KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

INVESTIGATING THE CHEMICAL CONSTITUENTS AND ANTI-DIABETIC ACTIVITY OF THE DRIED HUSK OF *ZEA MAYS* (CORN), POACEAE

BY

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MAY, 2016

DECLARATION

I declare that this thesis is my work. I further wish to declare that to the best of my knowledge, this thesis does not contain any material that has been previously published by anyone except where due acknowledgement has been made in the text.

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DEDICATION

This thesis is dedicated to my academic advisor Dr. (Mrs.) Abena Amponsaa Brobbey whom this research work is so dear to.

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Finally, to my lovely family, I say God bless you all.

ABSTRACT

This work was aimed at investigating the anti-diabetic activity and chemical constituents of the dried husk of *Zea Mays* (corn), Poaceae. The anti-diabetic activity of the aqueous extract was carried out by testing the fasting blood glucose levels of type 2 diabetes mellitus (DM) patients who were on their oral anti-diabetic medications for three weeks without administering the corn husk tea and the next 3 weeks, after administering corn husk tea (sample test). The fasting blood glucose test was carried out weekly for six weeks on another set of type 2 DM patients taking their oral anti-diabetic medications but not taking the corn husk tea (control test). The tea exhibited a significant anti-diabetic activity as compared to the control. The phytochemical screening conducted revealed the presence of flavonoids, saponins, alkaloids and glycosides in the aqueous and methanolic extracts of the corn husk. Ethylacetate: methanol: water: glacial acetic acid (60:20:20:2 %v/v) was the best solvent system used to develop a thin layer chromatography for the methanolic extract. Five fractions were obtained from column chromatography when petroleum ether, ethylacetate and methanol were combined in different ratios. Methanol: water (95:5) %v/v was used to develop a High Performance Liquid Chromatography (HPLC) fingerprint for all five fractions. Three pure isolates were separated from fraction one using preparative HPLC and Infrared spectra were obtained for each isolate.

The results from this research work has demonstrated the anti-diabetic activity of the dried husk of *Zea Mays* (corn) and also, the presence of chemicals that may be responsible for such activity. This makes it clear as to why DM patients would drink corn husk tea or the liquid obtained after cooking 'Ga' kenkey.

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ABBREVIATIONS

ATR -	Attenuated Total Reflection
CC -	Column Chromatography
DM -	Diabetes Mellitus
EA -	Ethyl Acetate
PE -	Petroleum Ether
HPLC -	High Performance Liquid Chromatography
IR -	Infrared
ME -	Methanol
IDF -	International Diabetes Federation
NMR -	Nuclear Magnetic Resonance
%T -	Percentage Transmitance
TLC -	Thin Layer Chromatography
F -	Fraction
FBGT -	Fasting Blood Glucose Test
GAA -	Glacial Acetic Acid
H_2SO_4 -	Sulphuric Acid
HbA1c -	Glycated Haemoglobin
UV-VIS -	Ultraviolet-Visible Spectroscopy
KOH -	Potassium Hydroxide
mg/dl -	Milligram per decilitre
mmol/l -	Millimole per litre

CHAPTER ONE

GENERAL INTRODUCTION

1.1 BACKGROUND OF STUDY

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia (an increase in blood glucose level). It is managed by medications and proper diets throughout the individual's life. Diabetes may result from the inability of the pancreas to produce sufficient insulin to mop up the excess glucose in the body or inability of the pancreas to respond to the insulin present in the body. DM is of type 1, type 2 and gestational diabetes.

Glucose is a vital component of the body, providing the energy needed by the body for its normal functions. It is produced from fats, proteins and mostly carbohydrates which form a major component of our daily meals or a major breakdown of body cells. After an intake of a meal, the carbohydrates, proteins or fats present are broken down into simple sugars (glucose). Insulin plays an important role in most metabolic processes. It aids in storing glycogen produced from excess glucose, in the liver and muscles. Again, it aids in storing fats in the adipose tissues and many more related functions. Insulin when bound to cell receptors aids in the uptake of glucose by the body cells. In this way, the cells are enriched with glucose for the production of energy. (Lemke L. T. et. al, 2007). Insulin therefore helps to control glucose in the blood. If sufficient energy is produced, the excess glucose is stored as glycogen in the liver. When glucose is high in the blood (hyperglycaemia) and there is no insulin to aid in its reuptake into the body cells, the cells become energy starved. The body compensates for the starvation of the cells by breaking down glycogen from all sites of storage

including the muscles and adipose tissues. This leads to massive complications and the symptoms that persist in the different types of diabetes.

Type 1 DM represents about 10% of patients with diabetes. This type can occur in people of varying ages but it is predominate in children and younger adults (IDF Diabetes Atlas, 2013). Type 1 DM is as a result of autoimmune destruction of the beta cells of the pancreas leading to no production of insulin. A major way of managing this deficiency is to administer insulin exogenously hence the name Insulin Dependent Diabetes Mellitus (IDDM) (Lemke L. T. et. al, 2007). Due to the breakdown of muscles and adipose tissues when glucose is high in the blood but less in the body cells, type 1 DM patients reduce in body weight and also produce ketones leading to ketoacidosis.

Type 2 DM, also known as noninsulin-dependent diabetes mellitus (NIDDM), is the most prevalent type. It represents approximately 90% to 95% of all DM diagnosed cases. It mostly occur in adults. This type is usually slow and progressive in its development and often is preceded by pre-diabetes.

Pre-diabetes is a state where an individual's blood glucose level is higher than the expected but the level is not high enough to be considered above normal (hyperglycemic). Individuals with abnormal fasting blood glucose (≥100 mg/dl and <126 mg/dL) and impaired glucose tolerance (≥140 mg/dl and <200 mg/dl) can be termed pre-diabetic (Lemke L. T. et. al, 2007). The development of prediabetes places the individual at high risk of eventually developing diabetes. The exact cause seems to be unknown but it assumed to be associated with nutrition. It may lead to long-term microvascular, macrovascular, and neuropathic complications.

In type 2 DM, there is a relative insulin deficiency and insulin resistance. This means that, the body produces insulin but the insulin produced may be insufficient or the receptors are resistant to the insulin produced. To resolve this, lifestyle modifications are encouraged along with moderate exercises and the intake of oral diabetic medications. Most people have type 2 DM unknowingly because symptoms take several years to manifest. Risk factors for type 2 DM include obesity, lack of exercise, family history, ethnicity and many more (IDF Diabetes Atlas, 2013).

During pregnancy, some women develop a high blood glucose level and a resistance to insulin. This condition is classified as **Gestational diabetes.** It normally occurs from the 24th week of pregnancy (www.diabetes.org/diabetesbasics/gestational). Major complications that may be observed in uncontrolled gestational diabetes is a difficulty in delivery due to shoulder dystocia and the delivery of abnormally large baby (foetal macrosoma). To manage this condition, the diabetic pregnant woman requires either oral medications or insulin administration or both, coupled with diet and exercise control (Harvey A. R., 2011).

Diabetes management require a knowledge of the type of diabetes. In traditional medicine practice, many natural products or herbs have been employed for the reduction of blood glucose in patients with the different types of DM. Some scientific research and clinical studies have proven the ability of some herbal products to have this anti-diabetic or hypoglycaemic effect even though the mechanisms of action for most of these herbal remedies remain unknown.

Amongst these herbal products include the use of corn silk in treating diabetes (Khairunnisa Hasanudin et al, 2012). Not only is corn silk used, many traditional medicine practitioners have been managing diabetes of any type with the aqueous extract (tea) of corn husk and the therapy has shown to be useful. Patients are made to take the tea together with their orthodox medicines.

Unfortunately, no scientific research has been carried out on this practice to prove the anti-diabetic activity or hypoglycaemic effect of the tea. One may ask if this tea actually has anti-diabetic activity. And if it does, what chemical constituents are present in the corn husk that may be contributing to the antidiabetic activity.

Hence, this project seeks to ascertain the anti-diabetic effect of the corn husk extract and identify any chemical constituents that may be present in the corn husk that can be linked to having an anti-diabetic effect.

The study would be in two parts, that is, the clinical and the laboratory analysis part. In the clinical aspect of the research, twelve (12) subjects who are type 2 diabetic patients would be selected from the tree of life clinic. Study questionnaires would be given to patients to complete. The questionnaire seeks to find out about the occupation, nationality and age group of the study subject. It asks about when the subject was diagnosed of his or her diabetes and the symptoms present. It continues to ask about other disease conditions the subject has, the type of diabetes (if known), and any medications being taken for the diabetic therapy and whether any herbal medicine has been taken before.

Subjects' prescriptions would be verified to prove that they are known diabetic patients. Study subjects would then be selected based on the fact that the subject

is a proven diabetic patient who is not taking corn husk tea before the start of the experiment and more importantly, one whose fasting blood glucose is 6mmol/L and above. A voluntary consent form would also be given to the patient to inform them of the purpose of the study and hence obtain their permission and the permission of any available relative.

The sample test begins with a 3-week measurement of the basal fasting blood glucose of subjects using OneTouch glucometer and as subjects continue with their orthodox diabetic medications. Subjects' identity would be coded. A specific quantity of the corn husk would be weighed and soaked in water and boiled for an hour. Subjects would be required to drink 100mls of the tea 8hourly after taking their orthodox medication, all after meals. The blood glucose levels would again be measured weekly for the next 3weeks. Fasting blood glucose levels before and after administration of the tea would be compared and recommendations made. Six (6) other subjects would be selected using the criteria as above to serve as a control for the study. The control subjects would have their fasting blood glucose levels measured weekly for 6 weeks as they continue with their oral anti-diabetics but the corn husk tea would not be administered.

In the analytical work, corn husk would be soaked in both methanol and water and boiled for an hour. The tea would be left to evaporate to dryness. The extract obtained would be taken through chromatographic techniques such as thin layer chromatography (TLC), column chromatography, high performance liquid chromatography and spectrophotometric methods such as and infra-red (IR) spectrophotometry for isolation, characterization and identification of specific functional groups.

1.2 PROBLEM STATEMENT

Diabetes is on the rise in most countries in the world. 382million people have been estimated to have diabetes worldwide. The prevalence now tends to be higher in younger people which can greatly affect the future. Also, there is an increasing number of pre-mature deaths due to diabetes. Even in Africa where the prevalence of DM is lowest, it has been estimated that in 2013, about 522,600 people died as a result of diabetes (IDF Diabetes atlas, 2013).

The management of DM has largely been by the use of orthodox medicines. The patronage of these medicines is of a challenge to patients due to the fact that they are expensive, the dosage regimen for most diabetes seem to be uncomfortable. The side effects of these medications also seem to pose as a problem as well as the length of onset of drug action.

1.3 RESEARCH OBJECTIVES

The research work seeks to investigate the chemical constituents and antidiabetic activity of the dried husk of *Zea mays* (corn), Poaceae. To achieve this, the following specific objectives have been aligned:

To investigate the anti-diabetic activity of the aqueous extract of corn husk on type 2 diabetic patients.

I. To identify phytochemical components of the corn husk extract
II. To fractionate the chemical constituents of methanolic corn husk extract using column chromatography (CC).
III. To develop thin layer chromatographic method of analysis
IV. To obtain fingerprint spectra for the various fractions from CC using certain spectroscopic methods and TLC.

V. To obtain pure isolates and identify specific functional groups present using infrared spectrometry.

1.5 SIGNIFICANCE OF STUDY

The findings from this research work would be of immense help to DM patients and the society at large considering the fact that the prevalence of diabetes is now not only high in older people but also increasing in younger adults. Notwithstanding the fact that orthodox medication cost keeps increasing as well.

The project seeks to provide an effective supplement but a more convenient (cost, dosage regimen and side effects) way of managing diabetes mellitus, especially type 2 DM that represents about 90-95% of DM cases.

1.6 JUSTIFICATION OF STUDY

The use of herbal medicines is increasing tremendously due to compliance and cost. People are all the more ready to resort to any therapy that promises a solution to their medical conditions at a convenient dose, fast onset of action, less side effects and above all, at a cheaper cost.

Traditional medicine practitioners use the aqueous extract from corn husk to lower blood glucose level to a normal. This has shown to be effective to them hence its continuous usage. The tea is inexpensive, tasteless, available and easily accessible. High cost of diabetes treatment with most orthodox medications is also catered for. The tea has a lesser side effect and this has led to an increased in compliance. The effectiveness of this medication needs to be scientifically proven and documented so that recommendations especially related to dosing and effectiveness of therapy, can be made based on scientific facts. With scientific analysis on the tea, the active pharmaceutical ingredient can be

identified and more improved formulations and dosage forms may be developed in future.

CHAPTER TWO

LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus is a chronic disease characterized by high blood glucose level (hyperglycaemia). It is a metabolic disorder that is managed with proper diets, moderate exercise and diabetic medications. The pancreas is an organ in the body that produces insulin and glucagon for the maintenance of blood glucose levels. When the functioning of the pancreas is altered, hyperglycaemia may occur, leading to diabetes mellitus. DM may occur if insufficient or no insulin is produced. An individual with diabetes mellitus basically has glucose circulating in the blood instead of it being absorbed into the body cells for energy production.

When glucose is high in the blood and the renal threshold for glucose reabsorption is exceeded, glucose moves into the urine leading to a condition termed glycosuria. This results in an osmotic pull of the body's water causing an increase in urination (polyuria) and thirst due to dehydration. The patient then drinks so much water (polydipsia). Because the glucose cannot reach the cells for energy, there is an automatic quest for food hence increased hunger (polyphagia). The body cells when deprived of energy result to muscle wasting. Glycogen is broken down from all storage sites. Adipose tissues breakdown fats and muscle proteins are all broken down into simple sugars (glucose) as a compensatory mechanism. This leads to a massive weight reduction. In the absence of aerobic carbohydrate metabolism, fat is broken down to acetone, acetoacetate and β-hydroxybutyrate which leads to an emergent condition known as Diabetic Ketoacidosis (Rang, H. P. et al, 1991).

The causes of diabetes remain unknown but a sedentary lifestyle, genetics, obesity, age and others predispose an individual to DM. Physiologically, DM results from the inability of the pancreas to produce sufficient insulin or inability of the pancreas to produce insulin to mop up the excess glucose in the body or the insulin resistance in the body.

Glucose is a vital element for providing the energy needed by the body for its normal functions. It may be produced from complex sugars like carbohydrates, fats and proteins. When glucose is in the blood, insulin is secreted to transport glucose to the body cells (smooth, heart and skeletal muscles) to provide the needed energy. The liver then prevents hyperglycaemia by storing the excess glucose in the form of glycogen and release it when needed.

Uncontrolled DM leads to micro and macro-vascular complications some of which include heart problems such as hypertension and heart attacks, stroke, nephropathies, neuropathies, blindness and etc. The types of DM are type 1, type 2 and gestational diabetes.

2.1.1 Type 1 DM

This represents about 5 to 10% of diabetic diagnosis and has a sudden onset in life. It occurs as a result of autoimmune destruction of the pancreatic beta cells which are responsible for insulin production. Hence, the pancreas is unable to produce insulin to balance the blood glucose levels. This leads to hyperglycaemia in which glucose in the blood cannot be converted to energy for the utilization of the cells. Typical symptoms of type 1 DM are abnormally increased thirst with dryness of mouth, increased hunger, increased urination, increased fatigue, sudden weight loss, poor healing of wounds, blurred vision,

increased risk of being infected and recurrence of infections (IDF Diabetes Atlas, 2013). When type 1 DM patients reduce in weight, the body resorts to producing ketones from the liver as an alternate source of energy. Ketoacidosis results and this is a serious condition that develops due to high amount of ketones produced. To curb this issue, patients in this category require insulin injection to manage the glucose levels in the blood. Hence the name insulindependent diabetes mellitus. They also require proper monitoring of their diets and a regular moderate exercise.

2.1.2 Type 2 DM

It occurs due to insulin resistance which may result from aging, obesity or other diseases like Cushing's syndrome (Lemke L. T. et. al, 2007). The prevalence of this type is about 90 to 95% of diabetic population and it occurs mostly in adults. In this type, the hyperglycaemia is due to the fact that insulin is unable to transport glucose to the body cells. Insulin resistance may be due to a decrease in insulin affinity to it receptors or a decrease in the number of receptors of insulin at receptor sites. Patients on type 2 DM usually need to manage their diets and engage in moderate exercise. Risk factors for this type include obesity, lack of exercise, unhealthy diets, genetics, increased age, race and many others. Oral diabetic medication, healthy diets and regular moderate exercise help in the management of type 2 DM.

2.1.3 Gestational Diabetes

Gestational diabetes occur when some women, during pregnancy experience a high blood glucose levels due to resistance to insulin. It usually occurs around the 24th week of pregnancy. Uncontrolled blood glucose in this state can affect

both mother and baby. Baby may grow in a bigger than normal size, a condition known as fetal macrosomia. Mother may have to deliver baby by caesarian section since normal delivery may be highly risky. To manage the condition can be managed effectively by healthy diets to control blood glucose and moderate exercises. Sometimes oral medications or insulin may be required. The good news is that this condition normally disappears after child delivery (IDF Diabetes Atlas, 2013).

2.1.4 Epidemiology

Diabetes mellitus (DM) is increasing worldwide due to the increasing rate of obesity as a result of poor nutrition and lifestyle, an increasing age, urbanization and high population growth. It is therefore prudent to study how alarming the present situation is and the future consequences on economic growth. This helps to put serious measures in place in order to protect resources (Wild, S.H. et al, 2004).

DM is one of the most challenging health problems in the 21st century. In high income countries, DM is about the 5th leading cause of death. Globally, in 2013, the prevalence of diabetes was 8.3% indicating 382million people had been estimated to suffer from DM by then (IDF Diabetes Atlas, 2013).

Diabetes mellitus remained the 7th leading cause of death in the United States of America as at 2010. In 2012, 29.1million Americans (9.3% of the population) had diabetes mellitus where, 21milion were diagnosed cases and 8.1million were undiagnosed. In terms of ethnic prevalence, DM cases are highest in the

American Indian or Alaskan natives, followed by the non-Hispanic blacks, Hispanics, Asian Americans and lowest in the non-Hispanic whites. The highest prevalence was still in the Caribbean regions and the North American.

Africa is a region with the lowest prevalence of DM (4.9%) in 2013. Reunion (15.4%), Seychelles (12.1%) and Gabon (10.7%) are the top three African countries with higher prevalence (Martinez R., 2013). Mali is the country with the lowest prevalence of DM (1.58%).

This DM prevalence is largely due to obesity and overweigh. Most people do not engage in physical exercise and also have poor diets. Most people take in diets with high amount of calories and salt. There is low intake of fruits and vegetables.

In 2014, there were 450,000 cases of diabetes in Ghana representing a prevalence of 3.3% and amongst individuals aged 20 to 79 years. Unfortunately, most people in Africa have undiagnosed DM.

2.1.5 Types of Blood Glucose Test

A blood glucose test measures the amount of a sugar (glucose) in a sample of your blood.

2.1.5.1 Glycated Hemoglobin (HbA1c) Test

This test is an indication of an individual's blood glucose level for the past two to three months. It is the measurement of the amount of blood glucose in percentage attached to the hemoglobin which carries oxygen in red blood cells. The higher the blood glucose level, the more glycated the hemoglobin will be.

On testing twice on different occasions, an A1c of ≥ 6.5% is an indicator of diabetes. Pre-diabetes is when the A1c is 5.7-6.4%. A normal or non-diabetic patient will have an A1c of ≤ 5.7%. During pregnancy, there is an uncommon form of hemoglobin hence A1c cannot be used to test for diabetes. Also, this test is not mostly available for usage hence the other tests may be employed.

2.1.5.2 Random Blood Glucose Test

In this test, an individual's blood glucose level is measured at random regardless of when last food was taken. Twice and above random blood glucose test of ≥ 200mg/dl or 11mmol/l is indicative of diabetes.

2.1.5.3 Fasting Blood Glucose Test

To diagnose diabetes, one of the tests to carry out is fasting blood glucose level tests. In this test, an individual should be devoid of foods and drinks excluding water for eight (8) hours which refers to the fasting period. During fasting, glucagon, a peptide hormone secreted by the alpha cells of the pancreas is stimulated as a response to the reduce blood glucose (due to hunger) to raise the blood glucose levels to normal. This is the body's compensatory mechanism to balance glucose in the body. Unfortunately, type 1 diabetic patients do not produce sufficient insulin to balance the raised glucose level and Type 2 diabetic patients have insulin but the body is unable to utilize it. Hence, when the fasting blood glucose levels are tested, diabetic patients will have higher blood glucose levels as compared to non-diabetics. A fasting blood glucose level of 3.95.5mmols/l is normal. DM is diagnosed when the blood glucose level is greater than 7mmols/l (diabetes.co.uk).

2.2 CORN DESCRIPTION

Zea mays (Corn) is from the kingdom Plantae and of the family Poaceae. Zea is a Greek word representing 'cereal' and meaning, sustenance of life whiles mays, is a Taino word meaning life giver. The monoecious corn plant has male flowers called the tassel while the axillary female plant forms cylindrical corn cobs. Each cob develops into corn kernels and a long protruding hair-like styles called the corn silk. The corn husk encloses the corn kernel or grains.

Corn is a staple food crop grown all over the world and amongst the top three cereal crops grown in the world, along with Oryza sativa (rice) and Triticum spp (wheat).

Maize is another name for corn and is believed to originate from Mexico and Central America. Corn is best grown in warm, tropical and sub-tropical regions. An important requirement for growing corn is to have a quality fertile soil with a pH between 6.0 and 6.8. It also requires a space, full day sunlight and moisture.

2.2.1 Benefits of Corn

Corn when ripe or matured maybe consumed fresh. It may be dried and grounded into flour for porridge, kenkey, banku etc. (Ghanaian dishes). The corn may also be boiled or roasted and eaten as a snack. Corn apart from being served as a meal has several other benefits.

Corn grains may be processed into starch powder or oil and used for cooking food, pharmaceutical productions and etc. In addition to the food component, the entire plant can be used as forage to feed livestock.

2.2.2 Corn Husk

Corn husk is the outer leafy covering of a corn grain. The husks before usage is either oven, sun or air dried. Corn husk has always had it use in the processing of food. It is also used to encase food as in the case of preparing kenkey (an African dish) or used in the presentation of a dishes. In other parts of the world, corn husk is used for making dolls of different types. Recently, traditional medicine practitioners have found use for these husks in the area of medicine. Corn husks are now been used in the treatment of diabetes mellitus.

2.3 HERBAL THERAPIES FOR DIABETES MELLITUS

Some herbal remedies have been studied in recent years to scientifically prove their ability to reduce blood glucose especially in patients with type 2 diabetes. Amongst such herbal remedies include the use of Aloe vera, Ginger, Okra, corn silk and many others. Their exact mechanism of action cannot be explained. Because of their improved blood glucose management, there is an increase in the use of natural or herbal remedies to manage diabetes.

The herbal remedies are cheap, easily available and easy to comply to and with less side effects as well. These remedies have a disadvantage of reducing the blood glucose to dangerously lower levels making the patient highly hypoglycemic and increasing the risk of some diabetic complications. This effect may be due to the fact that with herbal remedies, especially the liquid dosage forms, have a difficulty in taking a measurable dose for administration. It is therefore advisable to constantly monitor the blood glucose levels when on any of such herbal therapies.

2.4 PHYTOCHEMICAL SCREENING

Plants produce various organic compounds known as primary and secondary metabolites. The distinction between these two classes is somewhat blur.

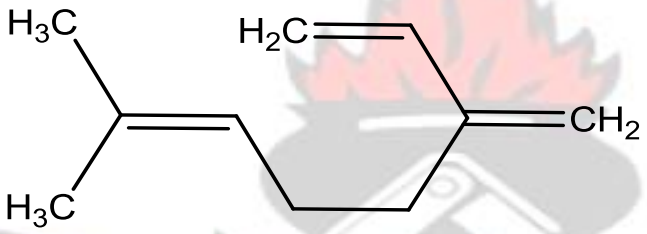
Primary metabolites are found in all plants and they partake in plant growth, development, photosynthesis and many more. Examples of primary metabolites are amino acids, nucleotides, organic acids and etc. Secondary metabolites on the other hand, are distributed differently in limited groups in the plant kingdom. They enhance plants interactions with their environment. The functions of secondary metabolites are gaining recognition as some of the metabolites seem to have antimicrobial activity, others protect plants from being consumed by herbivores. Most secondary metabolites have also offered usage as glues, dyes, flavouring agents, performs, and etc. (Croteau et al. 2000, Crozier et al. 2006).

Organic chemist have undertaken extensive research in this area and have found variety of uses of these phytochemicals. This has led to better development of separation and spectroscopic methods in the quest to isolate, elucidate or identify and characterize bioactive components in plant extracts. Natural products that may be obtained from plants are dyes, perfumes, drugs, oils, adhesives, fibers, flavouring agents etc. the study of phytochemicals maybe dependent on a scientist's search for new natural products e.g. drugs. Examples of the secondary metabolites are flavoinoids, alkaloids, terpenoids, saponnins, tannins, glycosides and etc. Below is an overview of these individual secondary metabolites.

2.4.1 Terpenoids

Terpenoids are secondary metabolites with various structural differences. Terpenoids are a form of terpenes (shown in *Figure 1*). Terpenes were derived from the word turpentine in the 19th century as a result of some research work carried out on turpentine. Terpene is a hydrocarbon structurally derived from isoprene unit (5 numbered carbons) and with a general formula of $C_{10}H_{16}$.

Terpenoids have insecticidal activity and some are used in perfumery and as flavouring agents as well.



Terpene

Figure 1: Basic Structure of Terpenes

2.4.2 Flavonoids

Flavonoids are polyphenolic secondary metabolites. They are found in high concentrations in the skin of fruits and the leaf epidermis. The flavonoid structural backbone is made of F carbons, two aromatic rings which are linked by a three-carbon forming a bridge. Hydroxyl substituents are usually found at carbon position 4, 5 and 7. Most flavonoids naturally exist as glycosides (sugars). The presence of the hydroxyl groups and the sugar moieties increase the solubility of flavonoids in water. They are subdivided into isoflavones, flavones, flavan-3-ols, flavonols, flavanones and flavanonol.

Flavonoids have structure dependent pharmacological activity. The presence of hydroxyl groups enables flavonoids to exhibit antioxidants properties. They have the tendency to scavenge free radicals and this occurs when they chelate with metal ions. When present in foods, they are of good benefit to the body since they mop up free radicals. Again, flavonoids have antimicrobial and antibacterial activities. Some examples of flavonoids include catechin, kaempferol, quercetin etc as shown in *Figure 2*. (Kumar S. et al, 2013)

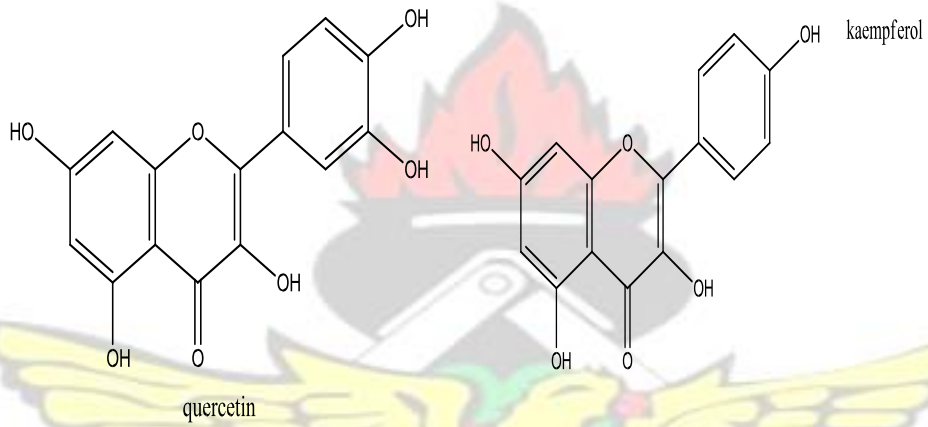


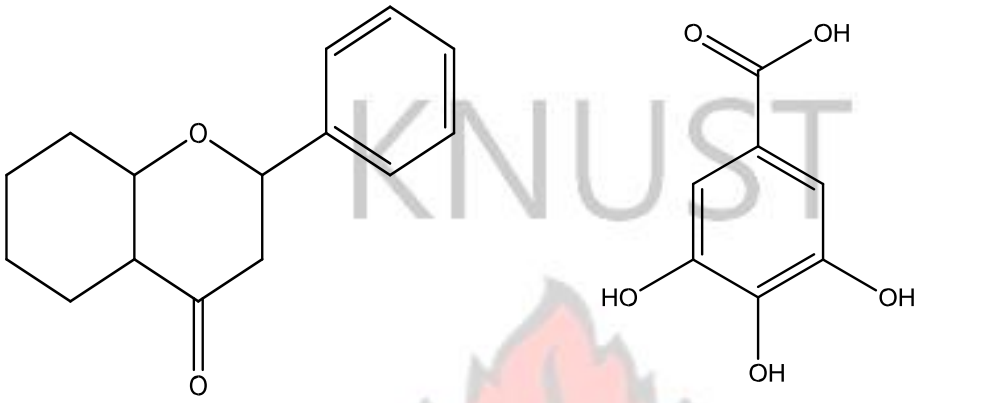
Figure 2: Structural examples of flavonoids

2.4.3 Tannins

There are two types of tannins namely; hydrolysable tannins and condensed tannins. Hydrolysable tannins are so called because upon treating them with dilute acid, they break down into ellagic acid (ellagitannins) and gallic acid (gallotannins). Condensed tannins on the other hand, do not break down when treated with dilute acid. Figure 3 shows an example each of the different types of tannins.

Tannins have an astringent taste making plants inedible. Because of this, they protect plants from being consumed by mammals. They are also used in the

texture industry for tanning clothes. High amount of tannins can be found in unripen fruits. Tannins are used in the production of beverages such as wines and certain foods but in smaller amount.



Flavone condensed tannin Gallic Acide hydrolysablebtannin

Figure 3: Structural examples of tannin

2.4.4 Alkaloids

These are secondary metabolites, basic in character and derived from plants. They are also heterocyclic compounds containing nitrogen. They are subgrouped into monocyclic, bicyclic and polycyclic alkaloids.

Alkaloids are of great pharmacological importance including antihypertensive, antiarrhythmic, anticancer and antimalarial effects. Some alkaloids have antibiotic, analgesic, antitussive effects and many more. Many common drugs are alkaloidal and of natural sources. Examples of alkaloidal drugs are Quinine and epinephrine as shown in *Figure 4* and acetylcholine, caffeine, scopolamine, cocaine, morphine etc. (Roberts F. M., et al). To test for the presence of Alkaloids, Dragendorff's test and Wagner's test may be employed.

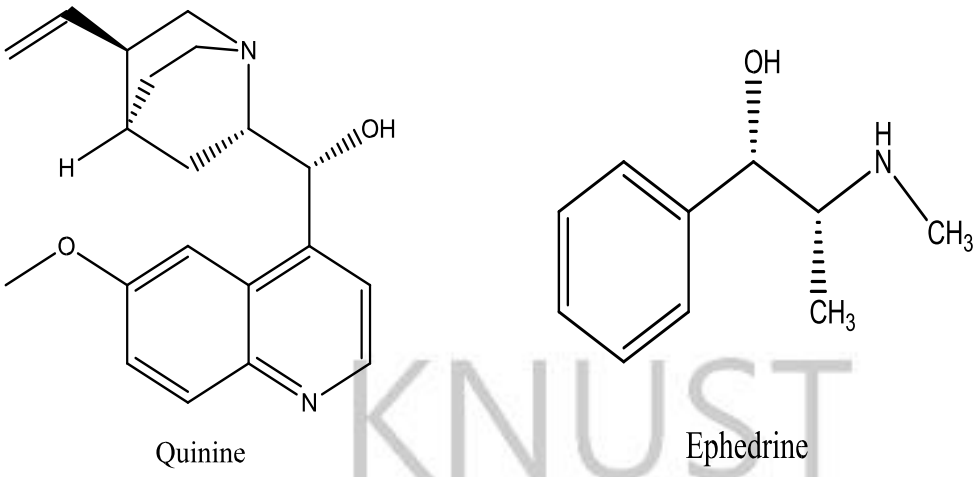


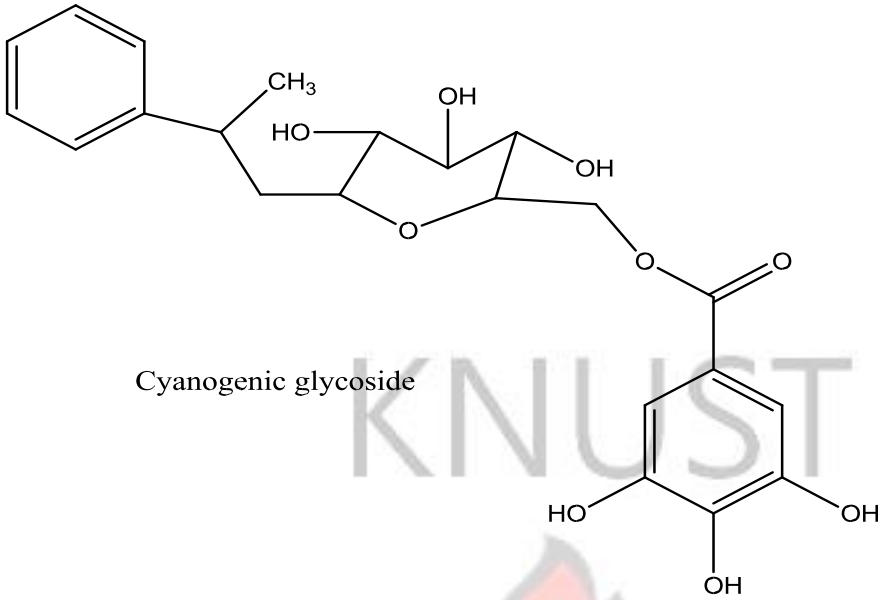
Figure 4: Structural examples of some alkaloids

2.4.5 Glycosides

Glycoside also known as reducing sugars are a complex group of secondary metabolites. They have two portions namely the non- sugar moiety called aglycone and the sugar moiety called the glycone. The chemical nature of the aglycone component form the basis of glycoside classification. Glycosides are classified into anthracene, steroid, phenol, flavonoid, coumarin, thio, cyanogenetic (example shown in *Figure 5*), saponin, aldehyde, cardiac glycosides and many more.

Glycoside are common in flowers and fruits. They have varying importance as some are used as dyes, antibiotics e.g. streptomycin. Others have heart stimulating activity and hence belong to the class of cardiac glycosides. Saponins, which reduce the surface tension of water are glycosidic in nature. Some nucleotides are also glycosides.

To test for the presence of cardiac glycosides, Kellar-Kiliani test may be employed. Ferric Chloride test may be used to test for the presence of phenols.



Cyanogenic glycoside

Figure 5: Structural example of glycoside

2.4.6 Saponins

The term saponin is from ‘sapo’, a latin word translated as soap. Saponins belong to the class of secondary metabolites with a high molecular weight. It is a glycoside which consists of a sugar moiety linked to an aglycone steroid (sapogenin) or a triterpene. Saponins have a soap lathering effect hence have detergent properties. Saponins are naturally bitter and are lethal to cold blooded animals. Saponins can be classified according to the structure into steroid glycosides, steroid alkaloid glycosides and Triterpene glycosides. Foam test or frothing test can be employed to determine the presence of saponins in compounds. A basic structure of saponin is as shown in Figure 6.

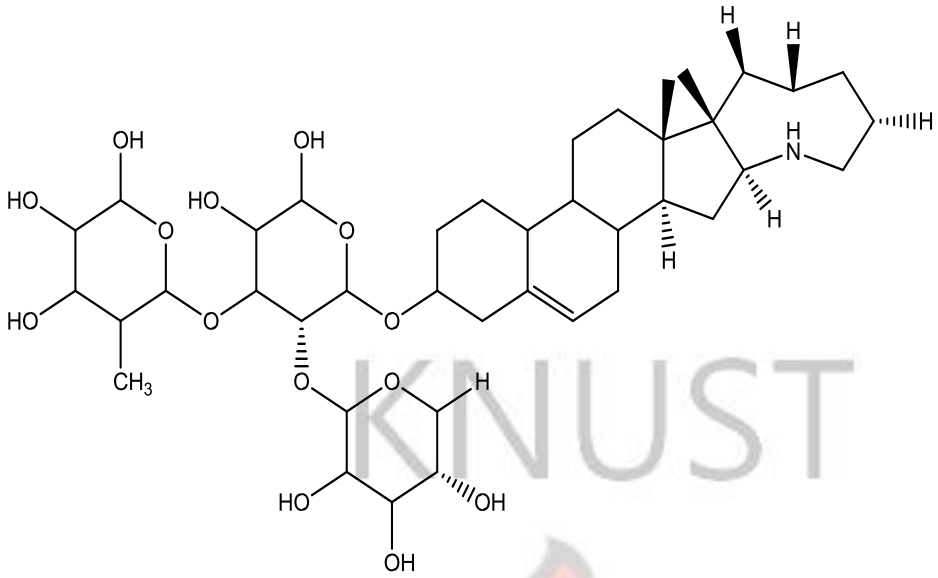


Figure 6: Basic Structure of saponin

2.5 IDENTIFICATION AND CHARACTERIZATION

Plants serve as one source of natural materials during drug discovery. They play a significant role in drug discovery and drug development. Plants contain secondary metabolites that provide special benefits to the health of living things. Some of these metabolites for example alkaloids, have antimalarial properties. Flavonoids are used as perfumes and also tend to attract agents for pollination. Tannins protect the plant against herbivores and other consumers.

To identify lead drugs from plant sources, certain processes and procedures should be followed in addition to analytical techniques. These processes and procedures are termed the ethno-pharmacological approach. Ethno-pharmacological approach in drug discovery involves extraction from the plant sample, sample preparation by solid-phase extraction, liquid-liquid extraction etc., biological assay including enzymatic assay, antibacterial assay etc. and many other processes (Brusotti G. et al, 2013).

Analytical techniques are employed for the isolation, identification and characterization of compounds that may be responsible for the biological activity on whose basis plant materials are used traditionally. These include separation of fractions by using column chromatography and the use of preparative TLC or preparative HPLC to obtain pure isolates. Spectroscopic and spectrophotometric methods like UV-Vis, NMR, IR, GC-MS and many others may be employed to identify and characterize isolates. Bioactive isolates may be identified by performing biological assays after isolation to determine which of the pure isolates give the speculated activity (Sasidharan et al., 2011)

2.5.1 Chromatographic Techniques

2.5.1.1 Column Chromatography

Column chromatography separates components of a samples into fractions. In this chromatographic technique, a glass column is packed with a solid stationary phase which may be silica gel (SiO_2) or alumina (Al_2O_3). The sample is poured onto the stationary phase layer and the liquid solvent, mobile phase, is poured onto the surface to cause separation. This is seen as the mobile phase flows from the top down the column. Separation is achieved based on the type of interaction between the sample, being carried by the mobile phase, and the stationary phase. Interactions may be due to adsorption or partition or both. The polarity of the mobile phase greatly affects how the compounds move through the column. Depending on the nature of the sample being separated, more than one solvent may be put together to help better the separation. Separation may take place either due to gravity hence gravity chromatography or by applying air pressure hence flash chromatography. Compounds (eluates) eluting from the column

may be coloured or colourless. Eluates may be collected in tubes and analyzed using thin layer chromatography.

2.5.1.2 Thin layer chromatography (TLC)

Thin layer chromatography is a chromatographic technique performed on a thin plate that is coated with a thin film of an adsorbent (silica gel, alumina or cellulose). The TLC plate may be made of glass, aluminum foil, metal or plastic material.

To perform TLC on a sample, the right solvent system is chosen to be the mobile phase. The stationary phase is the plate on which the sample is spotted with the aid of a capillary tube. The spotted plate is then placed in a chromatank containing the mobile phase. The solvent is then allowed to travel up the plate and in so doing, separation is achieved. To view the separated components, the plate may have to be sprayed with appropriate reagents or viewed under a UVVis light. The chromatographic process depends on the principle of adsorption, partition or both.

TLC is useful in separating components of mixtures and identification of impurities presents in mixtures. It is a versatile method because it promises the usage of simple equipment that are also easily available. The separation process is also rapid and easy to visualize. A major demerit in the usage of TLC method is the choice of the right mobile phase system. This choice depends on the nature of the components in the sample to be separated and the process involved (adsorption, partition or both) (Kar Ashutosh, 2005).

2.5.1.3 High performance liquid chromatography (HPLC)

HPLC analysis is amongst the most widely used techniques for quantification of drugs samples, determination of pka and partition coefficient of most drugs,

monitoring the stability of formulated drugs as well as pure drug samples and isolation of a natural pharmaceutically active plant components.

HPLC employs a liquid stationary phase, pumped under high pressure through a column packed with the stationary phase material and an analyte, containing the sample under study. The well prepared analyte is injected into the injection port, the mobile phase carries the components along. Separation is achieved by the adsorption, partition or both processes depending on the nature of compound, the mobile phase, type and nature of the stationary phase. The HPLC setup has detectors which can be used to monitor the different eluents that comes out of the column.

In HPLC, selectivity is very high due to the usage of a variety of columns and detectors that make the analysis adjustable.

2.5.2 Spectroscopic Techniques

2.5.2.1 Ultraviolet-Visible (UV-Vis) spectrophotometry

The UV-Vis spectrophotometry employs the ultraviolet light (200-400nm) and the visible light (400-800nm). This technique uses Beer-Lambert's law to determine concentrations of drugs in the pure state or in formulations. The law utilizes the concept that absorbance of a compound along a 1cm path length is directly dependent on its concentration. For a compound to absorb UV-Visible radiation, it should have chromophore (chemical that absorb light).

Auxochromes, functional groups attached to the chromophore, have the ability to modify the strength of absorption of the chromophore. UV-Vis spectrophotometry is applied mainly for quantification of drugs substances and identification of drugs based on their wavelength of absorption (Kar Ashutosh, 2005).

2.5.2.2 Infrared (IR) spectroscopy

Infrared refers to the portion of an electromagnetic spectrum between the visible region and the microwave region. The electromagnetic spectrum representing the infrared region extends from 0.8 to 200 μ. IR uses wavelength (μ) or wavenumber (cm^{-1}) in measuring the position of absorption. Its regions are classified in 3 regions namely: Ordinary Infrared with a wavenumber of 4000667 cm^{-1}, Near Infrared with a wavenumber of 12,500-4,000 cm^{-1} and Far Infrared with a wavenumber of 667-50 cm^{-1}. Another school of thought classifies the IR regions into two (2) namely; group frequency region with a wavenumber of 4000-1300 cm^{-1} and fingerprint region with a wavenumber from 1300-400 cm^{-1} (Kar Ashutosh, 2005).

When a sample absorbs infrared radiation, there is a conversion of radiation into an energy of molecular vibration. When the radiated energy matches a specific molecular vibration, there is absorption and bands are shown at the specific frequencies (wavenumbers). The wavenumbers at which samples absorb radiant energy give an indication of the particular functional groups present in the sample. The spectrum is always analyzed with the help of tables that match the wavenumbers to specific functional groups. The spectra is a plot of percentage transmittance (%T) as a function of wavenumber. Transmittance is the inverse of absorbajnce and vice versa. An increase in wavenumber relates to an increase in energy.

IR is used as an analytical tool to aid in structural identification of compounds and to determine the purity of the compounds as well. It is fast and very easy to run.

CHAPTER THREE

EXPERIMENTAL

3.1 COLLECTION OF SAMPLES AND AUTHENTICATION

The dried *Zea mays* (corn) husk were purchased at the Ayeduase market, a town in Kumasi, Ashanti-Region. The plants were identified at the herbarium at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology. The husk has KNUST/HMI/2015/L033 as the specimen voucher reference number in the herbarium.

3.2 SAMPLE PROCESSING

The husks were air dried, then coarsely milled and kept in the laboratory till when needed.

3.3 EXTRACTION OF PLANT MATERIAL

3.3.1 Extraction for anti-diabetic analysis

Approximately 65g of corn husk was weighed and soaked in 2.5 litres of water and boiled for an hour. The tea was then allowed to cool, decanted and filtered. The solution was then packaged in plastic bottles and labelled appropriately.

3.3.2 Extraction for analytical work

300g of the coarse powder was poured into a 5 litre conical flask. Two litres (2L) of methanol was added and left to for 48hrs. The mixture was then decanted and filtered using whatman no. 40 filter paper into a beaker**.** The solvent was evaporated over water bath at a temperature of 80°C until a viscous extract was obtained. The extract was kept in the refrigerator at a temperature of 8°C until

needed. The extraction process was repeated using distilled water as the solvent for extraction.

3.4 ANTI-DIABETIC ASSAY OF AQUEOUS EXTRACT

Twelve (12) subjects (males and females) who are type 2 diabetics were selected after assessment of their responses to questionnaires. The basal fasting blood glucose levels of the subjects were measured weekly using OneTouch glucometer and monitored for three (3) weeks as subjects continue with their orthodox diabetic medications. Subjects identified were coded. After the first 3 weeks, subjects were given the tea to take orally every week according to the dosage; 100mls, 8hourly for 7 days. This was followed for another 3 weeks. The tea was taken after meals alongside the orthodox anti-diabetic medications. The blood glucose levels were measured after the completion of the weekly dosage regimen.

Six (6) other subjects were selected using the criteria as above to serve as a control for the study. The control subjects had their fasting blood glucose levels measured weekly for 6 weeks as they continued with their oral anti-diabetics but corn husk tea was not be administered.

3.5 PHYTOCHEMICAL SCREENING OF EXTRACT

The crude methanolic and aqueous extract of the corn husk were phytochemically screened in the quest to identify secondary plant metabolites that may be present in both. The under listed standard procedures for the screening were carried out:

3.5.1 Wagner's test for Alkaloids

An appreciable quantity of the crude extract was treated with 3 drops of a mixture containing 1.27g of iodine and 2g of potassium iodide in 100mL of water (Wagner's reagent). The formation of reddish brown precipitate shows a positive result.

3.5.2 Keller Kelliani's test for Cardiac glycosides

To 5mL of the crude extract in a test tube, 2ml of glacial acetic acid was added followed by a drop of ferric chloride solution. 1ml concentrated sulphuric acid was carefully added to the inner walls of the test tube. A formation of a brown ring at the interface shows the presence of cardiac glycosides.

3.5.3 Alkaline reagent test for Flavonoids

To 2ml of the extract in a test tube, a few drops of 20% sodium hydroxide solution was added. A positive test is observed when there is a formation of an intense yellow colour which turns colourless when dilute hydrochloric acid is added.

3.5.4 Ferric chloride test for Phenols

To a quantity of the crude extract, a small amount of 5% aqueous ferric chloride was added. The formation of deep blue or black colour indicates a positive result.

3.5.5 Foam test for Saponins

6mL of water was added to 2ml of the crude extract in a test tube. The mixture was shaken vigorously. An observance of a froth/foam which persist for 5minutes upon standing indicates a positive result.

3.5.6 Liebermann-Burchard test for Sterols

To 1ml of the crude extract, drops of chloroform, acetic anhydride and concentrated sulphuric acid (H_2SO_4) were added. A positive test should show the formation of a dark pink or red colour.

3.5.7 Braymer's test for Tannins

2mls of the crude extract was treated with 10% alcoholic ferric chloride solution. The observation of a blue or greenish coloured solution indicates a positive result.

3.5.8 Salkowki's test for Terpenoids

1ml of chloroform was added to 2ml of the crude extract. A few drops of concentrated sulphuric acid was then added. An immediate reddish brown precipitate indicates the presence of terpenoids.

3.5.9 Test for Glycosides

Excess 20% Potassium hydroxide (KOH) was added to a quantity of the crude extract. Equal amounts of Fehling's solutions A and B was added and the mixture heated on a water bath for 2 minutes. The formation of a brick red precipitate indicates the presence of glycosides.

3.6 CHROMATOGRAPHIC TECHNIQUES

Chromatographic techniques employed in this work were Column chromatography, thin layer chromatography and High Performance Liquid Chromatography.

3.6.1 Column Chromatography

The crude methanolic extract from the corn husk was mixed with an appreciable amount of dry silica (Silica gel CC, 70-230 mesh, India) and left to dry for three days. After the third day, the glass column (CR 32/20, Quickfit England) was dry-packed with silica gel gently to about 15cm level of the column. 20ml volume of the initial solvent (100% petroleum ether) was poured into the column to wet the silica before use. The dried sample (silica-extract) was poured on top of the packed column to make about a 2cm level. A piece of cotton wool was then placed over levelled packing in order not to disturb the surface packing. Various solvent systems with specific proportions were poured into the column to separate the extract into different fractions and the eluates collected into glass beakers. This method helped to obtain the different fractions or isolates of the components of the plant extract.

3.6.2 Thin layer chromatography

3.6.2.1 Activation of Adsorbent

It is very important to eliminate as completely as possible any solvent imbedded into the coated adsorbent of the TLC plate (Pre-coated TLC ALUGRAM SIL G, Germany). This is achieved by air-drying the TLC plates for 30 minutes and then in an oven (hot-air) maintained at 110 °C for another 30 minutes. The plate is subsequently cooled in a desiccator. This drying process helps to render the TLC plate (adsorbent layer) active. In order to achieve very active layers, silica gel and alumina coated plates may be heated up to 150 °C for a duration of 4 hours and cooling them in a desiccator.

3.6.2.2 Development of TLC method of analysis

The order in the eluotropic series was employed to help develop a thin layer chromatogram. Fractions from column chromatography were individually spotted on one edge of the activated TLC plates. Aluminium pre-coated with silica gel, 0.25 mm thick) with the aid of capillary tubes. The TLC plate was then placed vertically in the glass chromatank, with the edge to which the spot was applied down. The mobile phase in the bottom of the container travels up the plate by capillary action, passes over the spot and carries the compounds in the fraction up the plate at different rates resulting in separation of the compounds. The separated compounds were identified by inserting the TLC plate in a chamber saturated with iodine vapour.

3.6.3 Development of High Performance Liquid Chromatography fingerprint

The nature of the sample and its solubility in methanol was a major point considered in order to start the separation process. Hence, an organic aqueous mixture (methanol: water), mobile phase, was selected in a ratio of 70:30%v/v. Based on how the sample elutes with the solvent front, several manipulations using the same solvents but at different ratios. The wavelengths of detection were adjusted several times. Wavelengths employed were 254nm, 230nm, 274nm and etc. Finally, a mobile phase system of methanol: water in a ratio of 95:5%v/v and a detection at 230nm was selected for the fingerprint chromatogram.

Preparative HPLC analysis was carried out on the first fraction (f1). The peaks that eluted around approximately 9mins, 11mins and 13mins were individually collected into vials repeatedly after each injection of the sample. This was done until a substantial volume was obtained for further analysis.

HPLC pump used for analysis was P100 isocratic LC-pump by thermos scientific, Waltham, USA. eDAD powetchrom Integrator, USA was also used with the detector being Jasco UV 2075 plus detector, Essex, UK.

3.7 SPECTROSCOPIC METHODS

3.7.1 Ultraviolet-Visible (UV-Vis) spectrophotometry

A standard solution of all fractions including the crude extract was prepared using methanol. 0.1g of each sample was weighed in a 25ml beaker and dissolved with a little quantity of methanol. The solution was transferred into a 50ml volumetric flask. The beaker was rinsed with methanol and solution transferred back into the volumetric flask. The solution was topped up to the 50ml mark and labelled appropriately.

Each solution was analysed by pouring them into the 1-cm cuvette and scanning them individually in the UV-Vis spectrophotometer (T90+ UV-VIS Spectrometer, PG Instruments LTD.) across a wavelength range off 200 to 800nm.

3.7.2 Infrared spectrometry

Three pure liquid isolates from the first fraction (F1) collected from the preparative HPLC were prepared for infra-red analysis. Using PerkinElmer Frontier MIR spectrometer equipped with a single diamond universal attenuated total reflection (ATR) accessory, IR spectra was obtained for the three isolates.

CHAPTER FOUR

RESULTS

4.1 ANTI-DIABETIC ASSAY

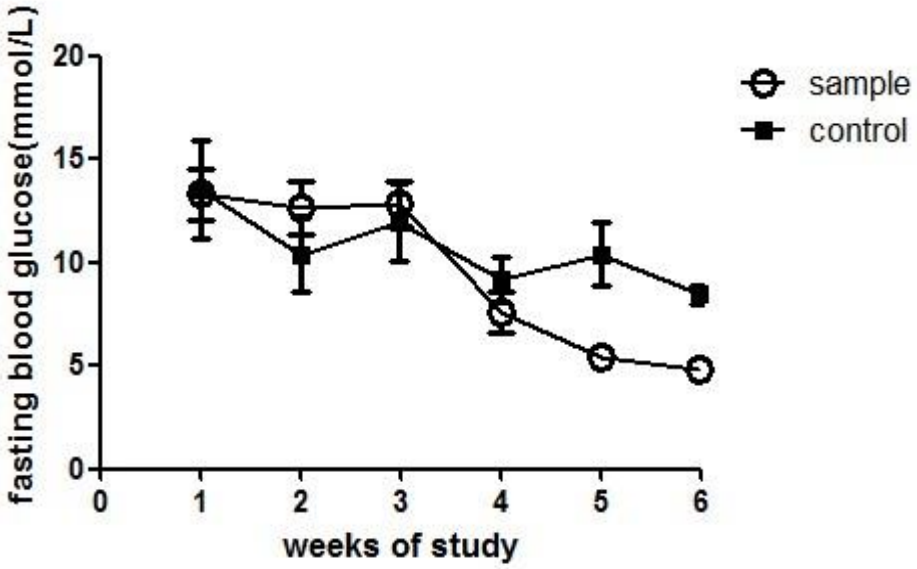


Figure 7: Study of corn husk tea on type 2 diabetics patients

4.2 PHYTOCHEMICAL SCREENING

Table 4.1: Results of the phytochemical screening of the methanolic and aqueous extract of the corn husk

Test	Methanolic extract	Aqueous extract
Tannins	+	–
Saponin	+	+
Alkaloids	+	+
Flavonoids	+	+
Steroid	–	–
Cardiac glycosides	–	–
Phenols	+	–
Terpenoids	–	–
Glycosides	+	+

4.3 CHROMATOGRAPHIC SEPARATIONS ON THE CRUDE EXTRACT

The brownish, oily methanolic crude extract was subjected to chromatographic analysis.

4.3.1 Column and Thin Layer Chromatography

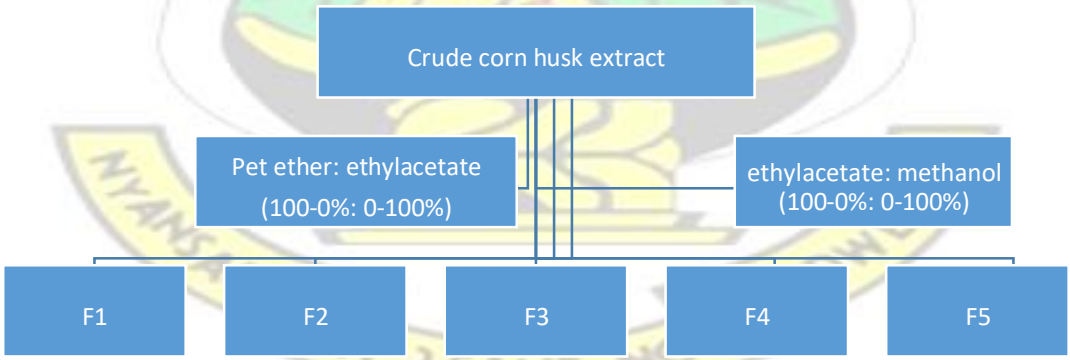


Figure 8: Fractions from column chromatography on crude methanolic extract

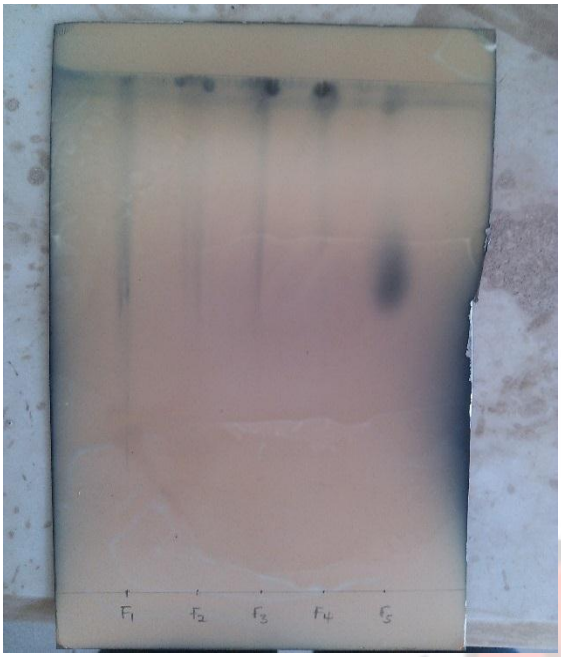


Figure 9: TLC of all five fractions

4.3.2 High Performance Liquid Chromatography

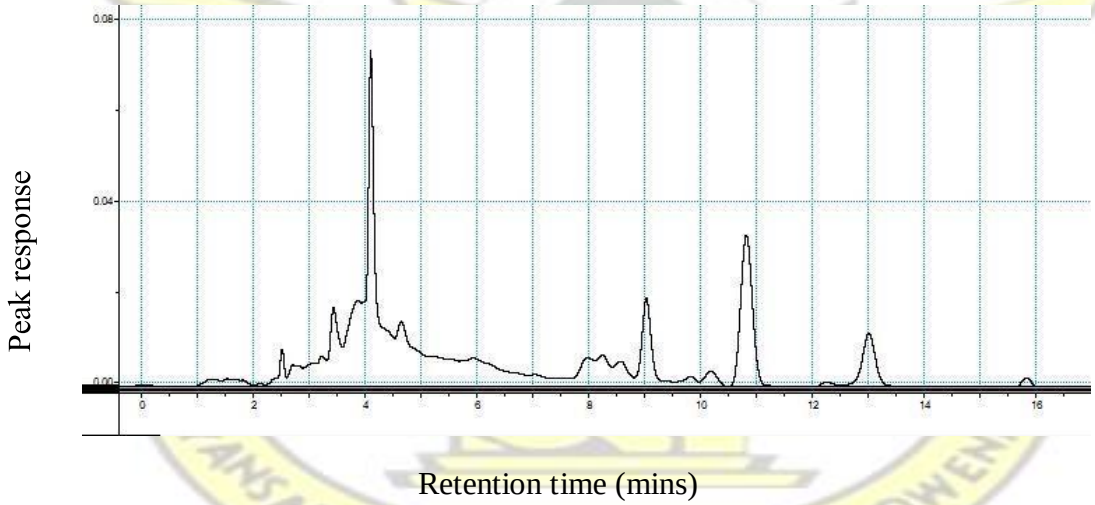


Figure 10: HPLC chromatogram for fraction one (F1) obtained from CC

4.4 SPECTROMETRIC ANALYSIS ON FRACTIONS AND PURE ISOLATES

4.4.1 Infrared Spectrometry

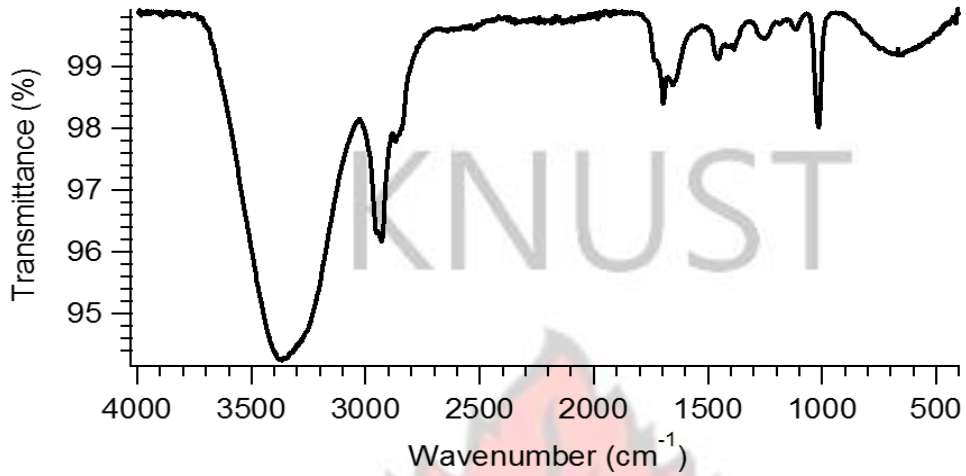


Figure 11: IR spectra for isolate 2

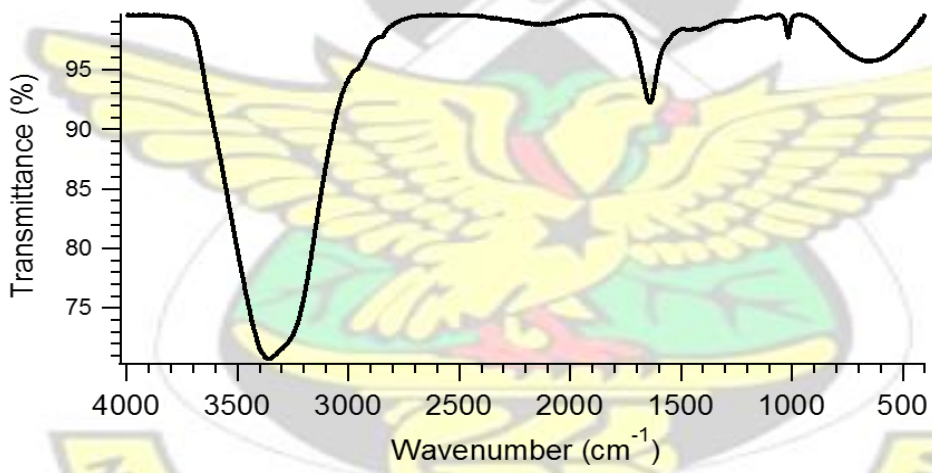


Figure 12: IR spectra for isolate 1

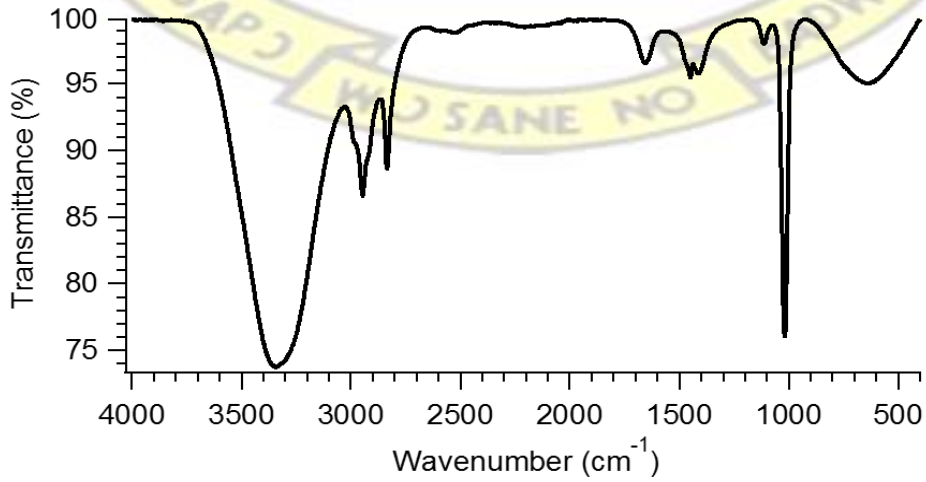


Figure 13: IR spectra for isolate 3

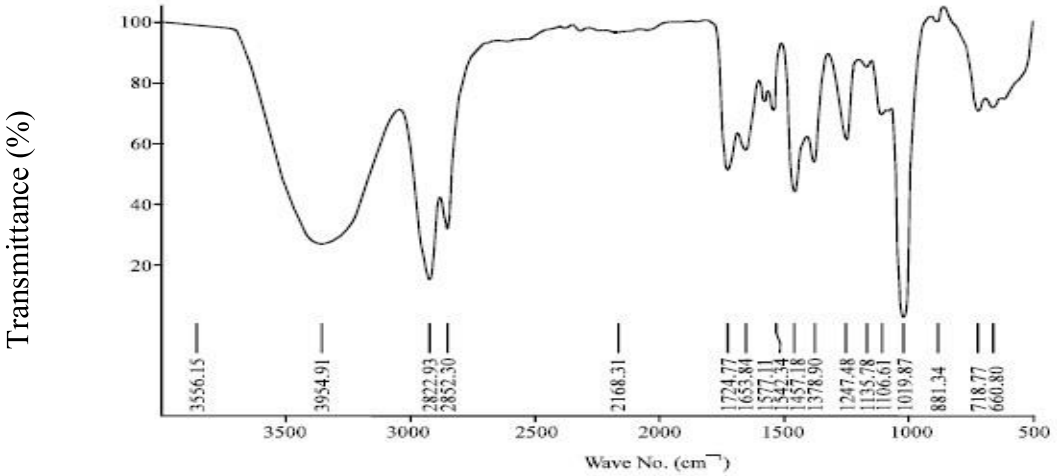
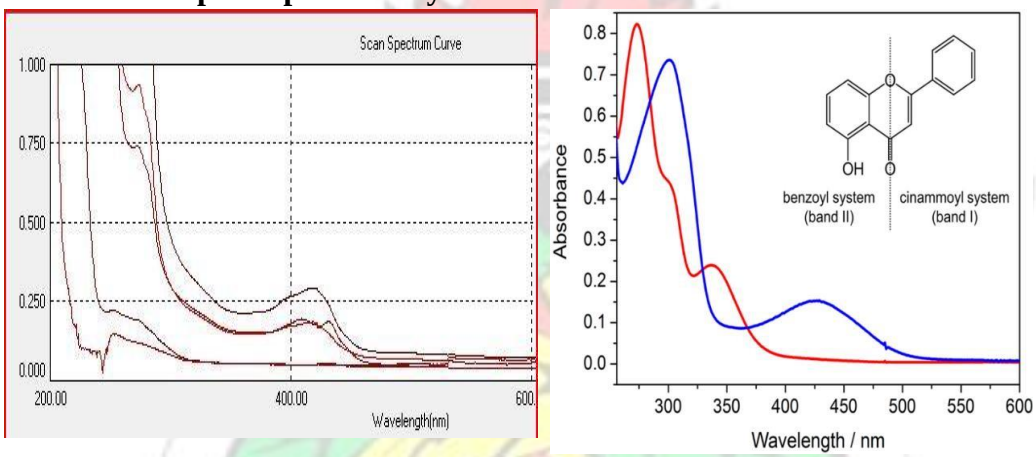


Figure 14: IR spectra of quercetin

4.4.2 UV-Vis Spectrophotometry



A **B**

Figure 15: (A) UV-visible spectrum of all 5 fractions and (B) UV-visible spectrum of primuletin

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

Zea mays (corn) husk has long been used to make dolls, wrapping 'Ga' Kenkey (an African dish), making paper, bags, lampshades and other decorative artefacts. The medicinal benefits of corn husk has not been highlighted and its anti-diabetic effects has not be scientifically proven.

This project seeks to provide scientific data and prove the anti-diabetic activity of corn husk and detect chemical compounds present in the aqueous and methanolic extract of the husk of Zea mays (corn) that may be responsible for the speculated activity.

The fight against diabetes mellitus has been on the rise for a long time. Several types of medications both orthodox and traditional are being employed. Corn silk, amongst the corn group, has been one of the traditional materials used to manage DM according to Khairunnisa Hasanudin et al, 2012. Corn husk promises to be effective in managing DM.

5.1.1 Anti-Diabetic Assay

This assay with corn husk tea was carried out to provide evidence of its glucose reduction ability and obtain a scientific data on the anti-diabetic activity of the corn husk tea. The assay was carried out using fasting blood glucose test. Fasting blood glucose test was selected because it helps to clearly tell how effective the different medications of the subject are since the subject by the end of the fasting period, would not have eaten any meal or taken any medication.

The basal glucose level was recorded to inform the extent of reduction of the blood glucose level by the orthodox medications whiles maintaining a healthy lifestyle. The anti-diabetic assay carried out in this experiment showed that corn husk tea reduces blood glucose levels as seen in Figure 7.

It can be observed that subjects within the first 3 weeks where the corn husk tea was not administered had their blood glucose levels high even upon taken their oral orthodox anti-diabetic medications. The following 3 weeks, where corn tea was administered with subjects still taking their oral anti-diabetics, saw the blood glucose levels drop to the almost normal, that is below 6mmol/l. the control, where subjects were not treated with the corn tea but maintained their oral anti-diabetics, showed no prominent decline in blood glucose levels. Blood glucose levels were being stabilized at the respective high levels but not necessarily being reduced to normal fasting blood glucose level of 3.9-5.5mmol/l (diabetes.co.uk).

Having an idea of the anti-diabetic activity of the corn husk tea, some chemical analysis were carried out with the quest to determine chemical components of the corn husk.

5.1.2 Phytochemical Screening

Phytochemical screening gives information on the nature of compounds including functional groups present in a plant extract and also helps to predict some biological activities that the sample may possess.

The aqueous and methanolic crude extract of the corn husk were screened for the presence of secondary metabolites (phytochemicals). Since the tea is prepared in the aqueous medium (water) in order to administer to the patients,

it was prudent to extract with methanol (with a polarity almost as water) to aid in a faster evaporation for analytical purposes. Both extracts showed the presence of alkaloids, saponins, flavonoids and glycosides as common constituents. The results are as shown in *Table 4.1*.

Methanolic extract showed the presence of phenols and tannins whiles that was absent in the aqueous extract. Methanol as a solvent for extraction has the capability of separating polyphenols. It is able to release polyphenols, of which flavonoids are example, from cells due to its high polarity (Tiwari et al, 2011). Flavonoids have been known to have antioxidant and anti-diabetic effect (Cherian S. and Augusti, 1995). This component of the corn husk tea may be exerting the anti-diabetic activity under investigation. The antioxidant ability of flavonoids make them health promoters since they mop up free radicals which DM patients accumulate a lot in the body. These observations support the usage of corn husk tea in the management of diabetes mellitus. Saponins have the potential to inhibit inflammation (Just M. J. et al, 1998). This activity of saponins would be of immense help to diabetics especially those with diabetic foot ulcers.

5.1.3 Chromatographic Separations on the Crude Extract

A TLC solvent system (mobile phase) was needed to help in detecting the components in the extract. Several solvent systems were employed but the components of the corn husk were not better resolved. To obtain better separation of fractions, the crude extract was subjected to column chromatography.

Separation of the compounds is based on the interaction between the stationary phase (silica gel) and mobile phase (solvent system). This method of elution

renders all components in crude extracts to be eluted based on their level of polarity. A non-polar solvent extracts non-polar compounds and vice versa.

The column was first eluted with 100% petroleum ether (a non-polar solvent), the polarity was gradually increased by addition of 10% ethyl acetate successively in an add-on manner and a 10% reduction of 100% petroleum ether till 0% petroleum ether was to 100% ethylacetate ratio. The same was repeated for ethylacetate in combination with methanol. Petroleum ether (PE) and ethylacetate (EA) gave three important fractions. Ethylacetate and methanol (ME) also gave two fractions at different proportions. Fractions obtained for PE: EA were of the ratios 80:20, 60:40 and 20:80%v/v respectively. That of ethylacetate and methanol (ME) were EA: ME in ratios of 80:20 and 40:60%v/v respectively. The labelled fractions are as shown in Figure 8.

Thin layer chromatography was then carried out again on the separate fractions but there was a poor development of the spots. Several solvent systems were tried by following solvent polarity states as seen in the eluotropic series. Two solvent systems were tried followed by three solvent systems, all in different proportions. It was observed that ethylacetate: methanol: water: glacial acetic acid (EA: ME: GAA: H_2O), a four solvent system in a ratio of 60: 20: 20: 2%v/v respectively gave a good development. Spots were seen visibly as a lot of streaks when exposed to iodine vapor.

The TLC of the individual fractions revealed the presence of many streaks as shown in Figure 9. This gave the idea that each fraction may be containing some other compounds.

Because of the poor development of the TLC, HPLC was carried out to detect the number of individual components present.

To carry out HPLC on any sample, it was expedient to develop a method of separation. To develop the method, methanol: water (H_2O) was chosen because the active ingredient(s) responsible for the said activity may be polar in nature. The choice of the solvent system depend greatly on the nature (polarity) of the sample under investigation. Since the tea is prepared in water and administered to patients for a reduction in blood sugar level, it can be concluded that the component or compound responsible for the activity is soluble in water hence polar.

The initial ratio of ME: H_2O chosen was 70:30%v/v respectively. The HPLC uses a UV detector hence detection with this system was done at 230nm, 274nm and a 0.002 range of detection. Detection was good at both wavelengths and appreciable but the eluted peaks were very close to the solvent front and peaks were closer to each other. This called for a change in the solvent system. A solvent system of ME: H_2O, 90:10%v/v was tried and the results was same as above. A good development was obtained when ME: H_2O, 95:5%v/v was used at a wavelength of 274nm and a range of 0.002.

The final solvent system used to develop the HPLC fingerprint was methanol: water in a ratio of 95:5%v/v at a wavelength of 274nm and a range of 0.002. The focus of the HPLC analysis on the five fractions, was to identify the number of peaks therein which gives the idea of the number of components present. Also, the analysis aimed at separating observables isolated peaks as pure isolates. From the Chromatogram, it was observed that the individual fractions

labelled F1 to F5, all were not pure fractions. Each fraction on analysis with the HPLC, gave a chromatogram showing the presence of other peaks that are clustered and not well resolved. The chromatogram of fraction one (F1) is as shown in

Figure 10 and that for the remaining fractions are as shown in Appendix C.

HPLC fingerprint for F1 showed distinct separation of some components with retention time of approximately 9mins, 11mins and 13mins. All the other major peaks with retention times between 2- 6mins were clustered and needed a better solvent system for separation. Preparative HPLC was carried out on the first fraction (F1) to separate the distinct peaks and hence three pure isolates from F1 was obtained.

5.1.4 Spectrometric Analysis on Fractions and Pure Isolates

Three (3) peaks with retention times approximately 9mins, 11mins and 13mins were observed in F1, F2, F3 and F4. But these peaks were prominent in F1, F2 and F3. Preparative HPLC was undertaken for the first fraction (F1) which had these three distinct peaks (as seen in

Figure 10) which could easily be collected. The peaks (pure isolates) were taken through infrared spectrophotometric analysis for identification of major functional groups present. Major observable absorption bands were identified in the spectra. This was compared to the IR spectra of quercetin (Karthishwaran K. et al, 2010), a flavonoid, shown in Figure 14.

From isolate 2, shown in Figure 11, it can be observed that the absorption spectrum consists of a few bands hence the substance is relatively simple. The presence of a hydroxyl –OH was indicated by the broad absorption at around 3300cm^{-1} (stretch) and at around 1020cm^{-1} (C-OH stretching or deformation). There is also a possibility of –OH hydrogen bonding which is demonstrated by a more intense and broader peak. This usually occurs when the sample is in the liquid state. The broad –OH band slips slights into the aliphatic –CH (sp3 hybridized) region of 2800cm^{-1}.

Aromatic absorption can be seen at its diagnostic regions of 1600, 1500 and 850-700cm^{-1}. A carbonyl stretch can be seen at 1700 cm^{-1} and it is confirmed by bands at 1280 cm^{-1} and 1310cm^{-1} (-C-O). These functional groups are characteristic of flavonoids.

Isolate 1, which is shown in Figure 12, is similar to the spectrum of isolate 3, seen in Figure 13. But there is a missing aliphatic –CH region at around 2800 cm^{-1} in isolate 1 and the absorption intensity of isolate 3 at around 1000 cm^{-1} is greater than in isolate 1. This makes the compounds different.

The spectra when compared to that of quercetin, a known flavonoids, revealed the fingerprint pattern of how a flavonoid IR spectra should look like. The IR spectra of all three isolates revealed that all spectra are flavonoids possessing a hydroxyl –OH that broadens due to the influence of hydrogen bonding. But may be having the same functional groups at different carbon positions.

5.1.5 UV-Vis Spectrophotometry

Ultra-Violet spectroscopy was also carried out on all fractions (F1 to F5) in order to determine the nature of the components there in. From the UV-Vis spectra

shown in Figure 15A, all the five fractions absorbed UV-Vis radiation and almost all the five fractions showed absorbance at prominent regions depictive of the presence of the flavonoid structural backbone.

Flavonoids show characteristic absorbance at certain wavelengths because of their characteristic benzoyl system and cinammoyl system. Based on the structural backbone of flavonoids, the cinammoyl system shows an absorbance at a wavelength around 300-400nm and that of the benzoyl system, around 240285nm (Anamaria D. P. Alexiou et al, 2015). The spectrum obtained from the analysis was compared to that of primuletin, a known flavonoid and it showed the similarity in how flavonoid UV-Vis spectra should look like. The spectrum of primuletin is as seen in Figure 15B.

5.2 CONCLUSION

The search for a more convenient, cost effective and a better complying way of managing diabetes mellitus is unending just as it is in the rise in the use of herbal medicines. The project aimed at investigating the chemical constituents and anti-diabetic activity of the dried husk of *Zea mays* (corn), Poaceae. It can be concluded that the corn husk tea has anti-diabetic activity. This anti-diabetic activity is potentiated by the presence of flavonoids, tannins and alkaloids. The phytochemical screening also revealed the presence of other secondary metabolites such as glycosides, saponins and phenols. From column chromatography, five fractions were obtained from CC. Also, ethylacetate: methanol: water: glacial acetic acid in a ratio of (60:20:20:2) %v/v was obtained as the best solvent system for detection of the chemical components of the fractions of the corn husk. Again, a simple fingerprint spectra was obtained for the five fractions using HPLC and the three isolates using IR.

From the IR, it can be observed that isolates have hydroxyl and carbonyl functional groups present, with some having aliphatic –CH and all having a conjugated system therein.

Finally, it can be deduced that the components of the corn husk tea are closely related of which flavonoids are included and may be exerting the anti-diabetic activity.

5.3 RECOMMENDATIONS

The following considerations demanding future studies are recommended:

i. Anti-diabetic assay should be extended to 6months for glycated hemoglobin (HbA1c) analysis to be done in addition to the fasting blood glucose test (FBGT).

ii. Identification of bioactive fraction (component) of corn husk should be carried out by using diabetes-induced rats in order to limit the analytical work to the bioactive fraction.

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APPENDIX A

Appendix A1 Anti-Diabetic Assay

Subjects/ Weeks	FBG Level Before Administration Of Tea (mmol/L)			FBG Level After Administration Of Tea (mmol/L)		
	1	2	3	4	5	6
A	17.2	10.5	15.8	7.2	5.7	2.7
B	21.6	22.6	20.6	17.5	6.1	4.7
C	17.1	17.4	17.0	6.1	6	5.4
D	12.7	12	12.5	5	5	2.7
E	8.8	8.6	9.2	7	5.6	5.1
F	9.2	8.3	9.6	9.2	5.9	4.5
G	9.4	8.8	9.4	7.2	6	6
H	11.1	12.1	11.6	5.2	5	6.5
I	16.5	16.5	16.3	5.8	6	6.1
J	16.3	14.8	12.2	5.7	4.1	5.8
K	8.2	8.2	8.2	7.7	4	6
L	11.6	11.6	11.6	7.2	5.8	2.8

Appendix A2 Control Test (Anti-Diabetic Assay)

Subjects / Weeks	Basal FBG Level (mmol/l)					
	1	2	3	4	5	6
A1	9.1	8.9	8.3	7.8	9.3	9
B2	17.2	5.9	7.3	5.7	7.5	7.8
C3	23.6	10.7	15.8	9.4	7.8	8.7
D4	10.1	19.1	11	13	17.4	9.1
E5	9	9.4	10	7.8	8.7	6.7
F6	12.1	8.4	19.2	11.3	11.6	9.4

APPENDIX B

Appendix B1 Description of Fractions from Column Chromatography

Fraction	Solvent	Ratio (%)	Observation of eluates
F1	PE:EA	80: 20	Light green band
F2	PE:EA	40: 60	Deep green band
F3	PE:EA	20: 80	Deep green band
F4	EA: ME	80: 20	Brown oily band
F5	EA: ME	40: 60	Brown non oily band

APPENDIX C

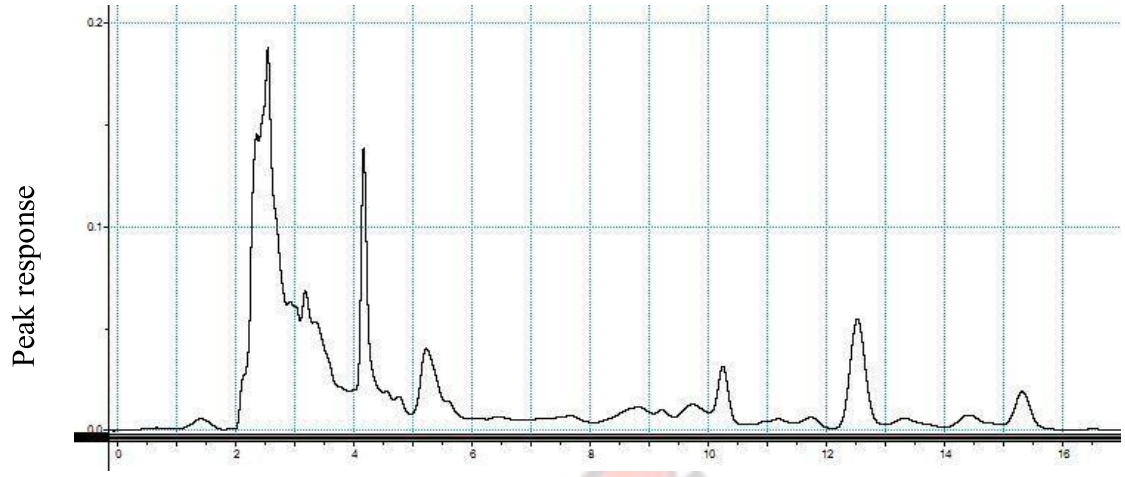


Figure 16: HPLC chromatogram of fractions F2

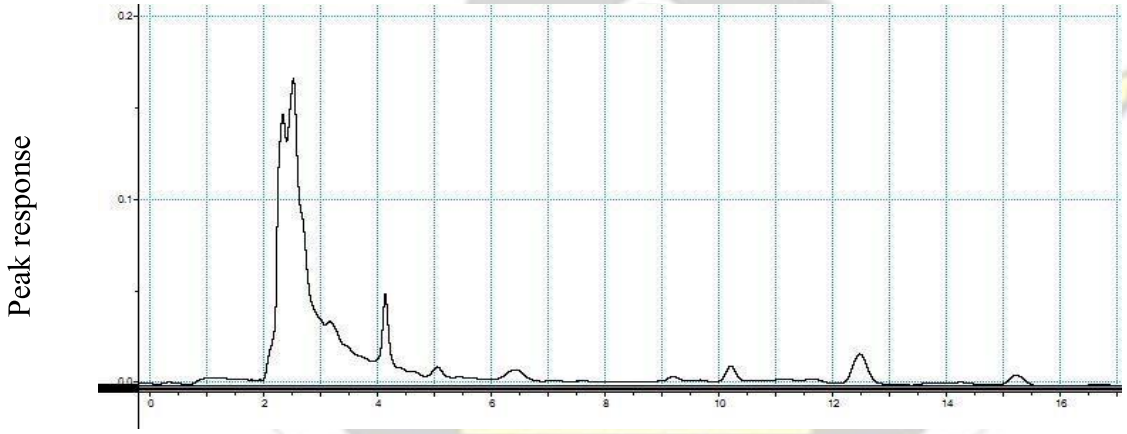


Figure 17: HPLC chromatogram of fractions F3

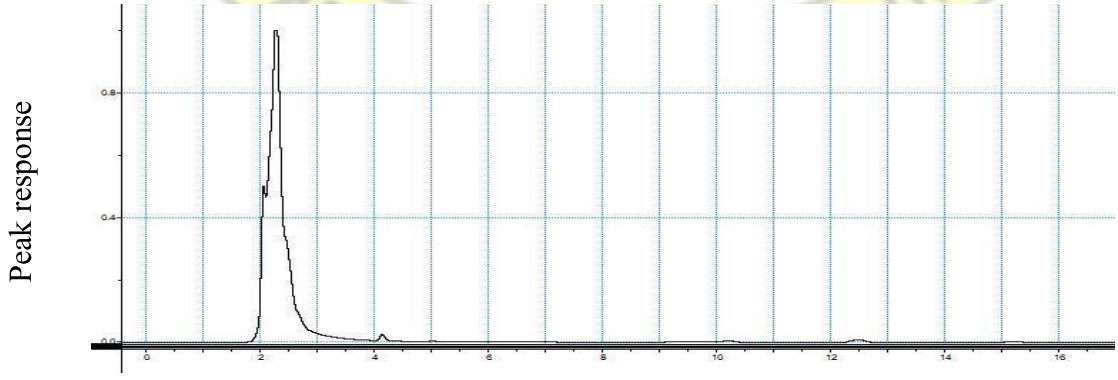


Figure 18: HPLC chromatogram of fractions F4

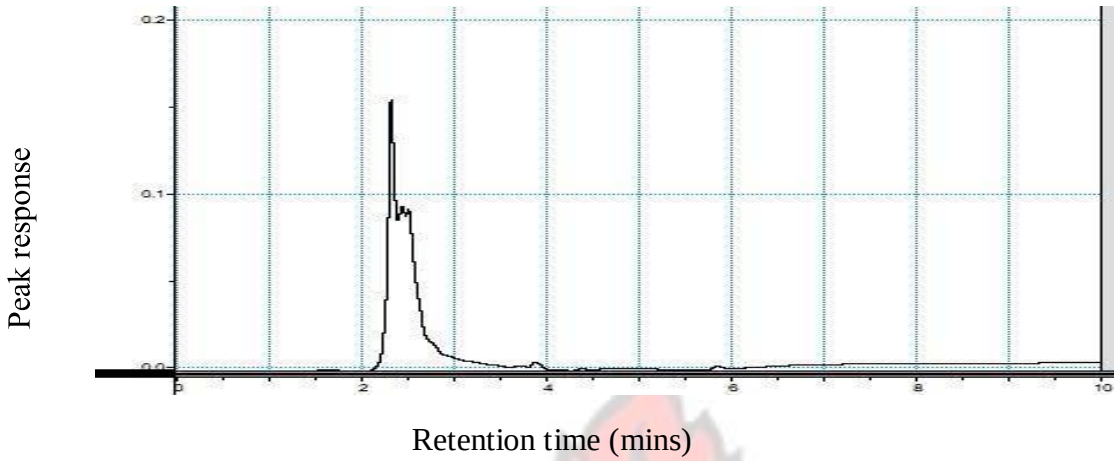
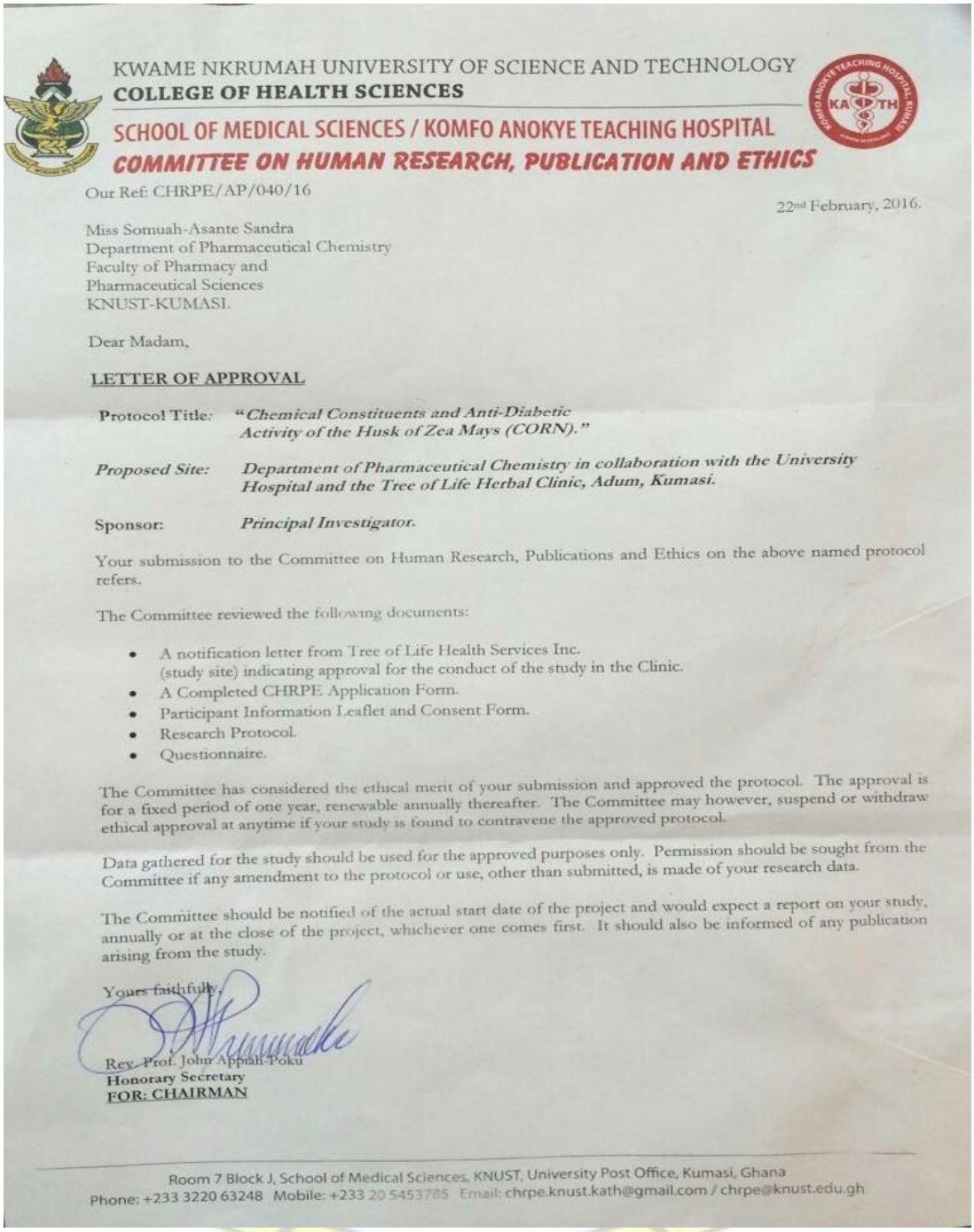


Figure 19: HPLC chromatogram of fractions F5

APPENDIX D



KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/040/16

22nd February, 2016.

Miss Somuah-Asante Sandra
Department of Pharmaceutical Chemistry
Faculty of Pharmacy and
Pharmaceutical Sciences
KNUST-KUMASI.

Dear Madam,

LETTER OF APPROVAL

Protocol Title: *"Chemical Constituents and Anti-Diabetic Activity of the Husk of Zea Mays (CORN)."*

Proposed Site: *Department of Pharmaceutical Chemistry in collaboration with the University Hospital and the Tree of Life Herbal Clinic, Adum, Kumasi.*

Sponsor: *Principal Investigator.*

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A notification letter from Tree of Life Health Services Inc. (study site) indicating approval for the conduct of the study in the Clinic.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Protocol.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at the close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Yours faithfully,

Rev. Prof. John Appiah-Poku
Honorary Secretary
FOR: CHAIRMAN

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Figure 20: Ethical Clearance Obtained for the Study

APPENDIX E

SAMPLE QUESTIONNAIRE FOR SUBJECTS

General

Date of birth: …… Sex: ◯Female ◯ Male

Address: …… Nationality: ……

Telephone: …….. …. Occupation: ……

Are you currently?

◯ married ◯ separated ◯ widowed ◯ single ◯ divorced

Age: ◯ under 45 years ◯ 45–64 years ◯ Over 64 years

Diabetes knowledge and History

In which year or age were you diagnosed of diabetes?

……..

Have you ever experienced any of the following symptoms?

a. Increased thirst..... ◯ Yes ◯ No

b. Dry mouth..... ◯ Yes ◯ No

c. Decreased appetite..... ◯ Yes ◯ No

d. Abdominal pain..... ◯ Yes ◯ No

e. Urinate 3 or more times a night? …….... ◯ Yes ◯ No

Please indicate below which chronic condition(s) you have:

◯ High cholesterol ◯ High blood pressure

Heart disease: Type of heart disease:

……

Lung disease: Type of lung disease:

……

Other chronic condition Specify:

……

Current diabetic state

What type of diabetes you have?

◯ Type 1 ◯ Type 2 ◯ Pre-diabetes ◯ Gestational ◯ Don't Know

Do you have a machine to measure your blood sugar (glucose) level?

◯ Yes ◯ No

What do you normally take for the following?

Breakfast ……..……..

Lunch ……..

Supper ……

How often do you eat vegetables and fruits?

◯ Everyday ◯ Often ◯ Once in a while

Medications

Have you been taking medications for diabetes? ◯ Yes ◯ No

Please specify……

About how often do you miss taking your medications as prescribed? …….

In the past 6 months, how many times did you visit your doctor at the hospital? ……

Have you ever been on insulin injections? ◯ Yes ◯ No

When last did you take a shot?

……

Have you tried any traditional medicine for diabetes treatment?

○ Yes ○ No

What was the results?

.....

What other medications are you on and for what indication?

..... for……..……..

…….. for……..…

Do you check your blood sugar level regularly? ○ Yes ○ No

Thank you for your availability!