2011; Soyiri *et al.,* 2008).

Contamination of food by food-borne pathogens may occur at various food production stage - in the field or during postharvest handling though bird droppings, fruit flies, fecal contamination, water, equipment or infected handlers are also responsible for contamination of food (Nou *et al.,* 2011; Jin and Niemira, 2011).

There are numerous physical and chemical methods for inhibiting or inactivating microorganisms in foods (Burris *et al.,* 2011; Ndagano *et al.,* 2011) and these have varying degrees of antimicrobial effects (Park *et al.,* 2011). Improvement in refrigeration has made international trade in perishable goods possible, but refrigeration alone cannot completely guarantee quality and safety. Hence most food products are packaged and stored under a modified atmosphere to lengthen their shelf life. However these procedures cannot successfully eliminate undesirable microorganisms that can cause the deterioration of the food as well as reduction of nutritional quality (Fratianni *et al.,* 2010). Certain compounds can be added to products to retard or minimize the deteriorative events. Synthetic sanitizers and antioxidants are common preservatives, however, their use has come to debate due to their opposed carcinogenic potential (Nou *et al.,* 2011; Jin and Niemira, 2011; Fratianni *et al.,* 2010). With increase in consumer concern about synthetic additives in food and feed and antibiotic resistance of food borne pathogens, food producers are searching for natural preservation methods that are not harmful to humans (Szczepaniak *et al.,* 2011). Therefore increased attention has been focused on herbal extracts which can preserve food in an effort to improve the sensory appeal of food and limit the growth of unwanted pathogenic bacteria and fungi (Fratianni *et al.,* 2010).

### 2.3.3 Major groups of antimicrobial compounds

Nature offers a wide spectrum of biologically active (phyto) chemicals that can be used as potential natural preservatives. Compounds with antimicrobial properties have been found in various plant parts including leaves, flowers, roots, fruits and other parts. These are mostly acids, alcohols, medium and long-chain organic acids, terpenic compounds and their derivatives. These compounds provide an alternative to conventional antimicrobial supplements (Szczepaniak *et al.,* 2011). Tannins, flavonoids, polyphenols, glycosides, saponins, alkaloids, terpenoids, essential oils, coumarins, lectin and polypeptide are the major groups of phytochemicals with antimicrobial activity and their mechanism of action vary (Tiwari *et al.,* 2011). Many edible plant foods contain phytochemicals and have antimicrobial activities. Some include wild mint, allspice, cinnamon, clove bud, garlic, oregano, thyme, balm, kiwi, peanut, sweet acacia, asparagus, lemon grass, pepper, artichoke, mango, basil, citrus, sea buckthorn, raspberry and grape (Michel *et al.,* 2012; Castillo *et al.,* 2011; Matemu *et al.,* 2011; Fratianni *et al.,* 2010; Mkaddem *et al.,* 2009; Du *et al.,* 2009). Antifungal activity of yam bean seed extracts against postharvest pathogens of papaya (Barrera-Necha *et al.*, 2004); and antiviral activity against herpes simplex virus (Phrutivorapongkul *et al.*, 2002) have also been reported.

### 2.3.4 Evaluation of natural plant products for antimicrobial activity

Full exploitation of benefits of natural products requires many well-designed and comprehensive screening programmes. Many researchers have adopted the procedure used in finding "hits" drugs for evaluating antimicrobial activity of plant products. This procedure is bioassay-guided isolation and considered the most successful experimental

approach in the quest for new pharmacologically active principles. According to Mata *et al.,* (2001), this method is systematic and involves selection of the plant materials of interest. The selection process may be according to the ethnomedical and chemotaxonomic criteria. Organic soluble extracts of the plant materials are then prepared and tested for biological activity using appropriate bioassay systems. Following experimental confirmation of biological activity, the active extracts are fractionated and their biological activity monitored at each step of the fractionation stage. This process continues until pure active compounds (leads) are isolated from crude extracts. The active isolates are then subjected to structure elucidation and, when possible, to further biological studies to confirm that leads are good candidates for drug development.

Often results of a study are compared with those of previous studies of the same or a similar plant extract. Differences in environmental factors, postharvest treatments (such as storage and maturity), different thermal processing techniques (if applicable) and many others can cause variation in results. Although all of these factors do play a role in the variation in results, the extraction technique and the antimicrobial evaluation assays selected significantly influence results. Therefore it is often difficult to make a meaningful comparison of results on antimicrobial activity from different studies on the same or different plant extracts (Das *et al.,* 2010; Ncube *et al.,* 2008; Valgas *et al.,* 2007; Tripoli *et al.,* 2007).

### *2.3.4.1 Extraction technique*

The extraction technique and especially the solvent used to extract the phytochemicals under consideration cause variation in results in many ways. It is well known that the

solvent type (based on polarity), pH, temperature, whether it is a mixture of solvents, or performing the extraction with different solvent in series will extract different phytochemicals (Tiwari *et al.,* 2011; Das *et al.,* 2010 and Ncube *et al.,* 2008). The choice of solvent is influenced by what is intended with the extract and the targeted compounds to be extracted (Tiwari *et al.,* 2011). Traditional healers use primarily water to extract plant products with antimicrobial activity but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extracts. This is because most antimicrobial active components that have been identified are not water soluble and thus organic solvent extracts have been found to be more potent (Parekh *et al.,* 2005). The most commonly used solvents for investigations of antimicrobial activity in plants are methanol, ethanol, and water (Castillo *et al.,* 2011; Ifesan *et al.,* 2009; Ennajar *et al.,* 2009; Parekh *et al.,* 2005) but due to issues of toxicity during ingestion, aqueous and ethanolic extracts are usually preferred in studying antimicrobial activity of plant food extracts (Michel *et al.,* 2012).

### *2.3.4.2 Antimicrobial assays*

Several methods are currently available to detect antimicrobial activity of natural plant products. Since not all of them are based on the same principles, the results obtained are influenced not only by the test method selected, but also, by the plant material used, the extraction method or the degree of solubility of each test-compound and the microorganisms used (Valgas *et al.,* 2007; Tripoli *et al.,*2007). This could make it difficult to make meaningful comparisons of results with works of other researchers. The broth and agar based methods are the conventional reference methods for antimicrobial susceptibility standard tests (AST) though other methods like agar well diffusion, agar

disk diffusion, broth dilution, TLC and poison food technique have been used in other studies to conduct AST (Das *et al.,* 2010; Ncube *et al.,* 2008). According to Ncube *et al.* (2008), the major limitation of the agar diffusion methods is that, it can only be used for AST of pure substances because when it is applied to mixtures containing constituents, which exhibit different diffusion rates, results may be unreliable. In contrast, the broth dilution method broth dilution method provides a potentially useful technique for determining the minimum inhibitory concentrations (MICs) of large numbers of test samples. Its advantages over diffusion techniques include increased sensitivity for small quantities of extract which is important if the antimicrobial material is scarce as is the case for many natural products; ability to distinguish between bacteriostatic and bactericidal effects; and quantitative determination of the MIC. This method can also be used for a wide variety of microorganisms; it is inexpensive and presents reproducible results. Due to the efficiency of the broth dilution method and the limited availability of yam bean materials for the study, this method was selected to evaluate the antimicrobial activity of yam bean plant parts.

## 2.4 Conclusion on literature review

Increasing the production, use and marketing of yam bean in Ghana, especially in rural areas, could be of high significance for food security and sustainable livelihood by diversifying agricultural production. The starch properties and phytochemical composition of yam bean may credit it as a source for new forms of starch for special purposes, and a new natural source of nutraceuticals, though the low production of the plant for industrial needs is acknowledged.

# CHAPTER THREE

# 3.0 MATERIALS AND METHOD

## 3.1 Materials

### 3.1.1 Plant materials

A yam bean (*P. erosus*) variety (EC 533 No. 209018) was sourced from the experimental field of the Crop Research Institute, Kumasi. The leaves, pods and roots of the yam bean were harvested in October 2011 when senescence started (~ 210 days after sowing). The plant materials used were free from mechanical and pathological injury. All chemicals and solvents used were of standard analytical grade.

### 3.1.2 Test organisms

A panel of microorganisms including food-borne and clinical microorganisms: two gram positive bacteria (*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922)), a gram negative bacteria (clinical isolate of *Salmonella typhi*) and yeast strains (clinical strain of *Candida albicans*) were obtained from the Faculty of Pharmacy, KNUST, Kumasi.

## 3.2 Sample preparation for starch extraction

Yam bean roots were received in the laboratory and processed immediately. Wounded or unhealthy roots were discarded. Afterwards, the roots were washed under running tap water to remove any soil residue and subsequently stored at room temperature (25 $^{o}$C) until further analysis.

### 3.3 Sample preparation for phytochemical and antimicrobial assays

Yam bean leaves, seeds and roots were received in the laboratory and washed under running tap water to remove any soil residue. Wounded or unhealthy parts were discarded. The leaves and seeds were air dried for 10 days prior to oven drying in order to prevent thermal degradation of their phytoconstituents by reducing moisture content at lower temperature than required for oven drying. Afterwards, the leaves were oven dried to constant weight for 1 h at 60 °C. The seeds were also initially oven dried to constant weight at 60 °C. The roots were prepared after washing by scraping off the thin epidermal layer on the roots and cutting the roots into small sizes of about 2 cm x 2 cm x 2 cm each. Subsequently, the roots were then oven dried to constant weight at 50°C. All the dried leaf, seed and root samples were milled in a laboratory mill to a moderately fine powder (particle size ≤ 0.5 mm), packaged in sterilized laminated polyethylene bags (Nasco Whirl Pak, 207 mL capacity, 9.5 cm x 18 cm, 0.076 mm thick, USA) and stored at 4 °C until further analysis.

### 3.4 Starch assays

The starch from yam bean roots was isolated and the yield, physicochemical characteristics, functional properties and pasting characteristics were analyzed as reported below:

#### 3.4.1 Starch isolation and yield

Starch was isolated from the fresh tubers as described by Benesi (2005). Fresh tuberous roots were washed with tap water, peeled, washed again, chopped to about 1 $cm^3$ cubes and about 500 g of the cubes were transferred into a heavy duty blender (Warring Commercial, model 32BL79 (8010), USA ). One litre of distilled water was added to the

cubes and pulverized at 3000 rpm for 5 min. The resultant suspension was filtered through a 250 μm sieve; the filtrate was allowed to stand for 4 h to facilitate starch sedimentation and the top liquid was decanted as waste. The sediment was suspended in 1 L of water and the whole process was repeated three times. The final sediment was air-dried for 48 h, ground in a mortar and stored in polyethylene bags prior to analysis.

Starch yield was determined as the percentage starch recovered after extraction from a weighed kilogram of sample.

### 3.4.2 Physicochemical characterization

#### *3.4.2.1 Moisture content*

Moisture content was determined according to established protocols (AOAC, 2000). Crucibles were cleaned and dried in an oven at 105 °C overnight, cooled to 25 °C in a desiccator with dry Silica gel for 40 min, and weighed to the nearest 1 mg ($W_0$). Triplicate starch samples of 2 - 3 g were weighed into the cooled crucibles ($W_1$). The crucibles and samples were dried for 24 h at 105 °C, and then dried samples and their containers were cooled down to 25 °C in a desiccator with dry Silica gel for 1 h and weighed immediately after removal from the desiccator to the nearest 1 mg ($W_2$). The moisture content in percent (% MC) of the samples (average results of three replicates) was calculated as follows:

$$\% MC = 100 - \left\{ \frac{(W_2 - W_0)}{(W_1 - W_0)} \times 100 \right\}$$

#### *3.4.2.2 Ash content*

The ash content was determined following established protocol (AOAC, 2000). Clean, dry ashing crucibles were heated for half an hour in a muffle furnace at 550 °C, cooled

in a desiccator containing a drying agent (Silica gel) to 25 °C for 30 min, and weighed on a balance to the nearest 0.1 mg ($W_0$). Starch samples of 2-3 g were weighed in triplicate to the nearest 0.1 mg and put in crucibles ($W_1$), placed in a muffle furnace at 550 °C for 5 h then cooled in a dessicator and weighed immediately to the nearest 0.1 mg ($W_2$). Ash content was determined by weight difference and expressed as percentage as follows:

$$\text{Ash content (\%)} = \left\{\frac{(W_2 - W_0)}{(W_1 - W_0)} \times 100\right\}$$

### *3.4.2.3 Protein content*

The starch protein content was analyzed by the method described by Suchy *et al.* (2003) using an automatic Protein / Nitrogen determinator (LECO FP-528, LECO Corp, St. Joseph, MI, USA). Starch samples of about 3 g were dried in an oven at 105 °C for 24 h, and then cooled in a desiccator containing dry Silica gel for 1 h. The dried starch samples (0.30 g) in triplicate were weighed immediately after removal from the desiccator and then loaded into the protein analyzer. The crude protein was calculated by multiplying the total nitrogen with 6.25, a customary conversion factor used when specific conversion factor for nitrogen content of food being analyzed is unknown or unavailable as is the case for yam bean.

### *3.4.2.4 Fat content*

Fat content of the starches was determined using Soxhlet extraction (AOAC, 2000). Starch samples of about 3 g were dried in an air oven at 105 °C overnight and cooled to 25 °C in a desiccator. Clean extraction pots were also dried in an oven at 105 °C

overnight, cooled to 25 °C in a desiccator, and weighed ($W_0$). The dried starch samples of about 3 g ($W_1$) were weighed in triplicate on a Whatman No. 1 filter paper, wrapped lightly and plugged with defatted cotton wool down the extraction thimble. Fat was extracted from the sample using 150 mL hexane for 3 h, after which the samples were removed from the extractor and most of the solvent was removed by evaporation. The extraction pots were then dried at 105 °C overnight, cooled to 25 °C in a desiccator, and reweighed ($W_1$). The fat content of the starches was calculated as follows:

$$\text{Fat content (\%)} = \left(\frac{W_2 - W_0}{W_1}\right) \text{x } 100$$

### *3.4.2.5 pH*

Triplicate starch samples, 5 g dry basis (db), were weighed into a beaker and mixed with 20 mL of distilled water. The resulting suspension was stirred for 5 min and left to settle for 10 min. The pH of the water phase was measured using a calibrated pH meter (Model PHS-25, Shanghai) (AOAC, 2000).

### *3.4.2.6 Amylose content*

Amylose content was determined using an Amylose / Amylopectin Assay Kit (Megazyme International Ireland Ltd., Bray, Ireland) by the method of Gibson *et al.* (1997). In this method, 20 – 25 mg starch samples were weighed into a screw cap tube and are completely dispersed in 1 mL 85 % dimethyl sulphoxide (DMSO). The tube was then heated at 100 °C in a water bath for 15 min, with intermittent stirring at high speed on a vortex mixer. The tube was allowed to cool to 25 °C for about 5 min and lipids were removed by precipitating the starch in the tube in 2 mL of 95 % ethanol. The

precipitated starch was recovered by centrifugation and dissolved in acetate buffer solution. Amylopectin was specifically precipitated by addition of concavanalin A (Con A) and removed by centrifugation. Amylose in the aliquot was determined using glucose peroxidase reagent (GOPOD) after enzymatic hydrolysis to glucose. Total starch in a separate aliquot was determined using GOPOD reagent after hydrolysis to glucose and the concentration of amylose was estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of Con A precipitated sample, to that of total starch sample. Duplicate starch samples of 25 mg (db) were weighed to the nearest 0.1 mg in 10 mL centrifuge tubes and 1 mL of DMSO was added while stirring at low speed on a vortex mixer. The tubes were then capped and the starch suspension heated in a boiling water bath for 1 min after which the contents were vigorously mixed and then heated for 15 min with constant stirring. After 15 min, the tubes were removed from the boiling water bath, cooled to 25 °C for 5 min and 6 mL of 95 % ethanol added. The tubes were left to stand for 1 h to precipitate the starch. After 1 h the tubes were centrifuged at 12000 x *g* for 5 min and the supernatant was decanted. The tubes were then drained on tissue paper for 10 min to ensure all ethanol was removed. To the starch pellet, 1 mL DMSO was added with gentle mixing and the mixture heated in a boiling water bath for 15 min with constant stirring. After the heating period, 2 mL of diluted Con A solvent was added and the mixture transferred quantitatively to a 25 mL volumetric flask and made up to the mark with Con A solvent.

The resulting starch solution was used to precipitate amylopectin, determine amylose and total starch as follows: amylopectin was precipitated from the starch solution by

mixing 1 mL of the starch solution with 0.50 mL of Con A solution (4 mg $mL^{-1}$) in a 2.0 mL Eppendorf micro centrifuge tube. The mixture was left to stand for 1 h at 25 °C and then centrifuged at 20000 x *g* for 10 min. Aliquots (1 mL) of the supernatant were transferred into the screw-capped centrifuge tubes and 3 mL of 0.1 M sodium acetate buffer (pH 4.5) added. The resulting mixture was heated in a boiling water bath for 5 min to denature the Con A. After 5 min, the tubes were placed in a water bath at 40 °C for 5 min, and 0.1 mL of amyloglucosidase / α-amylase enzyme mixture added and the mixture was incubated at 40 °C for 30 min. The resultant mixture was subsequently centrifuged at 12000 x *g* for 5 min. Amylose was determined by transferring 1 mL aliquots of the supernatant in triplicate into glass test tubes, adding 4 mL of the GOPOD reagent and incubating the mixture at 40 °C for 20 min. The absorbance of the samples and standard solution were measured at 510 nm against a reagent blank using a UV-Visible spectrophotometer (Spectronic Unicam, Helios, Cambridge, United Kingdom). The blank solution contained 1 mL of sodium acetate buffer and 4.0 mL of the GOPOD reagent, incubated for the same period as the samples. A standard control contained 0.1 mL of glucose standard solution (1 mg $mL^{-1}$), 0.9 mL of sodium acetate buffer and 4 mL of GOPOD reagent treated in the same way as the sample solutions. In order to determine total starch, 0.5 mL aliquots of the diluted starch solution were placed in clean test tubes, mixed with 4 mL of 0.1 M sodium acetate buffer (pH 4.5) and 0.1 mL of amyloglucosidase / α-amylase enzyme mixture and the mixture was incubated at 40 °C for 10 min. Aliquots (1 mL) of the digested solution were transferred in triplicate into glass tubes and 4 mL of GOPOD reagent was added. The mixture was incubated at 40 °C for 20 min. The incubation was performed concurrently with samples and

standards for amylose determination and the absorbance also read at 510 nm together with the amylose determination sample.

The amylose (% w/w) was calculated using the following equation:

$$\text{Amylose (\%)} = \frac{\text{Absorbance Con A supernatant}}{\text{Absorbance Total Starch aliquot}} \times \frac{6.15}{9.2} \times \frac{100}{1}$$

$$= \frac{\text{Absorbance Con A supernatant}}{\text{Absorbance Total Starch aliquot}} \times 66.8$$

where 6.15 and 9.2 are dilution factors for the Con A and Total starch extracts respectively.

### *3.4.2.7 Phosphorous content*

Phosphorus content of the starch samples was determined from the diluted digest by the ascorbic acid colorimetric method (Murphy and Riley, 1958). Triplicate aliquots (1 mL) were transferred to test tubes and mixed with 9 mL of Murphy-Riley solution. The mixture was left to stand for 15 min at 25 °C and absorbance at 880 nm read against a reagent blank. The Murphy- Riley solution was prepared by dissolving separately 0.40 g of antimony potassium tartrate, 4.3 g ammonium molybdate in 400 mL and 100 mL of distilled water, respectively, and then mixing the solutions in a 1000 mL volumetric flask. The flask was then placed in an ice bath and 54 mL of concentrated sulphuric acid was added slowly with stirring. The solution was cooled to 25 °C and made up to the 1000 mL mark with distilled water. An aliquot (100 mL) of this solution was mixed with 500 mL of distilled water and 0.526 g ascorbic acid and made up to 1000 mL with distilled water to make the final Murphy-Riley working solution. A calibration curve was prepared using phosphorus standards ranging from 0.01 to 2.0 mg P/L.

### 3.4.3 Functional properties

#### *3.4.3.1 Swelling power and solubility*

Solubility and Swelling power determinations were carried out at 95 °C based on the method of Leach *et al.* (1959). One gram of yam bean starch was dissolved with distilled water to a total volume of 40 mL using a weighed 50 mL graduated centrifuge tube. The suspension was stirred just sufficiently and uniformly at 25 °C, avoiding excessive speed since it might cause fragmentation of the starch granules. The slurry in the tube was heated at 85 °C in a thermostatically regulated temperature water bath for 30 min with constant gentle stirring. The tube was then removed, wiped dry on the outside and cooled to 25 °C. It was then centrifuged at 2200 rpm for 15 min. The supernatant was decanted into a pre-weighed moisture can. The solubility was determined by evaporating the supernatant in a thermostatically controlled drying oven at 105 °C and weighing the residue. The sedimented paste was weighed and swelling power was calculated as the weight of sedimented paste per gram of starch used. The swelling power and solubility were calculated as follows:

$$\text{Swelling power} = \frac{\text{Weight of sediment}}{\text{Sample weight} - \text{Weight of soluble}}$$

$$\% \text{ Solubility} = \frac{\text{Weight of soluble}}{\text{Weight of sample}} \times 100$$

#### *3.4.3.2 Water binding capacity*

Water binding capacity of yam bean starch was determined according to the method of Medcalf and Gilles (1965). An aqueous suspension of yam bean starch was made by dissolving 2.0 g (db) of starch in 40 mL of distilled water. The suspension was agitated for 1 h on a Griffin flask shaker (Shital Scientific, SS1-73, India) and centrifuged at

2200 rpm for 10 min. The free water (supernatant) was decanted from the wet starch, drained for 10 min and the wet starch was then weighed. The water binding capacity was calculated by difference as follows:

$$\% \text{ Water binding capacity} = \frac{\text{Weight of bound water}}{\text{Weight of sample}} \times 100$$

### *3.4.3.3 Paste clarity*

Starch suspensions (1% w/v) were gelatinised in a boiling bath for 30 min. After cooling to 25 °C for 1 h, the clarity of paste was measured with a spectrophotometer (CE 1021, Cecil Instruments, Cambridge, UK) as the percentage light transmittance (%T) at 650 nm, using water as a blank (Piyachomkwan *et al.*, 2002).

### *3.4.3.4 Pasting characteristics*

The pasting characteristics were determined using a Brabender viscoamylograph (Brabender Type 801203. Duisburg, Western Germany) equipped with a 1000cmg sensitivity cartridge. A smooth slurry prepared from starch (40 g) dissolved in 420 mL distilled water (8 % starch, db), was heated from 30 °C to 95 °C at spindle speed of 75 rpm. It was kept at this temperature for 30 min and then cooled to 50 °C and held at this temperature for 15 min. The heating and cooling rate was 1.5 °C / min. The viscosity profile indices recorded included the following: pasting temperature, peak temperature, peak viscosity, viscosity at 95 °C, viscosity after 30 min hold at 95 °C (95 °C - Hold), viscosity at 50 °C, viscosity after 30 min hold at 50 °C (50 °C - Hold), breakdown viscosity and setback viscosity. The paste stability ratio at 95 °C and setback ratio were estimated from the data generated.

## 3.5 Phytochemical screening, Total phenolics and Antimicrobial assays

### 3.5.1 Extraction and yield of yam bean crude extracts

The extraction of yam bean leaf, seed and root were carried out by wet extraction method (Tiwari *et al.*, 2011). About l0 g ($W_1$) of the ground powder of each yam bean leaf, seed, and root were each dissolved in a 100 mL 70 % ethanol inside a sealed glass jar for 4 days at 25 °C and then filtered using Whatman No 1 filter paper. The liquid extracts were then evaporated at 40 °C using rotary evaporator (Buchi Labortechnik AG, Switzerland) under vacuum and further concentrated at 40 °C for 3 h in an oven to obtain a dried crude extract ($W_2$), which was then protected from light by storing in a laminated polyethylene bags at 2 °C to prevent degradation of phytochemicals by light before use. Each extract was replicated thrice.

The yield (%, w/w) from all the dried extracts was calculated as:

$$\text{Yield (\%)} = \left(\frac{W_2}{W_1}\right) \mathbf{x}\ 100$$

### 3.5.2 Phytochemical screening

Three grams each of the various ground powder of yam bean leaf, seed and root were subjected to preliminary phytochemical screening using standard methods (Brain and Turner, 1975; Trease and Evans, 2004).

#### *3.5.2.1 Test for glycosides (General test)*

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction. A brick red precipitate indicated the presence of reducing sugars.

### *3.5.2.2 Test for terpenoids (Salkowski test)*

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated $H_2S0_4$ (3 mL) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

### *3.5.2.3 Test for flavonoids*

Three methods were used to test for flavonoids. First, dilute ammonia (5 mL) was added to a portion of an aqueous filtrate of each extract. Concentrated sulphuric acid (1 mL) was added. A yellow colouration that disappeared on standing for about 3 min indicated the presence of flavonoids. Second, 3 drops of 1% aluminium solution were added to a portion of the filtrate. A yellow colouration indicated the presence of flavonoids. Third, a portion of each extract was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids.

### *3.5.2.4 Test for saponins*

To 0.5 g of extract was added 5 mL of distilled water in a test tube. The solution was shaken and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken after which it was observed for the formation of an emulsion.

### *3.5.2.5 Test for tannins*

About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. About 3 drops of 0.1 % ferric chloride was added and observed for brownish green or a blue-black colouration.

### *3.5.2.6 Test for steroids*

To 20 mg of plant crude extract mixed with 1 mL methanol and filtrated, 1 mL chloroform and 1 mL concentrated $H_2SO_4$ were added. A yellow green fluorescent indicated the presence of steroids.

## 3.5.3 Determination of total phenolic content

The total phenolic content of the ethanolic crude extracts were determined by the Prussian Blue assay (Price and Butler 1977) as modified by Hagerman (2002). The crude extracts were dissolved in 50 % methanol. An aliquot of 0.1 mL was added to 50 mL of deionised water followed by the addition of 3.0 mL of 0.10 M $FeNH_4(SO_4)_2$ in 0.10 M HCl. After exactly 20 min, 0.008 M $K_3Fe(CN)_6$ was added. The absorbance was read after 20 min at 720 nm using a UV/VS spectrometer (PG Instruments, England). Eight times serial dilution were done for 1 mg / mL solution of each of the extracts. A standard curve was constructed using gallic acid (0.01 – 0.4 mM) and the results expressed as mg phenols / g sample.

## 3.5.4 Antibiotic susceptibility tests

Antimicrobial activities of ethanol extracts of the leaf, seed and root of yam bean were determined against two Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), one Gram-negative bacterium: *Salmonella typhi* and the fungus *Candida albicans*. Minimum inhibitory concentrations (MICs) were determined by broth macrodilution method in 24-wellplates, using a modification of methods reported by Bazzaz *et al.* (2011). Microbial suspension equivalent to 0.5 McFarland standard turbidity was prepared and further diluted to obtain a concentration

of $10^7$colony-forming units (CFU) / mL for the bacteria and $10^6$CFU / mL for fungi. The dried crude organicplant extracts were resuspended in 70% ethanol to a concentrationof 100 mg/ mL for root and seed extracts and 10mg/ mL for leaf extract.The concentrations selected were based on preliminary investigations carried out in this study. Plant extracts were serially diluted two-fold ranging from 1/2 up to 1/16 dilution from the crude extract. In each well already containing 0.5 mL of nutrient broth, 0.4 mL of each extract dilution was mixed with 0.1 mL of microbial suspension resulting in a final volume of 1 mL in each well. Control wells were prepared with culture medium, bacterial or fungal suspension only, plant extracts only and ethanol in amounts corresponding to the highest quantity present. Ciprofloxaxin (0.1mg/ mL) and ketoconazole (0.1mg/ mL) were used as positive control for bacterial and fungus strains, respectively. Plates were incubated for 24 h at 37°C for bacteria, and 48 h at 25°C for *C. albicans*. The growth of microorganisms was assessed by MTT (3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazoliumbromide, Sigma, USA) assay. 0.5 ml of MTT (1.25 mg / mL; dissolved in sterile water) was added to each well and the plates were incubated at 37 °C for bacteria and 25 °C for yeast for 3 h. Microbial growth were determined by observing the change of colour of MTT (bluish to violet formazan where there was growth and clear solution where there was no growth. MIC was defined as the lowest concentration of plant extract showing no colour change (clear). The experiment was repeated in triplicate.

### 3.6 Data analysis

All determinations were replicated three times, mean values and standard deviations were reported. Analysis of variance (ANOVA) was performed by Fishers Least Significance Difference (LSD) test ($p \leq 0.05$) using statistical software SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Physicochemical and functional properties of yam bean starch

#### 4.1.1 Physicochemical properties of yam bean starch from the roots

The starch yield, moisture, ash, lipid, protein, phosphorous and amylose contents and pH of starch from yam bean roots were determined and are presented in Table 4.1.

Table 4.1 Chemical composition of starch from yam bean root

| **Determination (unit)** | **Mean value*** | **Acceptable range / limit** |
|---|---|---|
| **Yield (% w/w db)** | 23.63± 0.02 | None |
| **Moisture (% w/w db)** | 12.47 ± 0.12 | 10-20%[a] |
| **Ash (% w/w db)** | 0.50 ± 0.00 | ≤0.5%[ab] |
| **Phosphorous (% w/w db)** | 0.06 ± 0.02 | <0.5[b] |
| **Protein (% w/w db)** | 0.41 ± 0.02 | <1%[c] |
| **Lipid (% w/w db)** | 0.69 ± 0.23 | <1%[d] |
| **Amylose (%)** | 23.25 ± 0.36 | None |
| **pH** | 4.95 ± 0.31 | 4.5 – 7.5[be] |

*mean values of 3 replicates determined at $p \leq 0.05$

[a]Mufumbo *et al.* (2011); [b]Abo-El-Fetoh *et al.* (2010); [c]Yusuph *et al.* (2003); [d]Hoover (2001); [e]Eliasson(2004).

### *4.1.1.1 Starch yield of yam bean root*

The starch yield of yam bean roots was 23.63 % (Table 4.1). This yield of the roots is in agreement with those reported Stevenson *et al.* (2007) who observed a yield of 22.1 %, although a higher value of 58.65 % was reported by Forsyth *et al.* (2002). Although starch comprises greater than 20% of the root dry matter, the starch content observed was modest because water is predominant in the roots (87.73 – 88.93 %) (Forsyth *et al.*, 2002). Differences in starch yield may be due to cultivar differences or environmental factors (Hoover, 2001).

### *4.1.1.2 Chemical composition of yam bean root starch*

The mean moisture content of yam bean root starch was 12.47 % (Table 4.1) and within the 10 – 20 % range recommended for commercial starches (Mufumbo *et al.*, 2011). Low moisture contents are required for longer storage life as higher moisture contents can lead to microbial damage and subsequent deterioration in quality (Moorthy, 2002). The ash content of the starch was 0.5% (Table 4.1) which is the maximum limit recommended for grade A industrial starches as high ash content indicate presence of impurities or contaminants in the starch (Mufumbo *et al.*, 2011). Phosphorous is an important parameter used to define the functional properties of starches. The phosphorous content of the starch was 0.006 % (Table 4.1), which is similar to value reported for yam bean starch (0.006 %) by Mélo *et al.* (1994) and within the acceptable range for starches (Abo-El-Fetoh *et al.*, 2010). The phosphorus content of starches has been reported to be influenced by growing conditions and storage (Hoover, 2001).The protein content of the starch was 0.41 % (Table 4.1) and according to Yusuph *et al.* (2003) starches with protein content of less than 0.45 can be considered very pure. The

lipid content of yam bean starch was 0.69 % (Table 4.1). This value is similar to report by Amaya-Llano *et al.* (2011) who reported 0.7 % lipid content for yam bean root starch. The lipid content was <1 %, therefore yam bean starch can be considered a low lipid starch which is similar to that of tuber starches (Hoover, 2001). High lipid content in starches is considered undesirable, since it could be responsible for off flavours, high turbidity, higher pasting temperatures and lower viscosity in starches (Roller, 1996).

### *4.1.1.3 Amylose content of yam bean root starch*

The amylose content measured for the starch was 23.25 % (Table 4.1) and similar to reports for yam bean starch by Stevenson *et al.* (2007) and Mélo *et al.* (1994) who observed amylose contents of 23.5 % and 23% respectively. Lower values for yam bean starch amylose have also been reported by other authors (Amaya-Llano *et al.*, 2011; Martınez-Bustos *et al.*, 2007; Forsyth *et al.*, 2002). The yam bean amylose content was lower than legume starches where the amylose content is generally in excess of 25 % (Hoover *et al.*, 2010) but slightly higher or similar to corn (21.40 – 22.5 %), rice (20.50 %), wheat (21.60 - 25.8 %) (Stevenson *et al.*, 2007), potato (25.4 %) (Hoover, 2001) and cassava (18.60 – 24.67 %) (Mufumbo *et al.*, 2011; Hoover, 2001). Generally, based on amylose composition starch may be classified as normal (20 – 30 %), waxy (0 %) or high amylose (50 – 70 %) starches and these starches differ in physicochemical and functional properties that makes them useful in certain food products (Hoover, 2001). Yam bean starch is therefore normal starch and may be used in relevant food applications. Amylose content is one of the characteristics that affect starch functional properties since amylose is an inhibitor of swelling, especially in

the presence of lipids. Therefore the swelling behaviour of starches has primarily been reported to be a property of their amylopectin content (Singh *et al.*, 2006).

#### *4.1.1.4 Yam bean root starch pH*

A mean pH value of 4.95 (Table 4.1) was recorded for the yam bean root starch was also within acceptable range for food starches (Abo-El-Fetoh *et al.*, 2010). The acidity of starch affects stability and taste of food therefore a neutral pH for starch is usually preferable in food systems. The pH considered neutral for starch is not 7. A measurable pH range of at least 4.5 up to 7.5 is considered neutral and acceptable (Abo-El-Fetoh *et al.*, 2010; Eliasson, 2004). Hence the pH of 4.95 recorded for yam bean when used in foods will not mask the flavours in food and hence maintain the taste of food (Eliasson, 2004).

### 4.1.2 Functional properties of yam bean root starch

#### *4.1.2.1 Swelling power and solubility of yam bean root starch*

The swelling power and solubility of yam bean starch were 15.23 g/g and 7.57 % respectively (Table 4.2). According to Schoch and Maywald (1968), starches can be classified, based on their swelling power; as high swelling, moderate swelling, restricted swelling or highly restricted swelling. High swelling starches have swelling power of approximately 30 % or higher at 95 °C. Their granules swell enormously and the internal bonds become fragile toward shear when the starch is cooked in water. Restricted swelling starches have swelling power in the range of 16 – 20 % at 95 °C. The cross linkages in their granules reduce swelling and stabilize them against shearing during cooking in water (Tulyathan *et al.*, 2005). The low swelling power measured for yam

bean is indicated that the starch extract obtained was highly restricted type according to Schoch and Maywald (1968).According to Tulyathan *et al.* (2005) formation of protein-amylose complexes in native starches may be the cause of decrease in swelling power. Additionally, high granule swelling permits the exudation of amylose resulting in high solubility and vice versa (Tulyathan *et al.*, 2005). Hence restricted swelling in the yam bean starch accounted for its low solubility. The results observed for the swelling power and solubility at 95 °C were much lower from those previously reported for yam bean starch (Mélo *et al.*, 2003). According to Mélo *et al.* (2003) a swelling power and solubility of 56.4 g/g and 27% respectively were observed. They also presented the swelling power and solubility of yam bean starch to be higher than those of cassava (48.0 g/g and 26%) and corn (24.0 g/g and 25.0 %). The disparity between swelling power and solubility of starches from different sources may be due to differences in the amylose to amylopectin ratio, the characteristics of amylose and amylopectin in terms of molecular weight / distribution, degree and length of branching and conformation, morphological structure of starch granules and variety (Singh *et al.*, 2003; Hoover, 2001; Li and Yeh, 2001). These factors to a large extent were not investigated in this study.

Table 4.2 Some functional properties of yam bean root starch

| Determination (Unit) | Mean value |
|---|---|
| **Swelling power (g/g)** | 15.23 ± 0.22 |
| **Solubility (% )** | 7.57 ± 0.32 |
| **Paste clarity (% T)** | 2.48 ± 0.09 |
| **Water binding capacity (%)** | 96.81 ± 0.93 |

### *4.1.2.2 Paste clarity of yam bean root starch*

The paste clarity index of yam bean starch was 2.48 % (Table 4.2). Paste clarity expressed as light transmittance provides information on the behavior of starch paste when the light passes through it and depends on the swollen and non-swollen granule remnants (Sandhu *et al.*, 2007). The low paste clarity index of the yam bean starch indicated an opaque paste making it suitable in finished food products like gravies, sauces, salad dressings, puddings and other savory foods (Eliasson, 2004). Higher paste clarity index values of 11.4 % and 7.26 % were reported for yam bean starch by Mélo *et al.* (2003) and Lopez *et al.* (2010) respectively. The lower paste clarity found in the yam bean starch compared to that reported in literature may be attributed to the presence of molecules less susceptible to retrogradation (Lopez *et al.*, 2010; Mélo *et al.*, 2003). Low retrogradation could make the starch useful for reducing stickiness of starchy foods, improving freeze-thaw stability, retarding staling in bread and biscuits and as dough conditioners and crumb softeners in bread (Copeland *et al.*, 2009).

### *4.1.2.3 Water binding capacity of yam bean root starch*

The water binding capacity of the yam bean starch was 96.81 % (Table 4.2), which is higher than that of potato (93 %) and cassava starches (72 – 92 %) but lower than sweetpotato starch (66.3 – 211.6 %) (Tan *et al.*, 2009). Water binding capacity is important in determining the quality and texture of some food products because it stabilizes them against effects such as syneresis which sometimes occur during retorting and freezing. High water binding capacities are desirable as they increase the unit yield of products (Zakpaa *et al.*, 2010; Ellis *et al.*, 2003; Oduro *et al.*, 2001; Baker *et al.*, 1994).

### *4.1.2.5 Pasting properties of yam bean root starch*

The Brabender viscoamylograph (BVA) paste viscosity analysis of the 8 % (db) yam bean starch slurry is presented in Table 4.3. Pasting temperature is indicated by the temperature at which the first detectable viscosity is measured by the amylograph. This index is characterized by initial change in viscosity due to the swelling properties of starches (Afoakwah and Sefa-Dedeh, 2002). The yam bean starch started viscosity at 71.45 °C which is similar to 72.30 °C obtained for yam bean starch in another study (Stevenson *et al.*, 2007). From Table 4.3, the pasting temperature of yam bean was slightly higher than that reported for cassava (68 °C) and lower than mung bean (75.8 °C). The implication is that yam bean starch which has relatively lower pasting temperature than mung bean would be easier to cook and would require less heat for gelatinization to start (Stevenson *et al.*, 2007).

Table 4.3 Pasting properties of yam bean, cassava and mung bean starches

| Parameters | Yam bean | Cassava[e] | Mungbean[f] |
|---|---|---|---|
| **Pasting temperature (°C)** | 71.45 ± 0.63 | 68.00 | 75.80 ± 0.30 |
| **Peak temperature (°C)** | 92.70 ± 0.00 | 78.00 | None |
| **Peak viscosity (BU)** | 563.50 ± 43.13 | 845.00 | None |
| **Viscosity at 95 °C (BU)** | 550.50 ± 38.89 | 590.00 | 795.50 ± 5.00 |
| **Viscosity at 95 °C -Hold (BU)** | 328.00 ± 25.46 | 320.00 | 797.50 ± 2.50 |
| **Paste stability ratio at (95 °C)[a]** | 1.7 | 1.8 | 1.0 |
| **Viscosity at 50 °C (BU)** | 474.00 ± 36.77 | 640.00 | 1715.0 ± 45.0 |
| **Viscosity at 50 °C-hold (BU)** | 429.00 ± 29.70 | NA | NA |
| **Breakdown viscosity (BU)[b]** | 235.5± 17.67 | 525 | NA |
| **Setback viscosity (BU)[c]** | 146 ± 11.31 | 320 | 917.5 |
| **Setback ratio[d]** | 1.4 | 2.0 | 2.0 |

BU - Brabender Units; NA – not available [a] Paste stability at 95°C: viscosity at 95 °C / viscosity at 95 °C-Hold; [b] Breakdown viscosity: peak viscosity - viscosity at 95 °C-Hold; [c] Setback viscosity: viscosity at 50 °C -viscosity at 95 °C-Hold; [d] Setback ratio: viscosity at 50 °C / viscosity at 95 °C-Hold.[e]Adapted from Nwokocha*et al.* (2009); [f] Adapted from Tulyathan*et al.* (2005)

Peak viscosity is a measure of the ability of starch to form a paste; it indicates the highest value of viscosity during the heating cycle and is also the ability of starch to swell freely before their physical breakdown (Sanni *et al.*, 2004) and is linked to the ease of cooking (Afoakwah and Sefa-Dedeh, 2002). Yam bean starch attained a peak

viscosity of 563.5 BU at 92.7 °C and a final viscosity of 550.5 BU at 95 °C. According to Hoover *et al.* (2010), legume starches generally exhibit a high pasting temperature and the absence of peak viscosity reflecting strong interaction between starch chains within the native granule and the orientation of amylose chains to one another. Tuber starches on the other hand are characterized by a peak in viscosity due to weak intergranular forces (Hoover, 2001). Hence, the presence of a rise in viscosity to peak of yam bean starch suggests the presence of weaker bonding forces within the granular interior. Since starches are used in food industries to impart viscosity, the high peak viscosity of yam bean starch makes it suitable as a thickener in food products.

The viscosity of yam bean starch decreased from 550.5 BU to 328 BU after holding temperature constant at 95 °C, for 30 min and had a paste stability ratio of 1.7. The viscosity after holding the temperature constant at 95 °C for 30 min gives an indication of the ease of breakdown of the cooked paste (Afoakwah and Sefa-Dedeh, 2002) while paste stability ratio during the 95°Calso reflects the strength of starch pastes (Oduro *et al.*, 2001). Generally, legume starches are known to increase in viscosity during the holding period and have a high thermal stability while the opposite holds for root and tuber starches (Hoover *et al.*, 2010; Hoover, 2001). A paste stability ratio of 1 for starch paste also implies that it possesses high shear and heat stability while lower or higher values indicate otherwise (Tulyathan *et al.*, 2005). The results therefore imply that yam bean starch paste is unstable and susceptible to shear thinning at high temperatures. This property is important and may be applicable as thin boiling starch in products like processed meat, coated foods such as bread or battered products, confectionery products, instant soups and imitation cheese (Eliasson, 2004).

The breakdown viscosity of yam bean starch was 235.5 BU. This value is twice lower than that of cassava starch (525 BU) (Table 4.3). Breakdown measures the ability of starch to withstand collapse during cooling or the degree of disintegration of granules or paste stability (Tsakama *et al.*, 2010). At breakdown, swollen granules disrupt further and amylose molecules generally leach into solution (Zaidul *et al.*, 2007). According to Adebowale *et al.* (2005), high breakdown in viscosity indicates low ability of the sample to withstand heating and shear thinning during cooking.

At 50 °C, the viscosity of yam bean starch increased from 328 to 474 BU. This increase was to a lesser extent than cassava starch which thickened from 320 to 640 BU (Table 4.3) and mung bean starch which thickened considerably from 797.5 to 1715 BU (Table 4.3). The viscosity after cooling the paste to 50 °C reflects the retrogradation tendency or setback viscosity (Afoakwah and Sefa-Dedeh, 2002). Starch retrogradation is defined as a process, which occurs when the molecular chains in gelatinised starches begin to re-associate in an ordered structure causing an increase in starch rigidity (Lopez *et al.*, 2010). Textural changes leading to undesirable properties such as staling in bread, skin formation, paste gelling and loss of clarity, in prepared starch pastes, has been associated with retrogradation (Afoakwah and Sefa-Dedeh, 2002). This result indicates that yam bean starch has a low tendency to retrograde in food products.

The setback viscosity and setback ratio of yam bean starch were 146 BU and 1.4 respectively (Table 4.3). Setback is defined as the degree of re-association between the starch molecules involving amylose (Yuan *et al.*, 2007). The setback values and setback ratio of yam bean starch paste were lower than that of cassava (320 BU and 2

respectively) and mung bean (917.5 BU and 2 respectively) (Table 4.3). A low setback value is also an indication of a non-cohesive paste while a high setback value is associated with a cohesive paste (Kim *et al.*, 1995).Low setback values increase stability paste in mechanical processes and reduce the tendency in retrograding during cooling, this being the case of starches with high swelling power and consequently high viscosity, such as the potato, cassava and waxy starches. Low setback values are useful for products such as weaning foods, which require low viscosity and paste stability at low temperatures (Oduro *et al.*, 2001) and as such yam bean starch may be useful for such products.

The viscosity of yam bean paste, after keeping the temperatures constant at 50 °C for 30 min (50 °C-Hold) decreased slightly from 474 to 429 BU. This viscosity, also referred to as cold paste viscosity, measures the stability of paste as it might be used. Cold paste viscosity is an important property if extruded starch is to be used as an ingredient in foods that require cold thickening capacity like instant creams, sauces or soups (Alves *et al.*, 1999). During the holding period, the viscosity of yam bean starch was quite stable; hence, yam bean starch paste will maintain its thickening power after cooking.

## 4.2 Phytochemical screening, phenolic composition and antimicrobial activity of yam bean extracts

### 4.2.1 Phytochemical screening of yam bean leaf, seed and root extracts

Investigations on the phytochemical composition of ethanol extracts of yam bean (*Pachyrhizus erosus*) revealed the presence of tannins, steroids, terpenoids and flavonoids in the leaf; tannins, saponins, steroids and terpenoids in the seed; and

saponins, glycosides and terpenoids in the root (Table 4.4). These compounds are known to elicit a broad spectrum of bioactivities (Tiwari *et al.*, 2011; Bejar *et al.*, 2000). The presence of various phytochemicals in yam bean organs illustrates its promising nutraceuticals value. Available information on phytochemical composition of yam bean from various studies show that the seeds contain saponins (Sørensen 1996), rotenoids (Sørensen 1996; Phrutivorapongkul*et al.*, 2002), isoflavonoids (Abid *et al.*, 2006; Phrutivorapongkul *et al.*, 2002), phenylcoumarin (Phrutivorapongkul *et al.*, 2002), pterocarpanoids (Al-Razimi and Alkhathlan, 2000) and an antifungal protein known as SPE-10 (Wu *et al.*, 2002; Song *et al.*, 2005);the tuberous root contains phenolics (Aquino-Bolanos *et al.*,2000; Heredia and Cisneros-Zevallos, 2009), isoflavonoids, pterocarpanoid and furans (Lukitaningsih, 2009); and leaf also contains pterocarpanoids (Al-hazimi and Alkhathlan 2000). The choice of solvent is influenced by what is intended with the extract and the targeted compounds to be extracted (Tiwari *et al.*, 2011). The only reported instance where ethanol was used for extraction was when yam bean seed was investigated for central nervous system depressant activity (Abid *et al.*, 2006) and isoflavonoids were found in the seed extract, which is consistent with results obtained for the seed extracts.

### 4.2.2 Phenolic composition of yam bean leaf, seed and root extracts

The total phenolic content (TPC) of yam bean ethanolic extracts ranged from 17.49 to 52.30 mg GAE/g (Table 4.4). The TPC of the root extract was significantly higher ($p \leq 0.05$) than the seed and leaf extracts. With the exception of the root, there is no available information on the phenolic contents of yam bean leaf and seed. A previous study (Lukitaningsih, 2009) comparing phenolic content of yam bean root extracts using

different solvents reported varying phenolic content in the root extracts depending on the solvent used. According to Lukitaningsih (2009) the TPC of methanol extract was 4.38 mg GAE/g, ethyl acetate extract was 140.76 mg GAE/g, ethyl acetate after hydrolysis was 11.68 mg GAE/g, n-butanol was 13.24 mg GAE/g and none was detected in petroleum ether extract. Phenolic compounds are secondary plant metabolites, which are important determinants in the sensory and nutritional quality of fruits, vegetables and other plants. They form a significant portion of our diet, and are among the most potent and therapeutically useful bioactive substances. Natural phenols in particular, have been reported to have excellent properties as food preservatives as well as having an important role in the protection against a number of pathological disturbances, such as atherosclerosis, brain dysfunction and cancer (Ignat *et al.*, 2011). Thus yam bean may find new application in the food industry and health market as a natural source of food preservative and nutraceuticals.

Table 4.4 Phytochemical constituents and total phenolic content of yam bean leaf, seed and root extracts

| **Phytochemicals** | **Plant part** | | |
|---|---|---|---|
| | Leaf | Seed | Root |
| **Tannins** | + | + | - |
| **Saponins** | - | + | + |
| **Glycosides** | - | - | + |
| **Steroids** | + | + | _ |
| **Terpenoids** | + | + | + |
| **Flavonoids** | + | - | _ |
| **Total phenolics (mg GAE / g dry extract)** | $17.49 \pm 0.04^{a}$ | $34.77 \pm 0.04^{b}$ | $52.30 \pm 0.06^{c}$ |

+ means present, - means absent;

Mean values of total phenolic content with different letters significant different at $p \leq 0.05$

### 4.2.3 Antimicrobial activity of yam bean leaf, seed and root extracts

The extraction yield, antibacterial and antifungal minimum inhibitory concentration (MIC) values of the leaf, root and seed ethanol extracts from yam bean are presented in Table 4.5. The extraction yield of yam bean leaf, seed and root extracts was from 7.18 to 26.75 %. The yield of the root extract was higher than leaf and seed extracts. The difference observed in their extraction yield could be attributed to the selectivity of extraction (Michel *et al.*, 2012). Ethanol is a polar solvent known to extract a wide range of molecules including sugar, glycoside and weakly polar compounds. Thus, ethanol was chosen for the extraction of bioactive compounds from yam bean organs. The

higher yield in root extracts may be due to higher content of more polar compounds in yam bean root than in the leaf and seed.

Table 4.5 Extract yields (%) and antimicrobial activity of *Pachyrhizus erosus* leaf, seed and root crude extracts expressed as MIC (mg/ml)

| **Sample** | **Yield (%)** | **MIC (mg/mL)** | | | |
|---|---|---|---|---|---|
| | | *S. aureus* | *E. coli* | *S. typhi* | *C. albicans* |
| **Leaf** | 7.18 | $5.00 \pm 0.00^{ab}$ | $5.00 \pm 0.00^{ab}$ | $0.625 \pm 0.00^{ab}$ | $5.00 \pm 0.00^{ab}$ |
| **Seed** | 7.46 | $50.00 \pm 0.00^{bc}$ | $50.00 \pm 0.00^{bc}$ | $50.00 \pm 0.00^{bc}$ | $25.00 \pm 0.00^{bc}$ |
| **Root** | 26.75 | $25.00 \pm 0.00^{cd}$ | $25.00 \pm 0.00^{cd}$ | $25.00 \pm 0.00^{cd}$ | $12.50 \pm 0.00^{cd}$ |
| **Ciprofloxacin (mg/ml)*** | | 0.1 | 0.1 | 0.1 | - |
| **Ketoconazole+ (mg/ml)[b]** | | - | - | - | 0.1 |

MIC – minimum inhibitory concentration. Means followed by different superscripts in a column denote values that are significantly different at $p \leq 0.01$.

*positive control for antibacterial assay + positive control for antifungal assay

Due to issues of toxicity during ingestion, aqueous and ethanolic extracts are usually preferred in studying antimicrobial activity of plant food extracts (Michel *et al.*, 2012). Moreover, plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extracts (Parekh *et al.*, 2005). The Minimum

Inhibitory Concentration (MIC) of yam bean ethanolic extracts against three bacteria and yeast is presented in Table 4.5. The result indicates that all the crude extracts depicted an antimicrobial activity with some difference between yam bean parts. The MIC was varied according to yam bean parts: leaf (0.625 – 5 mg/mL), seed (25 – 50 mg/mL) and root (12.5 – 25 mg/mL) across all organisms. This observation may be attributed to differences in phytochemical composition of the various organs extracts as well as differences in sensitivity of the different organisms tested (Ncube *et al.*, 2008). Only MIC values less than 1 mg/ml are considered sufficiently active for crude extracts (Ncube*et al.*, 2008). Therefore the best antibacterial activity was shown by the leaf extract with an MIC value of 0.625 mg/ml against *Salmonella typhi*. This result was unexpected since Gram negative bacteria are generally more resistant to antibiotics than Gram positive bacteria (Michel *et al.*, 2012). The MIC values shown by the leaf extract against *S. typhi* provides prospects for prevention or treatment of typhoid fever. Despite abundant information on phytochemical composition of yam bean, research on its antimicrobial activity is sparse. Antifungal activity of yam bean seed extracts against postharvest pathogens of papaya (Barrera-Necha *et al.*, 2004); and antiviral activity against herpes simplex virus (Phrutivorapongkul *et al.*, 2002) have been reported, though there is no available information on the antimicrobial activities of yam bean root and leaf. The results indicate that yam bean leaf, seed and root can be regarded as a natural source of antimicrobials. Thus yam bean parts may be considered in future for biofortification of foods, to replace synthetic preservatives in food and pharmaceutical products and for the treatment or prevention of microbial contaminations and infections.

# CHAPTER FIVE

# 5.0 CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

The physicochemical and functional properties of starch from yam bean root interestingly illustrate that though yam bean is a legume, its starch has some of its properties similar to that usually found in legume starches whiles some of its properties are similar to those in tuber starches. The starch properties indicate that yam bean root starch has a potential to be used in the food industry in products that require high bulk and for more opaque foods such as mayonnaise, sauces, gravies and dressings. Yam bean starch may also be used particularly, in food or other industrial application that require thick paste, short processing time and reduced heat.

Phytochemical screening of crude extracts from yam bean leaf, seed and root showed the presence of some bioactive compounds like tannins, steroids, terpenoids, flavonoids, saponins, glycosides and phenols. The phenolic contents of yam bean extracts varied among leaf, seed and root with the root having the highest phenolic content. Results of the yam bean phenolic contents indicate that extracts of its leaf, seed and root may be used in certain functional foods to boost their antioxidant capacity.

Antimicrobial analysis of ethanolic crude extracts of yam bean leaf, seed and root indicated that all the yam bean extracts inhibited the growth of bacteria and yeast, with the leaf being the most active part. Hence, yam bean extracts may have potential as

natural food preservatives and as additives in functional foods for prevention and treatment of food-related diseases.

## 5.2 Recommendations

In this study only a single yam bean cultivar was used to study yam bean starch properties, hence the data reported may not be truly representative of the entire yam bean (*P. erosus*) species. Further studies on the physicochemical and functional properties of yam bean starch involving many cultivars and different growing environments should be carried out to determine their full potential for diverse food applications.

Based on the unique phytoconstituents and antimicrobial activity of yam bean, it is necessary to confirm lack of toxicity in the root, seed and leaf extracts before investigating their possible use in functional foods and as food preservatives. Further works are needed to identify and develop extraction methods for the bioactive molecules responsible for antimicrobial effects of yam bean.

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# APPENDICES

## APPENDIX 1. ANOVA and Multiple Comparisons Tables

**Appendix 1A: ANOVA Table for Phenolic Content of *Pachyrhizus erosus* crude extracts**

| | Sum of Squares | df | Mean Square | F-ratio | P-value |
|---|---|---|---|---|---|
| Between Groups | 1817.731 | 2 | 908.866 | 4.459 | 0.000 |
| Within Groups | 0.012 | 6 | 0.002 | | |
| Total | 1817.744 | 8 | | | |

**Appendix 1B: Multiple Comparisons Table for Phenolic Content of *Pachyrhizus erosus* crude extracts**

| (I) Plant extract | (J) Plant extract | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Boundary | Upper Boundary |
| Leaf | Seed | -17.28024* | 0.03686 | 0.000 | -17.4169 | -17.1436 |
| | Root | -34.81092* | 0.03686 | 0.000 | -34.9476 | -34.6743 |
| Seed | Leaf | 17.28024* | 0.03686 | 0.000 | 17.1436 | 17.4169 |
| | Root | -17.53068* | 0.03686 | 0.000 | -17.6673 | -17.3940 |
| Root | Leaf | 34.81092* | 0.03686 | 0.000 | 34.6743 | 34.9476 |
| | Seed | 17.53068* | 0.03686 | 0.000 | 17.3940 | 17.6673 |

*. The mean difference is significant at the $p \leq 0.05$ level.

**Appendix 1C: ANOVA Table for Antimicrobial Activities of *Pachyrhizus erosus* crude extracts**

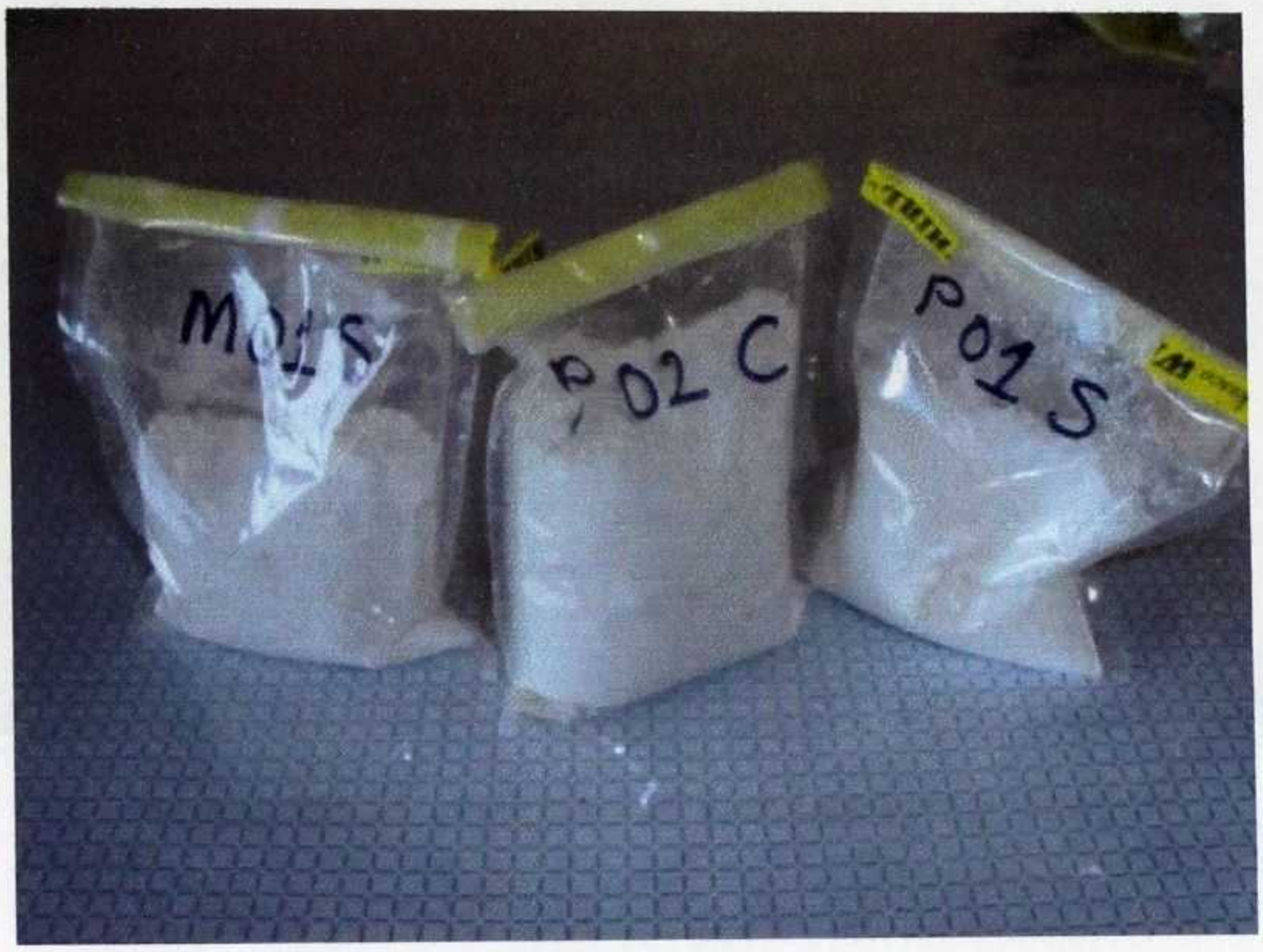
| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 9555.664 | 2 | 4777.832 | 87.551 | .000 |
| Within Groups | 1800.879 | 33 | 54.572 | | |
| Total | 11356.543 | 35 | | | |

**Appendix 1D: Multiple Comparisons Table for Antimicrobial Activities of *Pachyrhizus erosus* crude extracts**

| (I) Plant extracts | (J) Plant extracts | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Leaf | Seed | -39.843750* | 3.015849 | 0.000 | -48.08690 | -31.60060 |
| | Root | -17.968750* | 3.015849 | 0.000 | -26.21190 | -9.72560 |
| Seed | Leaf | 39.843750* | 3.015849 | 0.000 | 31.60060 | 48.08690 |
| | Root | 21.875000* | 3.015849 | 0.000 | 13.63185 | 30.11815 |
| Root | Leaf | 17.968750* | 3.015849 | 0.000 | 9.72560 | 26.21190 |
| | Seed | -21.875000* | 3.015849 | 0.000 | -30.11815 | -13.63185 |

*. The mean difference is significant at the 0.05 level.

## APPENDIX 2. Plates of *Pachyrhizus erosus* Starch, Powder and Crude Extracts

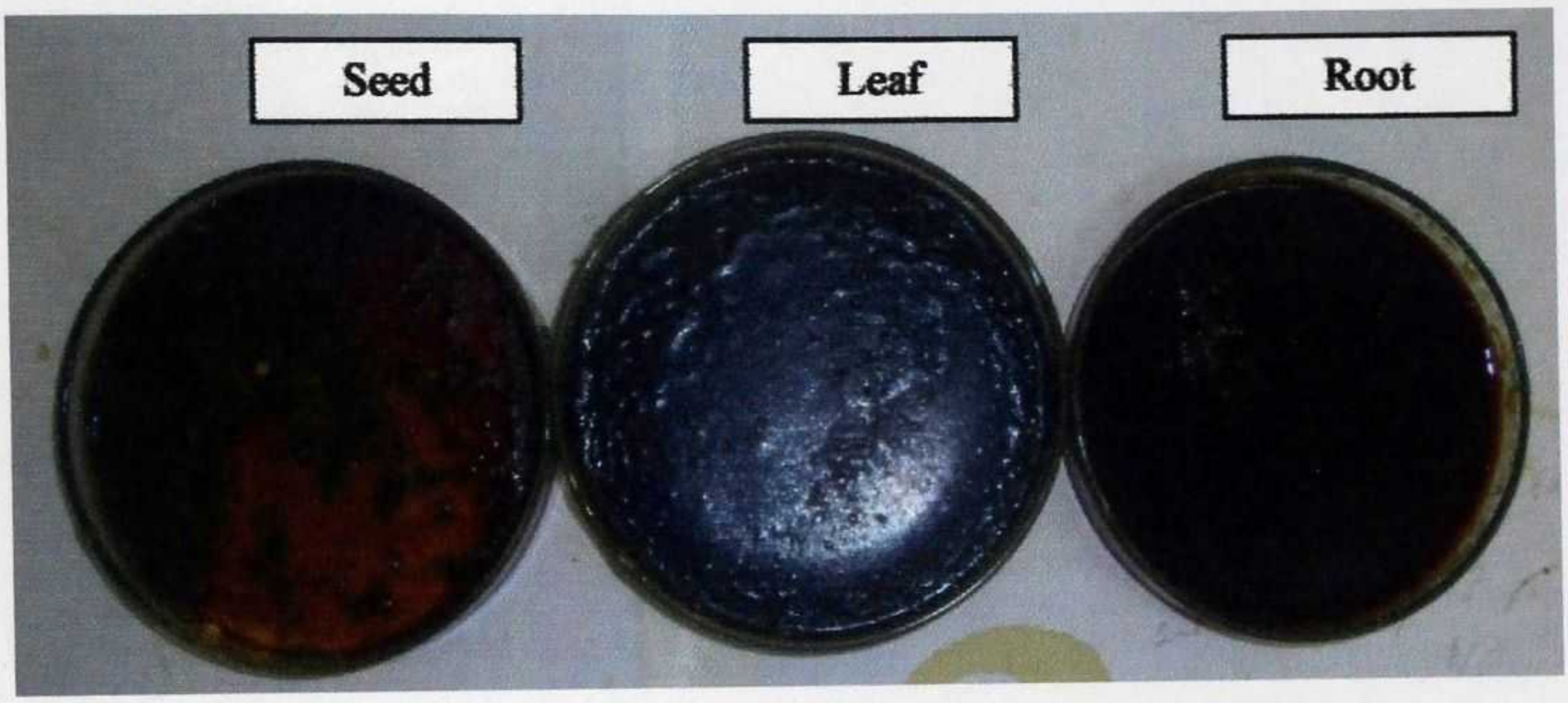



**Appendix 2A:** Samples of yam bean starch


**Appendix 2B:** Samples of yam bean root powder


**Appendix 2C:** Samples of yam bean seed powder


**Appendix 2D:** Samples of yam bean leaf powder




**Appendix 2E:** Samples of yam bean crude extracts