Component identification and starch extraction from (cassava) *Manihot esculenta* (Crantz).

A Thesis Submitted

By

Afroja Sultana

Examination Roll No: MS 070726

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Dedicated

TO MY FATHER

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Abstract

The experiment was conducted at Biochemistry Laboratory of Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh. This study was undertaken for component identification and starch extraction from Cassava (*Manihot esculenta* Crantz). Different parts of the plant were used as experimental material. The presence of different types of components was identified through different tests. In the extraction, two methods were employed viz alcohol extract and water extract. Alcoholic extraction method was superior to water extraction method. The phyto-chemical screening revealed the presence of alkaloids, reducing sugars, gums, sponins in leaf, bark and root extract of cassava. Tannins were identified in leaf extract but absent in root and bark extract. The result of steroids and flavonoids screening showed that no flavonoids were present but specific alkaloids cynogenic glycosides were present in the root of cassava. The Thin Layer Chromatography (TLC) silica gel revealed the presence of amino acids in the root of cassava.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIASTIONS

BCSIR	Bangladesh Council of Scientific and Industrial Resarch
et al	et alia = and other people
etc.	et cet. Era = and the others
pH	Acid Base Measurement
Kg.	Kilogram
m	Meter
cm	Centimeter
gm	Gram
mm	Milimeter
e.g.	exempli gratia = for example
°C.	Degree Celcius
TLC	Thin Layer Chromatography

Chapter One
Introduction

Chapter One

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the world's most important food crops (IFAD, 2001). Cassava crop has multiple and diversified uses in tropical world. Cassava roots are high in calories, an essential source of income and the leaves are a good source of protein and vitamins B and C (Diasolua *et al.*, 2003). Nutritionally, cassava contains potassium, iron, calcium, vitamin A, folic acid, sodium, vitamin C, vitamin B-6, and protein (Nwokoro *et al.*, 2002). It has been utilized as basic food for mankind and animals as well as in the starch industry for glue production and medicinal purpose (Mateles and Tannenbaum, 1968). Diversified because up to 600 derived products from the root of this crop can be processed by the starch industry (O'Hair, 1989, O'Hair *et al.*, 1983; Cabral *et al.*, 2000).

Fig 1: Cassava Plant (*Manihot esculenta* Crantz).

Cassava is applicable in many types of products such as food, confectionery, sweeteners, glues, plywood, textiles, paper, biodegradable products, monosodium glutamate, and drugs. Cassava chips and pellets are used in animal feed and alcohol production (Tan *et al.*, 1984).

Cassava originated in Brazil and Paraguay. Today it has been given the status of cultigens with no wild forms of this species being known (Indira and Kurian, 1977). Cassava is a

tropical root crop, requiring at least 8 months of warm weather to produce a crop (Mathews *et al.*, 1993). It is traditionally grown in a savanna climate, but can be grown in extremes of rainfall. In moist areas it does not tolerate flooding. In drought areas it loses its leaves to conserve moisture, producing new leaves when rains resume. It takes 18 or more months to produce a crop under adverse conditions such as cool or dry weather. Cassava does not tolerate freezing conditions. It tolerates a wide range of soil pH 4.0 to 8.0 and is most productive in full sun (Cabral *et al.*, 2000).

Starch is the major component of cassava flour. A starch that is granular in form and is naturally present in many plants such as grains (wheat or rice), pulses (corn), tubers (potatoes and cassavas), and numerous other plants (Rogers, 1965). Starch is a carbohydrate that is very similar to the carbohydrate cellulose, which is the major component of paper, wood and cotton (Thampan, 1979). Starch exists in tiny granules, which swell and break when boiled in water. This releases the starch molecules, which then all stick together to make a goopy, gluey mess (Vuilleumier, 1993).

Cassava starch is a very important raw material in making glue (International Starch Institute, Denmark, 2004). Cassava starch–based dextrates are excellent adhesives and are used in many applications including pre-gummed papers, tapes, labels, stamps, and envelopes (Rajeshwarisivaraj *et al.*, 2001). The glue is mainly used as adhesive. One of the most popular uses of glue is creating models. These can be model airplanes, model toys, model cars, boats, figurines, almost anything. Another important used for building models (Roble *et al.*, 2003). There are about 35 major chemical types of adhesives in use today, each with dozens of sub-types. Combine this with all the solid and semi-solid things that can think of

like wood, paper, glass, metal, rock, fiber, leather, skin, plastics, rubber etc. (Hong *et al.*, 2001).

The world glue production per year is about 8320 metric tons (Economic Statistical Report, 2004). Bangladesh requires a large amount of glue every year. But about 56% of glue import per year in Bangladesh (BBS, 1998). About 4% of world starch production moves in trade and most of this is cassava starch (Chadha, 1961).

Plant secondary metabolites have been used for mankind as remedies since the beginning of civilization. Now a day, they still play an important role in health care for about 80% of the world's population (Carvalho *et al.*, 2000). Cassava has diverse bioactive metabolites like alkaloids, reducing sugars, gums, saponins etc. They have formed the therapeutic basis of herbal medication (Jennings, 1959). The principal amino acids present in the protein are arginine, histidine, isoleucine, leucine and iysine in leaves and roots but they are low amount (Bolhuis, 1966).

Since, Cassava starch is a good source of glue production and it has the source of secondary metabolites, for this valid reason, I have tried to identify the secondary components and starch extraction for glue industries. Therefore, the present research has been undertaken with the following objectives:

1. to develop the effective starch extraction process and compare the amount of starch between Cassava and Potato.
2. to screen-out the present of amino acids, reducing sugar and different secondary metabolites in Cassava.

Chapter Two
Literature Review

Chapter Two

REVIEW OF LITERATURE

Common name / local name of Cassava are Yuca, Tapioca, Manioc, Cassava (Bangladesh). Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Malpighiales Family: Euphorbiaceae, Subfamily: Crotonoideae, Tribe: Manihoteae, Genus: Manihot, Species: *M. esculenta* and Binomial name: *Manihot esculenta* Crantz. Synonyms are *M. ultissima* Phol and *M. aipi* Phol (Indira and Kurian, 1977). Two types of cassava recognized are "bitter" and "sweet."

Cassava is a perennial shrub, with latex in all its parts, which produces enlarged tuberous roots. There are over 100 cultivars and there is great variation in the form of the plant. The height ranges from about 1 to 3 m or more. The stems are usually slender and glabrous, with leaves borne near the apex; the lower parts of the stems have nodes made conspicuous by prominent leaf scars. Branching is variable; some cultivars branch near the base and are spreading in form, others are erect and branch nearer the apex. Stems vary in colour, being grey or silvery, green, greenish-yellow, reddish-brown, or streaked with purple. The leaves, which are spirally arranged with phyllotaxis 2/5, have petioles 5-30 cm long, usually longer than the blades; the blades are deeply palmately divided with 5-7 (occasionally 3-9) lobes, each 4-20 cm long and 1-6 cm wide. They vary in colour from green to reddish; the petiole and midrib may be deep red. Older leaves are shed leaving the prominent leaf scars mentioned above. The flowers are borne in axillary racemes near the ends of branches, and are monoecious, pale yellow or red, 1-1.5 cm in diameter. The fruit is a six-winged capsule with 3 ellipsoidal seeds each about 12 mm long. Root tubers develop by a process of secondary thickening as swellings on adventitious roots a short distance from the stem (Jennings, 1959, Strasser *et al.*, 1970 and Subramanyam and Mathur, 1956).

Table1. Nutritional status of cassava tuberous root flesh.

Cassava nutrition components (%)	Tuberous root flesh
Water	62%
Carbohydrates	35%
Proteins	0.5-1.5%
Fat	0.3%
Fibre	1-2%
Mineral matter	1%

(Source: FAO, 2002).

Since 1990, UNICEF figures have consistently shown Nigeria as the world's largest cassava producer - moving from its fourth rank to beat Brazil, Thailand and Zaire to the second, third and fourth positions. Cassava is widely used in the following countries: Guyana, Barbados, Venezuela, Panama, Cuba, Peru and the Dominican Republic, Costa Rica, Brazil, Argentina, India, Thailand, China and some 31 countries in the continent of Africa (Grace, 1971).

Cassava (*Manihot esculenta*) is the most important staple crop in the tropics, where more than 800 million people eat this crop (FAO, 2002). Cassava has been the subject of worldwide efforts to develop its potential as a food, a feed and an industrial commodity (Nestel, 1975). The primary disadvantage of Cassava is very low protein content (less than 2%), but it yields the highest amount of starch, in that about 35% of its carbohydrate content. Cassava has another disadvantage; the fleshy roots contain poisonous compounds cyanogenic glycosides that must be removed before eating (Nassar, 1989). The roots are also used for animal feed and the starch is used for glue, laundry starch, and tapioca pudding (Manurung, 1974). The roots of the Cassava have to be left to grow for at least 10 months to provide high starch content (Rickard and Coursey, 1981).

An important food source:

More than two-thirds of the total production of cassava is used as food for humans, with lesser amounts being used for animal feed (Nwokoro *et al.*, 2002) and industrial purposes (Nestel, 1975). The future demand for fresh cassava may depend on improved storage methods, but the markets for cassava as a substitute for cereal flours in bakery products and as energy source in animal feed rations are likely to expand (Flaws and Palmer, 1968).

Starch is one of the most important plant products to man. It is an essential component of food providing a large proportion of the daily calorific intake (Chanseak, 1969). In West Africa, cassava flour and gari (a processed cassava product) are consumed in large quantities. Cassava starch is recommended for use in extruded snacks for improved expansion. It is also used as a thickener in foods that are not subject to rigorous processing conditions. Cassava starch, which is very bland in flavor, is used in processed baby foods as a filler material and bonding agent in confectionary and biscuit industries (Walters, 1983).

Cassava as an industrial base:

Paper industry

The paper industry uses various types of starches at different stages of the manufacturing process for different purposes. Currently, the most common starches used for paper manufacture are from maize, potato, and cassava (Williams, 1972).

Cassava starch has very good properties that are highly desirable for the paper manufacturer. Cassava starch, as a dominant source of starch in Nigeria, possesses a strong film, clear paste, good water holding properties, and stable viscosity (Williams, 1972). It should be the most suitable material for the paper industry in West Africa. In the paper and board industries, starch is used in large quantities as a coating agent and as an adhesive (Phillips, 1973).

Cassava starch has been widely used as a tub size and beater size in the manufacture of paper, in the past mainly on account of its low price (Nitis and Suarna, 1977).

Textile industry

In the textile industry, starch is used in three main areas: sizing, finishing and printing. Approximately 80% of the starch used in textiles is used in sizing (Scott *et al.*, 2000). Textile printing, or the impression of a design on fabrics, requires a carrier for the dyes and pigments and modified starches have found special uses in these applications (Leekokchoo and Hutagalung, 1972). Modified starches are frequently mixed with other industrial gums to give the required viscosity and paste characteristics (Phillips, 1973).

Glass fiber sizing is a special case among textile fibers sized with starches. Starches finding use as glass fiber sizes are those which have high film strength and are incompletely cooked under normal cooking conditions, but which still form a good film. Although most of the glass-sized yarn made goes into fabric uses, a portion is used for electrical insulation and in circuit boards (Phillips, 1973).

Adhesives industry

Starch is also the main raw material in glue and adhesive industries (International Starch Institute, Denmark, 2004). Starch is a popular base for adhesives, particularly those designed to bond paper in some form to itself or to other materials such as glass, mineral wool, and clay. Starch can also be used as a binder or adhesive for non paper substances such as charcoal in charcoal briquettes, mineral wool in ceiling tiles and ceramics before firing. The starches most commonly used for the manufacture of adhesive pastes are maize, potato, and cassava; of this cassava starch appears more suitable in several respects (Walters, 1983).

Cassava starch adhesives are more viscous and smoother working. They are fluid, stable glues of neutral pH that can be easily prepared and can be combined with many synthetic resin emulsions. Corn and rice starches take a much longer time to prepare and a higher temperature to reach the same level of conversion. For top-quality work, cassava starch is thought to be ideal, because it is slightly stronger than a potato starch adhesive while being odorless and tasteless, excellent as an adhesive for postage stamps, envelope flaps, and labels. Certain potato pastes have bitter tasting properties while cereal starches exhibit a cereal flavor (Walters, 1983).

Adhesives from cereal starches have poor mobility and are more suitable for purposes where short parts are required, for example, in the manufacture of corrugated boards. Wheat starch is usually used as a thick paste in the adhesive base for bill posting and the paper bag industry. Poster pastes used for billboards, wallpaper, and other pasting operations that the manual alignment of patterns or edges also tend to utilize high-viscosity starches (Phillips, 1973).

Cassava starch is also an important raw material for powder in the cosmetics industries. In detergent soap manufacture, starch is used to get better recovery and to improve the shelf life of detergents. While in the rubber and foam industries, starch is employed for getting better foaming and color (Scott *et al.*, 2000).

Cassava starch can be converted to maltotriose, maltose, and glucose as well as to other modified sugars and organic acids (Tan *et al.*, 1984). Starch from cassava can be used to make fructose syrups (Vuilleumier, 1993) and formulate gelatin capsules (Nduele *et al.*, 1993). The use of cassava as a source of ethanol for fuel is already being exploited and very promising. Recently, Roble *et al.*, (2003) demonstrated the production L-Lactic acid from

raw cassava starch in a bioreactor using *Aspergillus awamori* (fungus) and *Lactococcus lactis spp.* lactis (bacteria). Furthermore, cassava dregs could be employed for phytase production after the addition of a nitrogen source and mineral salts (Hong *et al.*, 2001), while activated carbons prepared from waste cassava peel are efficient as adsorbents for dyes and metal ions (Rajeshwarisivaraj *et al.*, 2001).

Furthermore, starch is also an important ingredient for the sugar industry, which was otherwise relying upon sugar cane and beet sugar (Kuhnlein and Receveur, 1996). Some of most common industrial uses of starch are: Ceramic Industry: binder; Rubber Industry: filler (Muller, 1977).

Two categories of methods typify cassava processing: methods that require a lot of water and those that do not require much water (Sreeramanamubry, 1945). Most traditional products have modest water input requirement (relatively low 5 m^3/tone product). For starch production, however, water is required at all stages irrespective of processing scale. Therefore, large volumes of water are needed - on average 2-6 times more than traditional production (Webb *et al.*, 1975).

Large factories possess the technological ability to efficiently use water, often incorporating recycling systems. The theoretical maximum water conservation rate is never achieved, as a minimum quantity of water is required for complete washing of starch. If this need cannot be satisfied, starch quality is adversely affected. (Tran, 1998; Howeler, 1996).

The starch extraction process of Cassava and Potatoes are near about same (Brook *et al.*, 1979). A potato is a starchy edible tuber native to South America and cultivated all over the world. The common white potato is classified as *Solanum tuberosum* (Mateles and Tannenbaum, 1968).

Potato starch has been produced in Denmark on an industrial scale since 1900. 75% of the potato crop is grown for industrial processing and the Danes produce per capita more starch than any other nation. (Hutagalung and Tan, 1976).

Table2: Various constituents of fresh potato.

Constituents	Typical analysis
Starch, dry substance	80%
Water	20%
Ash	0.3%
Sand	0.02%
Protein	0.09%
Phosphor, P	0.07%
Calcium, Ca	0.03%
Iron, Fe	3 ppm
Cold water soluble	0.1%

(Source: FAO, 2002).

Another source of starch production is from maize, rice, sweet potato, canna, lentils etc. (Rojas *et al.*, 1996). Vietnam is the first position of canna starch production (Roy, 2007). The Australian produce lentils starch (contain 44–45% starch) is the higher source (Roy, 2007).

Secondary metabolites, also known as natural products, are those products (chemical compounds) of metabolism that are not essential for normal growth, development or reproduction of an organism. In this sense they are "secondary". (http://www.open2.net/sciencetechnologynature/worldaroundus/chemicalpl nts.html).

Plants produce secondary metabolites as a response to adverse environmental conditions or in particular developmental stages. For example, exposure to UV (Ultra-violate light) radiation induces the biosynthesis of UV (Ultra-violate light) absorbing compounds (http://www.britannica.com/EBchecked/topic/531748/secondary-metabolites).

Plant secondary metabolites are a generic term used for more than 30,000 different substances which are exclusively produced by plants. The plants form secondary metabolites e.g. for protection against pests, as colouring, scent, or attractants and as the plant's own hormones. It used to be believed that secondary metabolites were irrelevant for the human diet. The importance of these substances has only recently been discovered by scientists (Alan *et al.*, 2006).

Secondary metabolites carry out a number of protective functions in the human body. Plant secondary metabolites can boost the immune system, protect the body from free radicals, kill pathogenic germs and much more (Alan *et al.*, 2006).

In contrast to the primary metabolites (carbohydrates, fats, proteins, vitamins and mineral nutrients) secondary metabolites do not have nutrient characteristics for human beings. They are usually found in very small amounts but have an effect on humans (White *et al.*, 1998).

Secondary metabolites have a scientifically proven effect on health. However many of these effects are unknown. A diet which is rich in plant foods contains a variety of secondary metabolites and contributes to protecting the body against cancer and cardiovascular illnesses. Secondary metabolites and their effects are currently being intensively researched (Claude and Denis, 1990).

Cassava has diverse bioactive metabolites like alkaloids, reducing sugars, gums, saponins etc. (Jennings, 1959). The principal amino acids present in the protein are arginine, histidine, isoleucine, leucine and iysine in leaves and roots but they are low amount (Bolhuis, 1966).

Chapter Three

Materials and Methods

Chapter Three

MATERIALS AND METHODS

Phytochemical screening:

Collection of plant materials:

The Cassava (*Manihot esculenta*) plant was collected from Bangladesh Council of Scientific and Industrial Resarch (BCSIR), Rajshahi, Bangladesh during April 2008.

Drying and grinding:

The collected plant parts (roots, leaves, and bark) were cleaned with water and cut into pieces. Plant parts (peeled roots, leaves, and bark) were sun-dried for fifteen days after cutting into small pieces. The plant parts were ground into a fine powder with the help of a grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Crude extraction:

Ethanol extract:

According to Harborne (1984), the powdered materials (400gm of leaves, 200gm of bark and 100gm of roots separately) were taken into three clean, flat bottomed glass containers (4 liters) and soaked in 1650ml, 1200ml and 500ml of absolute ethanol, respectively. The container with its contents was sealed and kept for a period of 12 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman 2 mm filter paper. The extract was concentrated by evaporation process.

Water extract:

According to Harborne (1984), the powdered materials (400gm of leaves, 200gm of bark and 100gm of roots separately) was taken in three clean, flat bottomed glass containers (4

liters) and soaked in 1650ml, 1200ml and 500ml of pure water, respectively and then kept for a period of 12 days accompanying occasional shaking and stirring. Then the whole mixture underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman 2 mm filter paper. The extract was concentrated by evaporation process.

Evaporation of the solvent:

The filtrate (ethanol or water extract) was taken in the glass jug and evaporated by air at room temperature. The leaf extract rendered a dark green color and the bark extract turned into brown color and root extract white color. The concentrate was designated as crude extract (ethanol or water extract).

Reagents and other preparation:

The following reagents were prepared and used for the different chemical group tests.

- **Mayer's reagent**

1.36 gm mercuric iodide in 60 ml of water was mixed with a solution contains 5 gm of potassium iodide in 20 ml of water.

- **Dragendroff's reagent**

Two stock solutions were prepared:

Stock solution 1: 0.6 gm bismuth sub- nitrate in 2 ml conc. HCL and 10 ml water

Stock solution 2: 6 gm potassium iodide in 10 ml water.

These stock solutions were mixed together with 7 ml conc. HCL and 15 ml water and the whole solutions were diluted with 400 ml water.

- **Fehling's reagent:**
- **Fehling's solution A**

34.64 gm copper sulphate was dissolved in a mixture of 0.50 ml of sulfuric acid and sufficient water to produce 500 ml.

- **Fehling's solution B**

176 gm of sodium potassium tartarate and 77 gm of sodium hydroxide were dissolved in sufficient water to produce 500 ml.

Equal volume of solution A and B were mixed at the time of use.

- **Benedicts reagent**

1.73 gm cupric sulphate, 1.73 gm sodium citrate and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

- **Molish reagent**

2.5 gm of pure α-naphthol was dissolved in 25 ml of ethanol.

- **Libermann-Burchardr reagent**

5 ml acetic anhydride was carefully mixed under cooling with 5ml concentrated sulfuric acid. This mixture was added cautiously to 50 ml absolute ethanol with cooling.

- **Sodium picrate saturated filter paper:**

Prepared by dipping a filter paper strip in sodium picrate solution. Three gm of sodium picrate was added in 15 ml of water to make the sodium picrate solution.

- **Ninhydrin solution preparation:**

0.10 gm of ninhydrin was taken in a test tube. Ten ml of 2-propanol was taken in the test tube. The end of the test tube was sealed with parafilm and shaked.

Thin Layer Chromatography (TLC):

TLC test was conducted to know the presence of amino acids in the leaf, bark of root extracts of cassava. Each TLC silica gel (The Silica Gel 60 F 254, Mark, Germany) was cut horizontally into 16 small plates which were 6 cm tall by 2 cm widths; which used for ninhydrin colors of amino acids. The chromatographic was consisting of two phases; stationary phase (the silica gel) and the mobile phase (the mixture of solvents such as chloroform: methanol; 3:2). (Fowden, 1970).

- **Equipments/ materials used:**
  - Bowl
  - Motor and Pestle
  - Sieves
  - Water
  - Knife
  - Sprit Lamp
  - Beaker
  - Filter Paper
  - Test Tube

Tests for reducing sugar:

The following tests were done to confirm the presence of reducing sugar in the leaf, bark and root samples of cassava. Three tests have been carried out for reducing sugar. They are given below.

- **Benedict's test**

0.5 ml of aqueous extract of the plant materials (roots, leaves, and bark extract) were taken in a test tube. Five ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously. A red color precipitate of cuprous oxide was formed in the presence of a reducing sugar.

- **Fehling's test (Standard test)**

Two ml of an aqueous extract of the plant material (roots, leaves, and bark extract) were added to1 ml of a mixture of equal volumes of Fehling's solutions A and B. Boiled for few minutes. A red or brick red color precipitate was formed in the presence of a reducing sugar.

- **Combined reducing sugar test**

One ml of aqueous extract was boiled with 2ml of dilute hydrochloric acid for 5 minutes. Cooled and neutralized the mixture with sodium hydroxide solution and perform the Fehling's test as described above. A brick-red precipitate was formed in the presence of a reducing sugar due to the release of reducing sugars on hydrolysis.

Tests for tannins:

The following tests were performed weather the tannins are present in the leaf, bark and root of cassava.

- **Ferric chloride test**

5 ml solution of the ethanolic extract (roots, leaves, and bark extract) was taken in a test tube. Then 1 ml of 5% ferric chloride solution was added. Greenish black precipitate was formed in the presence of tannins. No greenish-black precipitate was formed from the balk and root extract; so the bark and root did not contain any tannin.

- **Potassium dichromate test**

Five ml solution of the ethanolic extract (roots, leaves, and bark extract) was taken in a test tube. Then 1 ml of 10% potassium dichromate solution was added. precipitate was formed in the presence of tannins. No Yellow precipitate was formed from the balk and root extract; so the bark and root did not contain any tannin.

Lead acetate test

One ml. of 10% lead acetate solution was added to 5 ml of extract (roots, leaves, and bark extract) solutions. A yellow precipitate was formed. It was indicated the presence of tannins. No Yellow precipitate was formed from the balk and root extract; so the bark and root did not contain any tannin.

Test for flavonoids:

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract (roots, leaves, and bark extract) of the plant material. An immediate red color was not developed which indicates the absence of flavonoids.

Test for saponins:

One ml solution of the extract (roots, leaves, and bark extract) was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. A layer of foam was formed in the presence of saponins.

Test for gums:

Five ml solution of the extract (roots, leaves, and bark extract) was taken and then molish reagent and sulphuric acid were added. Red violet ring was produced at the junction of two liquids in the presence of gums and carbohydrate.

Test for steroids:

- **Libermann-Burchard test**

One ml solution of ethanolic extract (roots, leaves, and bark extract) was taken and then 2 ml Libermann-Burchard reagent was added. Reddish purple color was not produced which indicated the absence of steroid.

- **Sulphuric acid test**

One ml solution of ethanolic extract (roots, leaves, and bark extract) was taken and then 1ml sulphuric acid was added. Red color was not produced in the absence of steroid.

Test for alkaloids:

- **Mayer's test**

Two ml solution of the extract and 0.2 ml of dilute hydrochloric acid was taken in a test tube. Then 1 ml of Mayer's reagent was added. Yellow precipitate was formed in the presence of alkaloids.

- **Dragendroff's test (general lab test)**

Stired about 0.5 gm of extract (roots, leaves, and bark extract) with 5 ml of 1% HCL on a steam bath (100°C) and filtered. Treated 1 ml of the filtrate with a few drops of Dragendroff's reagent. Showed orange-red or orange precipitate.

- **Wagner's test**

Two ml solution of the extract and 0.2 ml of dilute hydrochloric acid was taken in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added. Reddish brown precipitate was formed in the presence of alkaloids.

Specific alkaloids test:

Two mg of dried power materials was taken in a small conical flask and moistened with water. Added 4 drops of chloroform. A sodium picrate saturated filter paper was hanged at the neck of the flask with the helped of cork. The flask was warmed in the temperature range of 30-35 °C. The filter paper was turned into brick red/maroon color because the release of HCN vapors due to hydrolysis of cynogenic glycosides. This test was specific test for cynogenic glycosides. The roots of cassava contain this alkaloid. (Elmore, 1968).

Ninhydrin colors of amino acids:

One piece of small silica gel plate was marked near the top of the plate with the helped of blade. Then two drops of plant materials (dissolved in ethanol or methanol or water) was placed in the bottom of the plate. The drops of the plant materials were not too much bottom or too much side and dried it. Then the dried plate was placed in the solvent of chloroform:methanol (3:2). The solvent ran up to the mark of the plate. When the solvent ran to the mark, the Chromatography was allowed to dry and was then sprayed with a solution of ninhydrin and heated by sprit lamp. Ninhydrin reacted with amino acids to give light purple colored compounds (Brenner *et al.*, 1969).

Extraction of starch from cassava (*Manihot esculenta*)

The roots of cassava were collected from Bangladesh Council of Scientific and Industrial Resarch, Rajshahi, Bangladesh. About 2 Kg cassavas were collected to produce starch. The roots of cassava were peeled by hand.

The peeled roots were then washed with water for removal the fiber and other unwanted materials. Grated the cassava roots with pestle and mortar for fine particles to easily sieving and then added water with the grating materials.

The grated roots were filtered to separate watery portion and solid particles. Only the watery particles contained starch. The watery precipitates were settled in the bowl. This step was permitted to stay for about 4-6 hours. The watery supernatant was drained off and the final precipitates were dried under the sunlight. The dried starch was then milled to produce starch. After milling the starch was collected in a bag for storage.

Extraction of starch from potato (*Solanum tuberosum*):

The potatoes were collected from Gallamari Market, Khulna. Potatoes were coarsely cleaned for removal of soil and stones. The potato was washed before grating. The purified potatoes were mashed by means of a pastel and mortal to produce fine particles. Added with water.

The grated particles were filtered to separate watery portion and solid particles. Only the watery particles contained starch. The watery precipitates were settled in the bowl. This step was permitted to stay for about 4-6 hours. The watery supernatant was drained off and the final precipitates were dried under the sunlight. The dried starch was then milled to produce starch. After milling the starch was collected in a bag for storage.

Chapter Four

Results and Discussion

Chapter Four

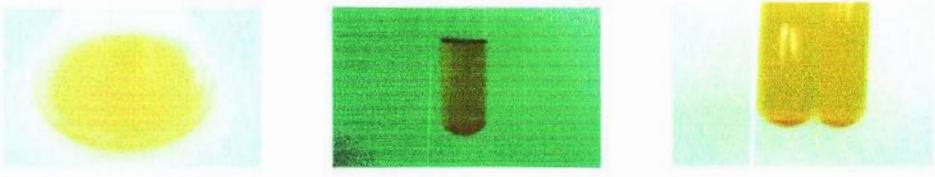
RESULTS AND DISCUSSION

Phytochemical screening:

The crude ethanolic extract of *Manihot esculenta* was subjected for chemical group test. Alkaloids, saponins, reducing sugers, tennins and gums were found in the leaf extract. Ethanolic bark extract was found to contain alkaloids, reducing sugars, saponins, tennins and gums. In root extract alkaloids, saponins, reducing sugers and gums were found. The results are summarized below in tables.

Table 3: The presence of alkaloids in leaf, bark and root extract of cassava.

Sample Materials	Observation	Inference
Leaves	Reddish brown precipitate was observed	Presence of alkaloid
Barks	Red-orange precipitate was observed	Presence of alkaloid
Roots	Red-orange precipitate was observed	Presence of alkaloid



(A) (B) (C)

Fig 2: The presence of alkaloids in different parts of cassava plant (A) leaf, (B) bark and (C) root.

Alkaloids were identified in all of the plant parts. Reddish brown precipitate was observed of leaf extract. Red-orange precipitate was observed in both of barks and roots extract. The other plants of this family euphorbiaceae contain a large variety of phytotoxins (toxic substances produced by plants), alkaloids, glycosides, and ricin-type toxins (Okezie and Kosikowski, 1999). According to Wagner's test, Dragendroff's test and Mayer's test (Kupchan *et al.*,

1973), the extract was observed red-orange precipitate that indicated the presence of alkaloids.

Flavonoids were not identified in any parts of cassava. Immediate red color was not formed of leaves, barks and roots extract. According to Haner *et al* (1961), different plants parts showed red color immediately to indicate the presence of flavonoids. The other plants such as *Markhamia zanzibarica* and *Mariscus hemisphaericus* of the family euphorbiaceae showed red color precipitates and indicated presence of flavonoids in leaf, root and fruits (Okezie and Kosikowski, 1999). From the discussion, it verifies the absence of flavonoids in most plants of the family euphorbiaceae.

Table 4: The presence of secondary product (saponins) in leaf, bark and root extract of cassava.

Sample Materials	Observation	Inference
Leaves	One centimeter layer of foam was formed	Presence of saponins
Barks	One centimeter layer of foam was formed	Presence of saponins
Roots	One centimeter layer of foam was formed	Presence of saponins

Saponins were identified in all of the plant parts. One centimeter layer of foam was formed of leaves, barks and roots extract. The other plants of the family euphorbiaceae such as *Markhamia zanzibarica* and *Mariscus hemisphaericus* also contain saponins (Okezie and Kosikowski, 1999). Different types of plants such as *Chenopodium procerum* and *Euclea natalensis* (family euphorbiaceae) (www.worldbotanical.com/ african_plants.htm) was not found sponins.

Steroids were not identified in any parts of the plant. Ethanolic layer did not produce reddish purple color of leaves, barks and roots extract. The other plants such as *Markhamia zanzibarica* and *Mariscus hemisphaericus* of the family euphorbiaceae; ethanolic layer was

not acquired reddish purple color and not indicated presence of steriods in leaves, fruits (Okezie and Kosikowski, 1999). Most of the plants of family euphorbiaceae were found not steroids (www.worldbotanical.com/ african_plants.htm).

Table 5: The presence of reducing sugars in leaf, bark and root extract of cassava.

Sample Materials	Observation	Inference
Leaves	Brick red colored was precipitate	Presence of carbohydrates
Barks	Brick red colored was precipitate	Presence of carbohydrates
Roots	Brick red colored was precipitate	Presence of carbohydrates

(A) (B) (C)

Fig 3: The presence of reducing sugars in different parts of cassava plant (A) leaf, (B) bark and (C) root.

Reducing sugars was identified in all parts the plant. Brick red colored precipitate of leaves, barks and roots extract was formed. The pulpy portion of fruit, dried and freed from the *Adenium obesum* (family euphorbiaceae) contains up to 36% reducing sugar (Okezie and Kosikowski, 1999). The other plants such as *Markhamia zanzibarica* and *Mariscus hemisphaericus* of the family euphorbiaceae; were found to contain resucing sugar (Okezie and Kosikowski, 1999).

Tannins were identified in leaf extract but not found in bark and root extract. Greenish black precipitate was formed in leaves extract and yellow precipitate was not obtained in bark and root extract. The pulpy portion of fruit, dried and freed from the *Adenium obesum* (family euphorbiaceae) contains tannins up to 36% (Okezie and Kosikowski, 1999). The other plants such as *Markhamia zanzibarica* and *Mariscus hemisphaericus* of the family euphorbiaceae; was found to contain tannins in their leaves, fruits. (Okezie and Kosikowski, 1999).

Table 8: Summary of different chemical group tests in different plant parts of cassava.

	Plant Parts	Steroid	Alkaloid	Flavonoid	Reducin sugar	Tannin	Gum	Saponin
Ethanolic extract	Leaves	-	+	-	+	+	+	+
	Roots	-	+	-	+	-	+	+
	Barks	-	+	-	+	-	+	+

+ =Presence; - =Absence.

In our study, steroids and flavonoids were absent in all parts of the cassava plant. The root and bark extract of plant was absent of tannins. All other components such as reducing sugars, tannins, gums, alkaloids and saponins were identified in all parts of the plant.

Cassava was not indicated for the presence of flavonoids and steroids. The plants such as *Markhamia zanzibarica* and *Mariscus hemisphaericus* of the family euphorbiaceae were showed red color precipitates and indicated presence of flavonoids (Okezie and Kosikowski, 1999). In the plants *Markhamia zanzibarica* and *Mariscus hemisphaericus* of the family euphorbiaceae, ethanolic layer was not acquired reddish purple color and not indicated for presence of steriods (Okezie and Kosikowski, 1999). Tannins were identified in leaf extract

but not found in root and bark extract. The pulpy portion of fruit, dried and freed from the *Adenium obesum* contains tannins 36.10% (Okezie and Kosikowski, 1999).

All other components such as reducing sugars, gums, alkaloids and saponins were identified in all parts of the plant. The other plants of the family euphorbiaceae contain a large variety of phytotoxins (toxic substances produced by plants), alkaloids, glycosides, and ricin-type toxins (Okezie and Kosikowski, 1999). The pulpy portion of fruit of *Adenium obesum* (family euphorbiaceae) contains gums and reducing sugar (Okezie and Kosikowski, 1999). The other plants of this family euphorbiaceae such as *Markhamia zanzibarica* and *Mariscus hemisphaericus* also contain saponins (Okezie and Kosikowski, 1999).

Test for cynogenic glycosides:

The root flour is one of the staple foods of the some African and Latin American countries (Ha and Swain, 1996). The specific alkaloid such as cynogenic glycosides is necessary to destroy before eating because it is toxic to human. The large amount of cynogenic glycosides is present in the roots. The fruit of *Ricinus communis* and *Boophone disticha* (family euphorbiaceae) contain the toxic compound cynogenic glycosides (http://www.hort.purdue.edu/newcrop/duke_energy/Ricinus_communis.html#Chemistry).

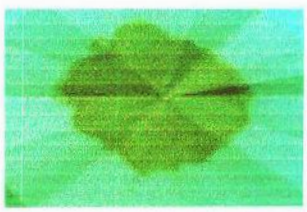


Fig 5: Presence of cynogenic glycosides of cassava root flour.

The brick red color was presented the cynogenic glycosides in the samples. (http://www.hort.purdue.edu/newcrop/duke_energy/Ricinus_communis.html#Chemistry).

Ninhydrin color of amino acids:

TLC was performed to identify the amino acids. On the TLC silica gel, ninhydrin solution was sprayed and the characteristics color was obtained. Light purple color was indicated the presence of amino acids. The presence of amino acids in cassava extract is also supported by Hanes *et al,* (1961). The fruit of Alma (Family: Euphorbiaceae) also contains considerably higher concentration of most minerals and amino acids. Glutamic acid, proline, aspartic acid, alanine, and lysine are 29.6%, 14.6%, 8.1%, 5.4% and 5.3% respectively of the total amino acids.

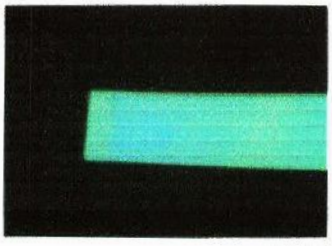


Fig 6: Ninhydrine color of amino acids of root flour.

Some amino acids in the tuber (edible portion) of other cassava's cultivars namely, TMS 30572, (Afisiafi), TMS 50395 (Gblomaduade) and TMS 4 (2) 1425 (Abasa fitaa) were determined of light pulped color on the TLC plate (Brook *et al,* 1979).

Extraction of Starch from Cassava (*Manihot esculenta*) and Potato (*Solanum tuberosum*):

The starch is the key part of Cassava roots. About 200 gm of extract was found from 1 Kg of Cassava's root. According to Diasolua Ngudi (2006) Cassava has many advantages for starch production.

- High level of purity
- Excellent thickening characteristics
- A neutral (bland) taste
- Desirable textural characteristics
- A relatively cheap source of raw material containing a high concentration of starch (dry-matter basis) that can equal or surpass the properties offered by other starches (maize, wheat, sweet-potato, and rice)

The potato is available in our country all over the year. Significant quantities of potatoes are destroyed during the period of harvesting and the prices of potatoes are not enough during the period of harvesting. If we store the potatoes during the period of harvesting; we can easily use them for starch production.

Comparative Analysis:

Table 9: Comparative analysis between cassava and potato starch.

Cassava starch	Potato starch
200 gm of starch was found from 1 Kg of cassava.	280 gm of starch is found from 1 Kg of potato.
The production cost of cassava is very low	The production cost of potato is high than that of cassava.
Cassava production period is long that is about 8 months.	Potato production period is short than that of cassava production
The flour of cassava is not well-known foods.	The potato is one of the most important carbohydrates containing food all over the world.

About 200 gm of starch was found from 1 Kg of cassava. In case of potato about 280 gm of starch was found from 1 Kg. The production cost of cassava is comparatively lower than that of potato. During the process of cassava growing, there is no need for fertilizers and pesticides (Mathews *et al.*, 1993). In potato growing process, different types of fertilizers are used and also different types of pesticides and herbicides are used. So potato production is costly (Mathews *et al.*, 1993). Cassava production period is long than that of potato production. The time of Potato production is about 4 months but the time of Cassava production is about 8 months (Nassar, 1989). The flour of cassava is not well-known foods but the potato is one of the most important carbohydrates containing food all over the world (FAO, 2002).

The starch of cassava is used in industry mainly for glue production (International Starch Institute, Denmark, 2004). For this reason; the production of starch from cassava is important for our industrial use. In our country annually 56% of glue is collected from foreign countries (BBS, 1998). If we can use the glue obtained from the starch of cassava in the industry we will be able to save a large amount of currency.

Chapter Five

Summary and Conclusion

Chapter Five

SUMMARY AND CONCLUSION

The plant leaves, barks and roots were collected, washed, dried and extracted with absolute ethanol and water. This extract was filtered and the solvent was evaporated to get very concentrated crude extract.

The ethanolic extract was tested for the presence of different chemical groups and the following groups were identified- alkaloids, reducing sugars, gums, steroids in leaves, barks and root extract. Tannins were identified in leaves and barks extract but it was not found in roots extract.

The large amount of cynogenic glycosides was presented in the roots flours. The root flours were tested for the presence of cynogenic glycosides was very important because cynogenic glycosides are toxic for human body (Ha *et al.*, 1996).

On the TLC silica gel, ninhydrin solution was sprayed and light purple color was obtained that indicated the presence of different amino acids (Hanes *et al.*, 1961).

Cassava contains the highest amount of starch, in that about 35% of its carbohydrate content. First of all about 1 Kg of roots of cassava was taken and about 200 gm of extract found of 1 Kg of cassava's root. A relatively cheap source of raw material containing a high concentration of starch (dry-matter basis) that can equal or surpass the properties offered by other starches (maize, wheat, sweet-potato, and rice) (Diasolua, 2006). And another source of

starch production in our country from potato. About 280 gm of starch found from 1 Kg of potato.

Comparatively the production cost of cassava is lower than that of potato; there are no fertilizers are needed and no herbicides and pesticides are needed. The starch of cassava is used in industry mainly for glue production (International Starch Institute, Denmark, 2004). For this reason; the production of starch from cassava is important for our industrial use. In our country annually 56% of glue is collected from foreign countries (BBS, 1998). If we can use the glue obtained from the starch of cassava in the industry we will be able to save a large amount of currency.

Chapter Six
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Appendixes

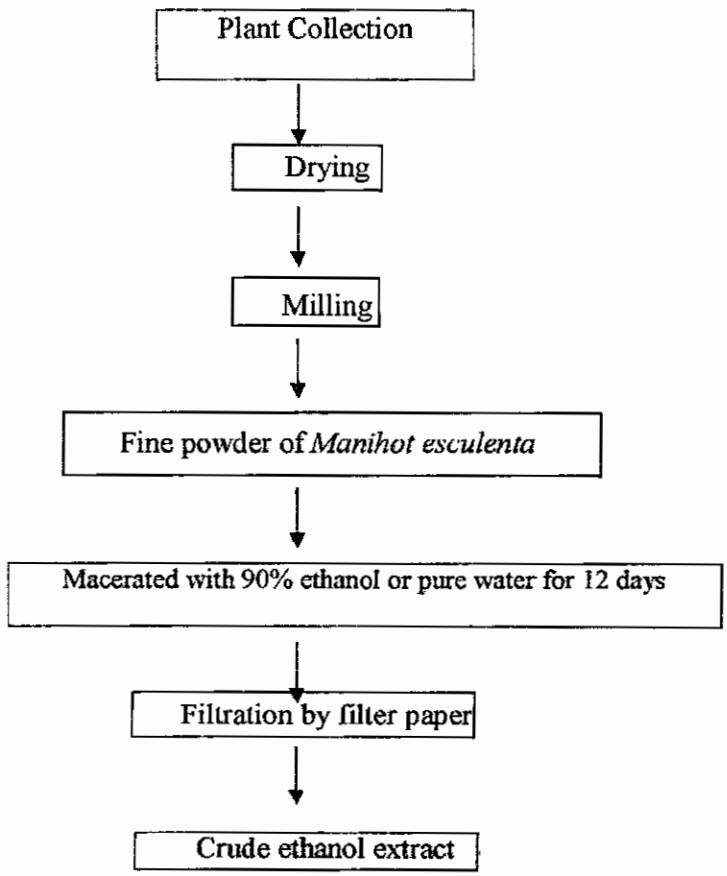

Fig 7: Flow diagram of ethanol extraction procedure of crude extract of cassava.