PHYTOCHEMISTRY AND ANTIDIABETIC ACTIVITY OF THE SEED EXTRACTS OF *Eleusine coracana* Linn (POACEAE)

BY

IRENE ONOJIMENEJE OSEGHALE

(PG/PHA0502774)

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY

JUNE, 2019

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A THESIS WRITTEN IN THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE OF THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA

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CERTIFICATION

This is to certify that this work was done by Irene Onojimeneje Oseghale in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

Signature_____ Date _____

Prof. Abiodun Falodun

(Supervisor)

Signature _____ Date _____

Dr. Ighodaro Igbe

(Co-Supervisor)

Signature _____ Date _____

Dr. Patrick Igbinaduwa

(Head of Department)

CERTIFICATION OF THESIS/DISSERTATION ON PLAGIARISM

We the undersigned attest and declare that the thesis of Irene Onojimeneje Oseghale, titled: Phytochemistry and Antidiabetic Activity of the Seed Extracts of *Eleusine coracana* Linn (POACEAE), has successfully passed the anti-plagiarism test and does not violate any copyright regulations.

_____	_____
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(Chief Supervisor)	(Co-Supervisor)

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Dr. Patrick Igbinaduwa

(Head of Department)

DEDICATION

This work is dedicated to the Almighty God and to all who have lost a dear one to diabetes.

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LIST OF ABBREVIATIONS

ABTS	-	2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid
ALB	-	Albumin
ALP	-	Alkaline phosphatase
ALT	-	Alanine aminotransferase
ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemists
ASP	-	Aspartate aminotransferase
ATP	-	Adenosine triphosphate
CAT	-	Catalase
CB	-	Conjugated bilirubin
CNS	-	Central Nervous System
CO	-	Combination
CO_2	-	Carbon iv oxide
CUPRIC	-	Cupric reducing antioxidant capacity
DCM	-	Dichloromethane
DMPD		N,N-dimethyl-p-phenylene diamine dihydrochloride
DNA	-	Deoxyribonucleic acid
DPPH	-	1, 1-diphenyl-2-picrylhydrazyl
DW	-	Distilled water
EA	-	Ethylacetate
EA: MeOH	-	Ethylacetate : methanol
EDTA	-	Ethylenediamine tetraacetic acid
EIMS	-	Electron Impact Mass Spectrometry
eV	-	electron Volt
FCR	-	Folin-Ciocalteu
FRAP	-	Ferric reducing-antioxidant power
FTC	-	Ferric thiocyanate
GAE	-	Gallic acid equivalents
GC	-	Gas Chromatography

GC-MS	-	Gas Chromatography-Mass Spectrometry
GGT	-	Gamma-glutamyl transpeptidase
GIP	-	Glucose-dependent insulinotropic polypeptide
GLO	-	Globulin
GLP 1	-	Glucagon-like peptide 1
GLP 1R	-	Glucagon –like peptide 1 receptor
GR	-	Granulocyte
$GSHP_X$	-	Glutathione peroxidase
GSt		Glutathione-S-transferase
HCT	-	Haematocrit
HDL	-	High density lipoprotein
Hgb	-	Haemoglobin
HORAC	-	Hydroxyl radical scavenging activity, hydroxyl radical averting capacity
HPLC	-	High Performance Liquid Chromatography
IDF	-	International Diabetes Federation
IR	-	Infra-Red
LD_{50}	-	Median lethal dose
LDL	-	Low-density lipoprotein
LPO		lipid peroxidation
LY	-	Lymphocyte
MCH	-	Mean corpuscular haemoglobin
MCHC	-	Mean corpuscular haemoglobin concentration
MCV	-	Mean corpuscular volume
MeOH	-	Methanol
mMFSE	-	Millimolar ferrous sulphate equivalent
MO	-	Monocyte
MS	-	Mass Spectrometry
PLT	-	Platelet
PPM	-	Parts per million
QE	-	Quercetin equivalent
RDW	-	Red cell distribution width.
RNA	-	Ribonucleic acid

RNS	-	Reactive Nitrogen Species
ROS	-	Reactive Oxygen Species
RSS	-	Reactive Sulphur Species
SEM	-	Standard Error of Mean
SOD	-	Superoxide Dismutase
TB	-	Total bilirubin
TBA		Thiobarbituric acid
TC	-	Total cholesterol
TG	-	Triglyceride.
TLC	-	Thin Layer Chromatography
TP	-	Total protein
TRAP	-	Total radical-trapping antioxidant parameter
UV	-	Ultra-Violet
UV-VIS	-	Utraviolet- visible
WBC	-	White blood cell
WHO	-	World Health Organisation

ABSTRACT

Eleusine coracana is commonly found in the arid regions of Asia and Africa. The seeds are used locally in the management of various diseases such as diabetes, osteoporosis, anaemia, ulcer, depression and insomnia. This study was carried out to determine the antidiabetic activity of different fractions of *Eleusine coracana* seeds and identify compounds present in the active fractions.

Proximate analysis, phytochemical screening and mineral analysis were carried out using standard methods. The powdered seed (5.5 kg) was extracted with methanol in a Soxhlet apparatus. The concentrated crude extract (100 g) was subjected to vacuum liquid chromatography using solvents of increasing polarity (n-nexane, dichloromethane, ethylacetate, 50% ethanol/ethyl, 100% methanol, 20% water/methanol, 50% water/methanol and water) to obtain different fractions. The median lethal dose was determined using Lorke's method. Antioxidant screening, total phenols and flavonoid content determination were also carried out on the extract and fractions using 1,1, diphenyl 2 picryl hydrazyl (DPPH) free radical scavenging method, ferric reducing antioxidant power (FRAP) method, folin ciocateau and aluminium chloride test respectively. The crude extract and selected fractions at a dose of 200 and 400 mg/kg/day were screened for antidiabetic activity using streptozotocin-induced diabetic rats. The ethylacetate fraction was chromatographed to obtain fractions. Fractions with similar TLC profile were combined and selected combinations were further purified. The ethylacetate and n-hexane fraction were subjected to infrared spectroscopic (IR) analysis and Gas Chromatography-Mass Spectroscopic (GC-MS) analysis to determine the chemical constituent(s) present.

The moisture content and total ash of the powdered seed were 0.38 ± 0.14 and 2.23 ± 0.13, respectively. Phytochemical screening revealed the presence of carbohydrates, proteins,

alkaloids, flavonoids, tannins, reducing sugars, saponins, phenols and terpenoids. Mineral analysis revealed the presence of essential minerals such as calcium (362.20 ± 9.01), sodium (11.66 ± 1.17), potassium (19.72 ± 0.39), magnesium (1.06 ± 0.00) and zinc (0.35 ± 0.01). Heavy metals such as nickel and cadmium were not present in the sample while the others fell below WHO recommended limit for heavy metals. The median lethal dose was observed to be 5000 mg/kg/day, no mortality was observed after 24 hr and 14 days of monitoring. Antioxidant screening revealed that the 50% ethylacetate: methanol fraction had the highest antioxidant activity (IC50 = 29.65 μg/mL) as well as the highest total phenol (132.40 ± 0.51 mgGAE/g) content compared to the crude extract and fractions. The crude extract and fractions at 200 and 400 mg/kg/day dose were able to significantly ($P < 0.05$) reduce the blood glucose level of the diabetic rats at varying degrees compared to the diabetic untreated animals. Photomicrograph of the pancreas revealed that the crude extract and fractions reduced degenerative changes in the beta cells of islet of Langerhans at varying degrees compared to the diabetic untreated animals. At 400 mg/kg/day, the n-hexane fraction showed the least protective effect on the islets cells. IR spectroscopic analysis revealed that carbonyl groups are the major functional groups present in the tested samples. GC-MS analysis of the ethylacetate and n-hexane fractions revealed that the compounds contained in these fractions are non-polar, long chain fatty acids and esters, few alcohols, long chain saturated alkanes and unsaturated alkenes. This study has shown that the various fractions of *Eleusine coracana* can significantly reduce blood glucose level in diabetic rats and these fractions contain compounds which may attribute to their antidiabetic effect.

CHAPTER ONE

INTRODUCTION

1.1 PLANTS AS A SOURCE OF MEDICINE

From time immemorial plants have been used as source of medication for the treatment and management of various ailments (Monier and El-Ghani, 2016). Medicinal plants can be defined as any plant that contains any substance in one or more organ (leaf, fruit, flower, stem or root) that could be used for treatment and as precursors for the synthesis of new medicines (WHO,1976). Many plants are being used in the treatment of various ailments all over the world (Mahmoud, 2013). Up to 80% of people living in the world use medicinal plants as their primary health care source (Sandhya *et al*., 2006). Natural products contain several chemical constituents or secondary metabolites which work either individually, additively or synergistically to produce therapeutic effects (Mahomoodally, 2013). Due to their diverse chemical constituents, they are a good platform for drug discovery and to achieve this, different processes such as; extraction using various solvent systems, pharmacological screening of extracts, isolation and characterization of active principles are carried out. Also screening of the isolated compounds for toxicity and clinical evaluation are also done (Sasidharan *et al*., 2011). Some plants being used for managing diseases are; *Veronia cinerea* for the management of kidney stones and management of conditions such as stones contained in urinary bladder (Thenmozhi *et al*., 2016), *Sida acuta* used for the management of wound, sores and bleeding conditions (Edeoga *et al*., 2005), *Zingiber officinale* used in treating malaria, *Ocimum gratissimum* for the treatment of pile (Borokini *et al*., 2013), *Polyalthia longifolia* used in the treatment of gout (Singh *et al*., 2010), *Arctium lappa* used in the treatment of cancer (Kharb *et al*., 2012). Others are; *Marrubium vulgar* for the treatment of liver disorder (Mohammad *et al*., 2016) and *Eleusine coracana* for the

treatment of diabetes (Oseghale *et al.*, 2017). Also, some drugs obtained from natural sources include; atropine from *Atropa belladonna*, benzyl penicillin from *Penicillium chrysogenum*, codeine from *Papaver somniferum*, Digoxin from *Digitalis lantana*, quinine from *Cinchona succirubra*, vinblastin and vincristin from *Cathranthus roseus* and lovastatin from *Aspergillus terrus* (Edeoga *et al.*, 2005). Drugs such as artemisinin, etoposide and mevastatin are also derived from plants (Maridass and Britto, 2008). Plants contain a lot of minerals and vitamins necessary for metabolic processes in the body. They also contain secondary metabolites such as cardiac glycosides, phenols, alkaloids, saponins, tannins, steroids, flavonoids and other plant nutrients which have been discovered to be of therapeutic benefits when used appropriately. The development of chronic diseases in the body such as; atherosclerosis, cancer, ageing and diabetes is associated with the presence of excess free radicals in the body system (Sreeramalu *et al.*, 2009). These plants could be explored with the aim of getting newer agents that will be beneficial in managing disease conditions. A lot of plants contain antioxidants and exhibit antioxidant potentials (Duduku *et al.*, 2011). Antioxidants prevent or delay the oxidation of macromolecules like enzymes, genetic materials, proteins and fat in the body by removing free radicals and reducing the level of oxidative stress (Sulaiman *et al.*, 2013).

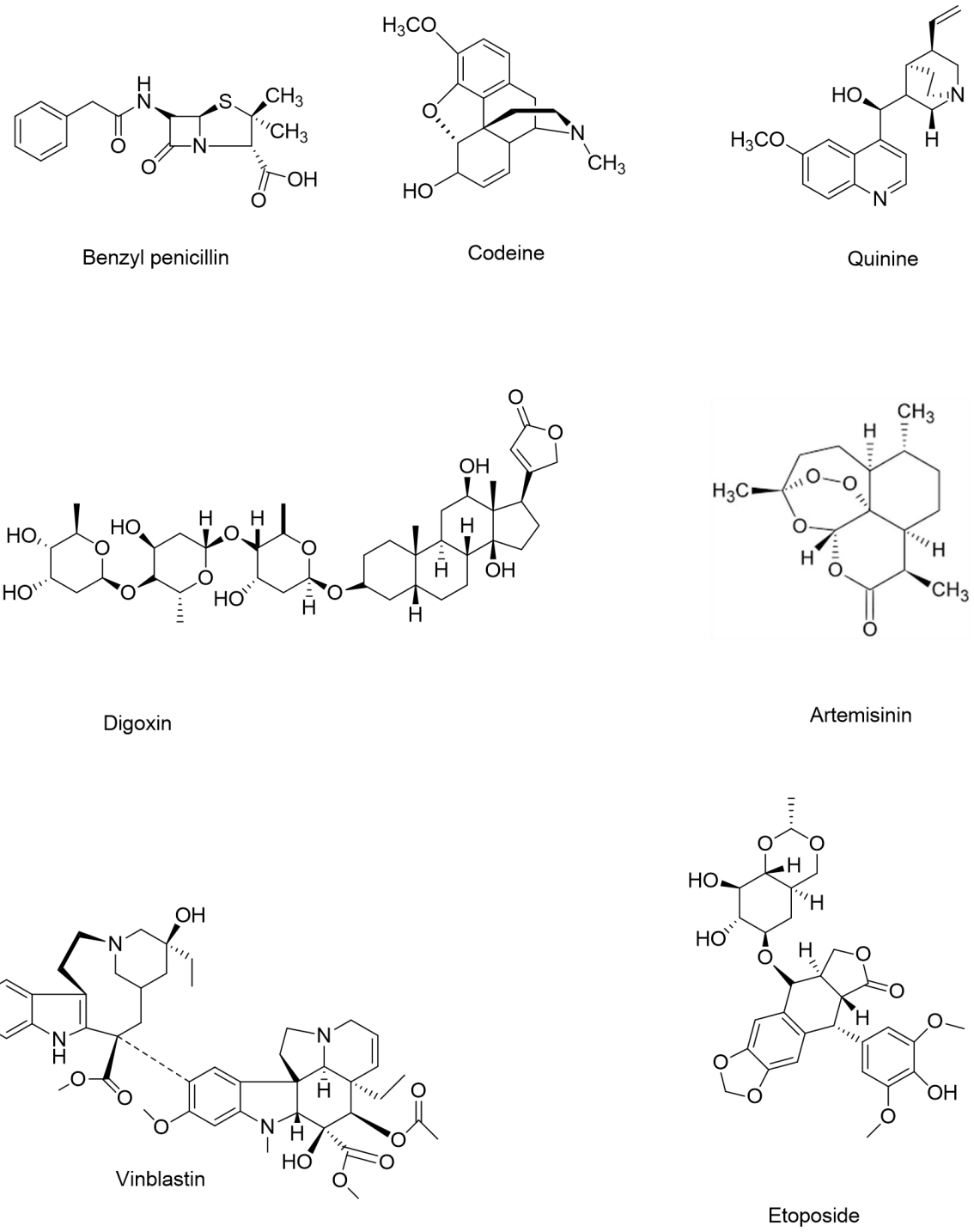


Figure 1.1: Structures of some drugs obtained from natural sources

1.2 PLANT SECONDARY METABOLITES

Secondary metabolites found in plants are either intermediates products or products of plant metabolism. Plant secondary metabolites perform different functions in the plants they are produced. These functions include; pigmentation of the plant to improve the chances of pollination, protection from UV radiation, protection against pathogens, animals and other factors that are threats to the plant (Joash and Yau, 2014). Plant secondary metabolites are organic compounds which do not directly take part in important processes such as reproduction, growth and development in plants. Examples are alkaloids, phenols (flavonoids, coumarins and stilbenes), terpenes, (Ruby and Rana, 2015). Some of the phytoconstituents that have antioxidant activities are; tannins, coumarins, lignans, monoterpenes, diterpenes, cinnamic acids, phenylpropanoids, flavonoids and triterpenes (Larkins and Wynn, 2004). Plant secondary metabolites also include; glycosides and saponins (Kabera *et al*., 2014).

1.2.1 ALKALOIDS

Alkaloids are secondary metabolites which have low molecular weight and many alkaloids are known to have a heterocyclic ring structure with a nitrogen atom attached making it alkaline in nature (Helio and Arthur, 2015). Alkaloids in plants serve as defense mechanism against predators and pathogens. Examples of alkaloids present in nature are; indole alkaloids, monoterpenoid alkaloids, peptide alkaloids, betalain alkaloids, phenethylamines, methylxanthines, amaryllidaceae alkaloids, imidazole alkaloids, diterpenoid alkaloids, pyrrolizidine alkaloids, quinolizidine alkaloids, tropane alkaloids and steroidal alkaloids (Anurag *et al.,* 2014). Some alkaloids may be toxic (e.g. Nicotine) while others may be of therapeutic benefit (e.g. quinine) (Helio and Arthur, 2015). Alkaloids can be classified based on the structure of their heterocyclic ring into; Pyrrolidine alkaloids e.g.; hygrine contained in the leaves of *Erythroxylum coca*, Pyridine alkaloids e.g.; piperine, Pyrrolidine-pyridine

alkaloids e.g.; Nicotine, Pyridine-piperidine alkaloids e.g.; Anabasine, Quinoline Alkaloids e.g.; Quinine and Isoquinoline alkaloids e.g.; Opium alkaloids such as morphine (Saxena *et al.*, 2013).

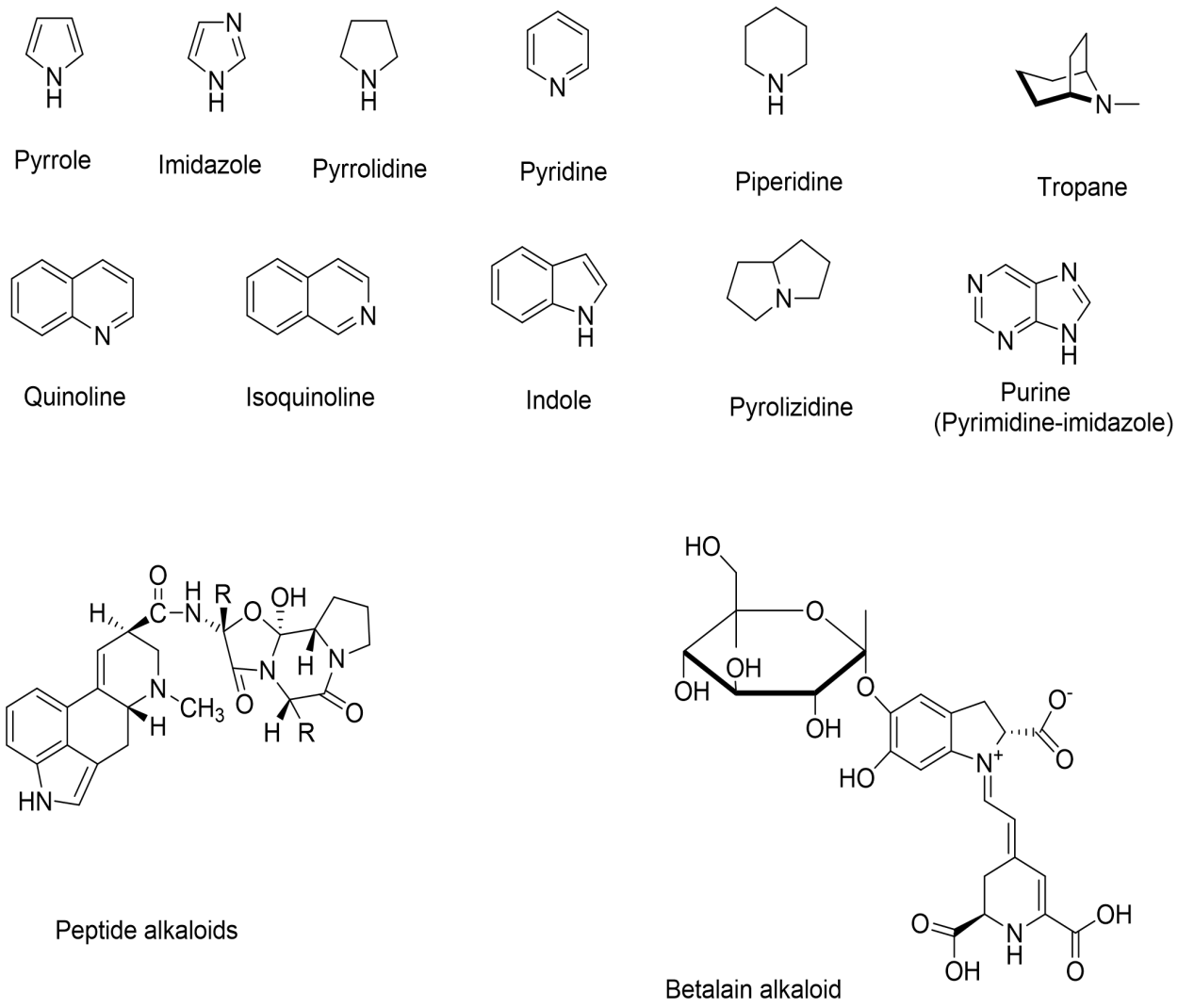


Figure 1.2: Examples of alkaloids

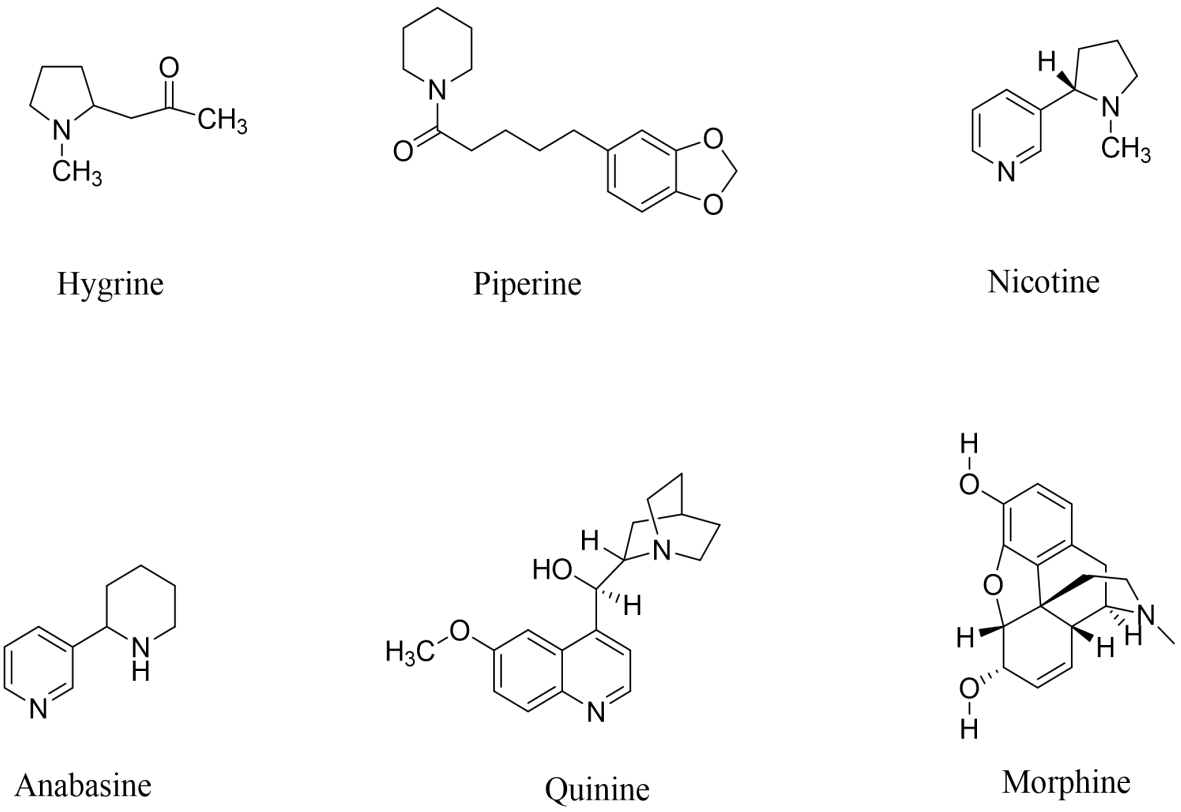
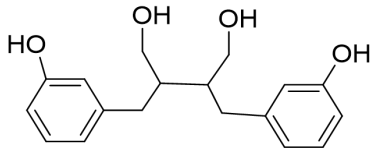


Figure 1.2: Examples of alkaloids

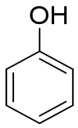
1.2.2 PHENOLICS

These are the most frequently occurring metabolites in plants. Their structure is made up of benzene nucleus with hydroxyl group(s) attached. Phenolic acids, flavonoids and anthocyanins make up the phenolics with flavonoids being the most common in plants. Plant phenols are usually involved in plant defense mechanism and serves as powerful antioxidants for human beings which helps to prevent oxidative stress linked to with different ailments such as diabetes, diseases that affect the neurons, atherosclerosis, central nervous system (CNS) related diseases and cancer (Obrenovich *et al*., 2011). Fruits and vegetables contain a lot of phenols (Derong *et al.*, 2016). Generally, plant phenols are classified according to the number of catechol rings found in the structure as well as the nature of substituents attached

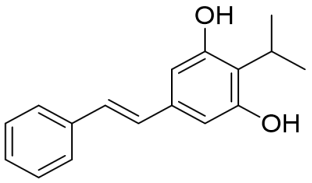
to the ring. The two major groups of polyphenols that exists are; the flavonoids (chalcones, flavonols, dihydroflavonols, flavan-3-ols, isoflavones, flavones, flavanones, proanthocyanidins and anthocyanidins) and the non-flavonoids (simple phenols, hydrolysable tannins, cinnamic acids, benzoic acids, acetophenones, xanthones, secoiridoids coumarins, phenylacetic acids, benzophenones, stilbenes and lignans) (Shahriar and Robin, 2010).



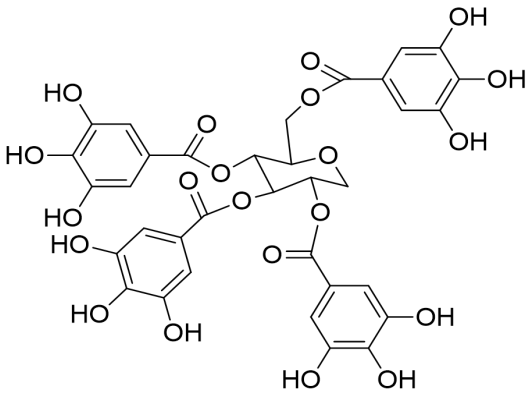
Lignans



Simple phenol



Stilbenes



Hydrolysable tanins

Figure 1.3: Examples of phenols

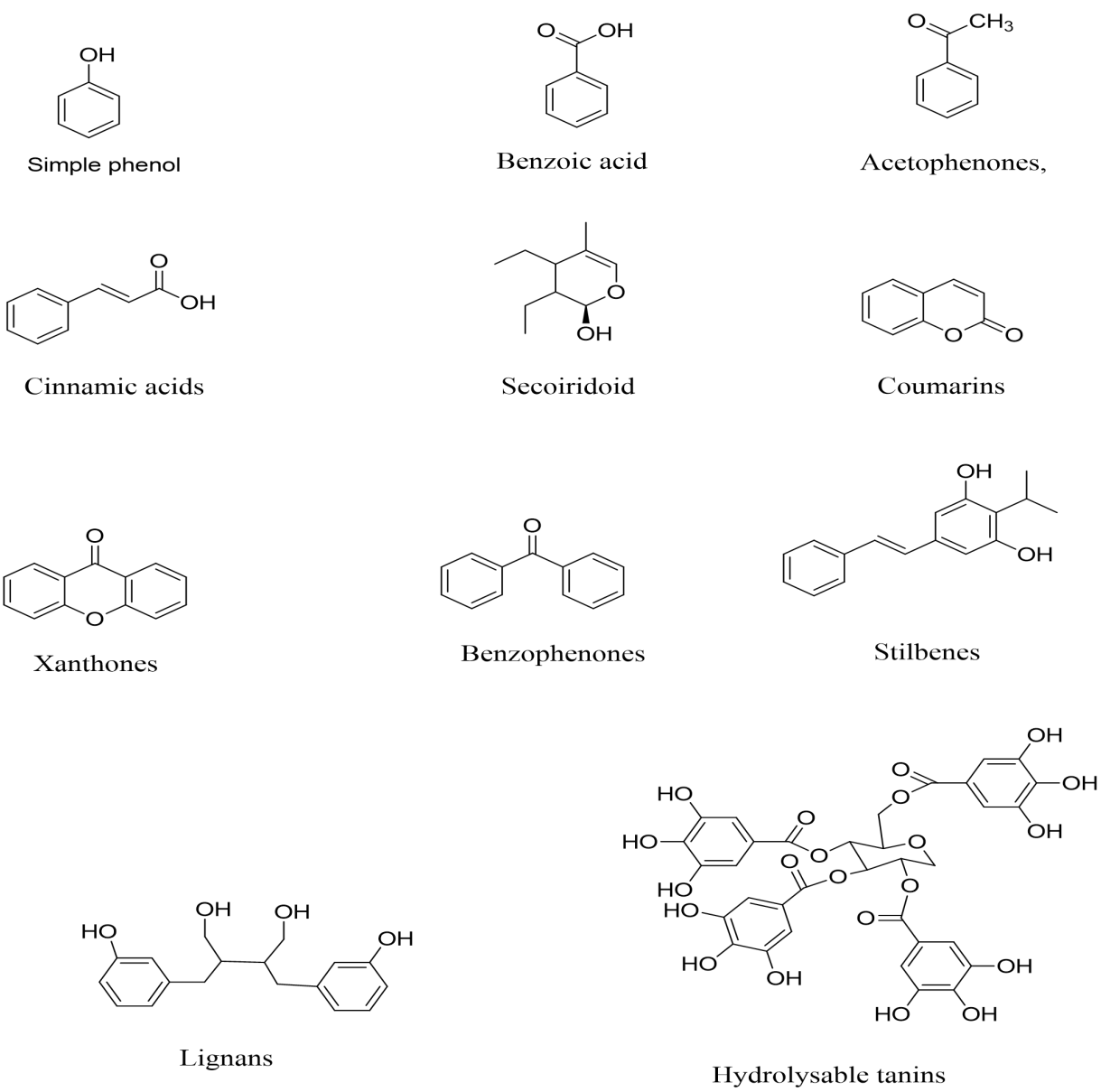


Figure 1.4: Examples of flavonoids

1.2.3 TERPENES

Terpenes are secondary metabolites that show a wide range of biological activities (Villar *et al*., 2003). They are made up of 5 carbon isoprene units which are linked together. They vary in their level of unsaturation, power of oxidation, the functional groups attached and also the type of ring closure hence the different terpenoids found in nature. They are both classified based on the number of isoprene units they contain. They are; hemiterpenes e.g. isoprene (C_5H_8), monoterpenes e.g. limonene ($C_{10}H_{16}$), sesquiterpenes e.g. zingiberene ($C_{15}H_{24}$), diterpenes e.g. vitamin A1 ($C_{20}H_{30}$), sesterpenes e.g. ophiobolin A ($C_{25}H_{40}$), triterpenes e.g. squalene ($C_{30}H_{48}$), tetraterpenes e.g. carotene ($C_{40}H_{64}$) and polyterpenes which are terpenes with carbon atoms greater than 40 e.g. ubiquinones (Singh and Sharma, 2015).

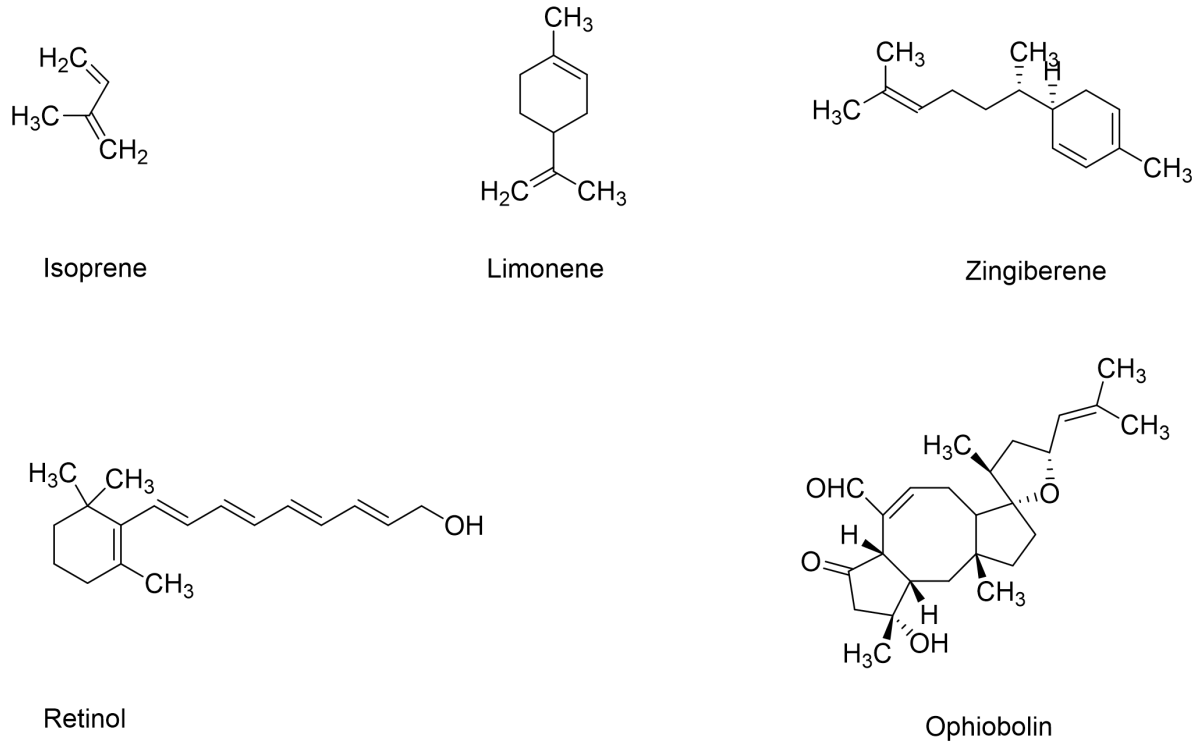
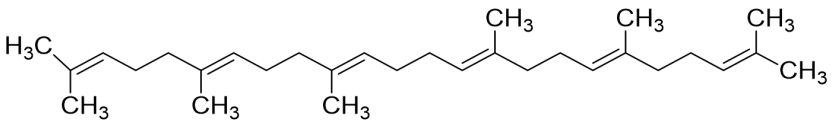
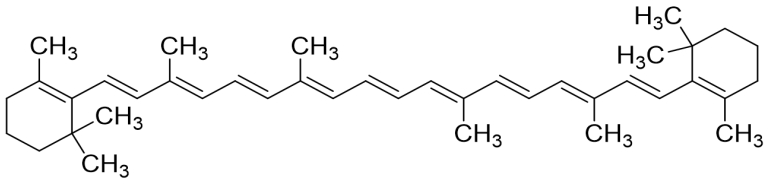


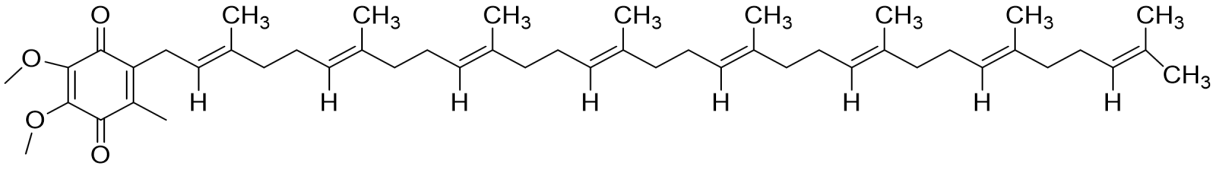
Figure 1.5: Examples of terpenes



Squalene



Carotene



Ubiquinone

Figure 1.5: Examples of terpenes

1.2.4 PLANT GLYCOSIDES

Glycosides are secondary metabolites made up of two parts which are the glycone and the aglycone part linked together by a glycosidic bond which are unstable and can be hydrolysed by enzymes and acids. Based on the glycosidic bond present, the glycosides can be classified as; C-glycosides (Carbon), S-glycosides (Sulphur bond), O-glycosides (Oxygen bond) and N-glycosides (Nitrogen bond). The glycone part of glycoside are sugars which may be monosaccharide, disaccharides, trisaccharides or tetrasachharides. The main types of glycosides found in plant are the anthraquinone glycosides, cardiac glycosides, coumaric

glycosides, cyanogenic glycosides, chromone glycosides, flavonoid glycosides, phenolic glycosides and thioglycosides (Bartnik and Facey, 2017).

1.2.4.1 Anthraquinone glycosides

Anthraquinones are secondary metabolites that are structurally related to anthracene. They have 1, 4-diketo-cyclohexa-2,5-diene as the central structure with two phenyl groups attached to both sides. Anthraquinone glycosides are known for their numerous activities such as laxatives, for the treatment of psoriasis, prevention of kidney stones and antiviral activities (Bartnik and Facey, 2017).

1.2.4.2 Cardiac glycosides

Cardiac glycosides contain a sterol and lactone ring as the aglycone portion of the glycoside. They have a 5 membered (cardenolides) or a six membered (pentadienolides) lactone ring at the 17- β position while the sugar moiety is attached to the 3 - β position. (Todt and Fozzard, 1997). Cardiac glycosides are usually obtained from digitalis plants and are known for their use in circulatory disorders (Swati *et al*., 2015).

1.2.4.3 Coumaric glycosides

Coumarins have the benzo *α* pyrone basic structure where a pyrone is attached to a benzene ring. Coumarins combine with sugars to form glycosides. They can be classified into; simple coumarins, furanocoumarins, dihydrofuranocoumarins, pyranocoumarins, phenylcoumarin and bicoumarins. Coumarins have several biological activities such as blood thinning and

smooth muscle relaxation. They occur in the fruits, leaves, stems and roots of different plant families such as; solanaceae and rosaceae. (Vaidehi and Rajesh, 2016).

1.2.4.4 Cyanogenic glycosides

Cyanogenic glycosides contain α-hydroxylnitriles which may be aliphatic or aromatic as their aglycone portion. They release hydrogen cyanides which are poisonous on hydrolysis. They cut across the different families of plant such as; linaceae, compositeae, papavaracea, mimosaceae and rosaceae. (Vetter, 2000). Cyanide poisoning can occur at a dose of 0.5-3.5 mg of hydrogen cyanide per kg bodyweight of animal. They function as powerful defense mechanism for plants. Foods such as cassava, cocoyam, bamboo shoot, sorghum, apples and apricots contain cyanogenic glycosides. Some toxic effects of cyanogenic glycosides are; tropical ataxic neuropathy, growth retardation, goiter and cretinism (Islamiyat *et al*., 2016).

1.2.4.5 Chromone glycosides

Chromones or 4H-chromen-4-one, 4H-1-benzopyran-4-one are plant metabolites which belong to the flavonoid family. Chromones have various derivatives and sometimes bind to sugars to form chromone glycosides. They belong to the flavonoid family having a benzo- γ -pyrone ring. They exhibit numerous pharmacological properties such as; antiinflammatory, antifungal, antibacterial, anticancer and anti-ulcer properties (Keri *et al*., 2014).

1.2.4.6 Flavonoid glycoside

Flavonoids are secondary metabolites that have shown very good antioxidant activities. They are commonly found in fruits and vegetables as flavonoid glycosides where they are combined with sugars via β-glycosidic bond. Experiments conducted have shown that the combination of flavonoids to sugars does not affect the absorption of the flavonoid. (Hollman *et al*., 1999).

1.2.4.7 Saponin glycosides

Saponin glycosides are secondary metabolites which yield sapogenins (aglycone) and sugars (glycone) on hydrolysis. Saponin glycosides are classified into acidic or neutral depending on the nature of aglycone attached to the sugars. The neutral saponin glycosides contain aglycone which are steroidal in nature while the acid saponin glycosides contain aglycone structure which are triterpenoids (Sapna *et al*., 2009).

1.2.4.8 Thioglycosides

Thioglycosides occur less frequently in nature compared to other glycosides. Most members are glucosinolates which can be found in plants such as; *Eruca sativa, Arabidopsis thaliana, Barbarea vulgaris and Isatis tinctoria* other thioglycosides apart from the glucosinolates are; Lincomycin A which is used as an antibiotic, afrostyraxthioside A, Salacino and raphanuside. (Lian *et al*., 2014).

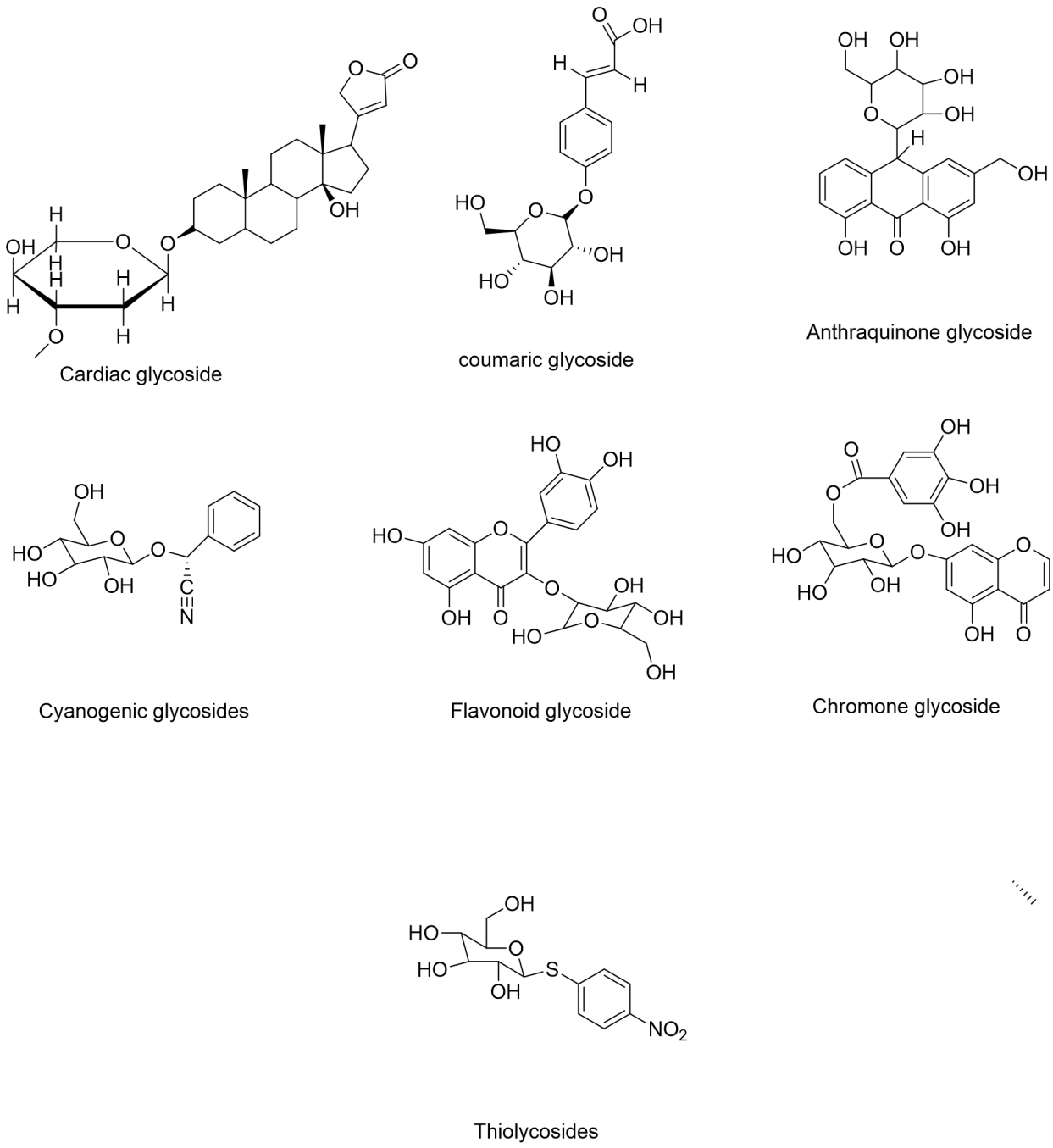


Figure 1.6: Structures of glycosides

1.2.5 SAPONINS

These are plant metabolites that appear frequently in nature. They are mostly found in combination with one or more sugars in which the aglycone portion is either a triterpenoid or a steroid. The steroidal saponins are mainly gotten from the monocotyledonous plants while the triterpenoid saponins are mainly gotten from the dicotyledonous plants. Saponins are surface active agents with foaming properties. They have numerous biological activities such as; cytotoxic, sedative, diuretic, anti-allergic, antiviral and antiulcer activities. (Ozlem and Giuseppe, 2007).

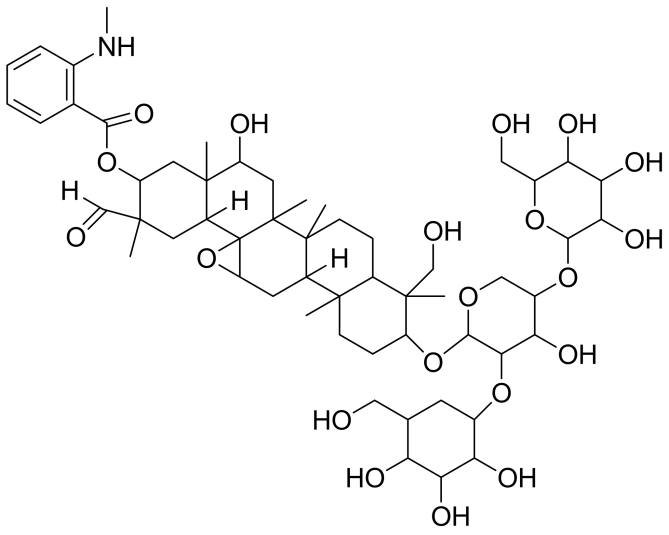


Figure1.7: Structure of Avenacin A1 (a saponin)

1.2.6 TANNINS

Tannins are polyphenolic plant secondary metabolites having a very high molecular mass of up to 20,000 (Karamali and Teunis, 2001). Tannins are abundant in leaves, roots, stems and fruits of plants where they take part in defending the plant from harsh circumstances and diseases. They have a characteristic smell and taste and appear as white or yellow powder.

They can be used for their astringent, diuretic, and anti-inflammatory properties. Tannins can be divided into different classes such as; condensed tannins, complex tannins, ellagitannins and gallotannins, (Karamali and Teunis, 2001). They are able to cause precipitation of gelatin and other proteins from solutions. They also react with ferric chloride like other polyphenols to give a blue color (Apak *et al*., 2007).

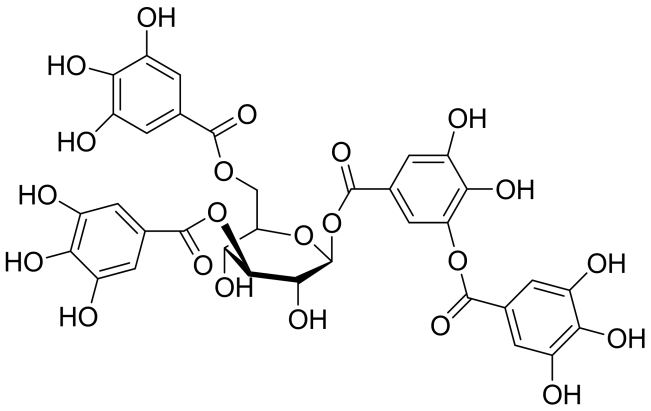


Figure 1.8: Structure of tannic acid

1.3 DIABETES MELLITUS

Diabetes is a disorder involving high levels of blood glucose which occurs due to insufficient insulin or resistance to insulin or both (Chauhan *et al*., 2010). It can also be described as a chronic disease that affects the metabolism of carbohydrates, proteins and fat characterized by increased blood glucose levels (Jayaprasad *et al.*, 2012).

1.3.1 PREVALENCE OF DIABETES

The International Diabetes Federation (2015), revealed that 415 million adults (1 in 11 adults) were living in the world with diabetes and predicted that by 2040, the number will increase to about 642 million adults (1 in 10 adults) will come down with diabetes (IDF, 2015). It is a common misconception that diabetes is a disease for the rich but on the contrary, 80% of people diagnosed with diabetes come from low- and medium-income countries (IDF, 2013). In 2013, 5.1 million deaths were recorded as a result of diabetes which meant that every six seconds, a person died of diabetes around the globe. It is also sad that a lot of diabetic cases are still undiagnosed. Data obtained from IDF 2017 shows that 425 million people are now living with diabetes compared to the 415 million as at 2015 and the number of people under 20 years diagnosed with diabetes has increased to over a million (IDF, 2017). In Africa, 16 million people were diagnosed with diabetes in 2017 and the number is expected to increase to 41 million in the year 2045 (IDF, 2017). Nigeria which is the most populous African nation as at 2014, had up to 4 million people living with diabetes unfortunately, 70% - 80% of the diabetic cases were undiagnosed (IDF, 2014). About 95% of people diagnosed with diabetes in Nigeria have type 2 diabetes while people with type 1, gestational and other types of diabetes form the remaining 5% (Olufemi and Samuel, 2015).

1.3.2 ETIOLOGY OF DIABETES

The human pancreas is a gland located under the stomach which has four major types of endocrine cells. These endocrine cells are; the beta cells which secret insulin, the alpha cells which contain glucagon, the delta cells which contain somatostatins and the polypeptide containing pancreatic cells (Murray, 1993). Insulin which is the hormone that regulates glucose uptake is produced by the beta islet cells in the pancreas and stored within vacuoles. These hormones are released when there is an increase in the level of glucose in the blood but

in cases where there is reduced blood glucose, the pancreas secretes glucagon from the alpha cells of the pancreas which converts glycogen to glucose (Lin and Sun, 2010). Failure of the pancreas to secret enough insulin or resistance of the target cells to the insulin secreted, or a combination of both cases gives rise to the metabolic disorder called diabetes mellitus (Jayaprasad *et al*., 2012).

1.3.3 CLASSIFICATION OF DIABETES

According to the American Diabetes Association, there are different types of diabetes which includes;

1. Type 1 or juvenile onset diabetes which has to do with autoimmune destruction of beta cells of the pancreas leading to insulin deficiency and accounts for 5-10% of the diabetic population.
2. Type 2 or non-insulin dependent diabetes which has to do with insulin resistance and accounts for 90-95 % of the diabetic population.
3. Gestational diabetes which occur in pregnant women.
4. Other types caused by genetic defect of the beta cells, genetic defect in insulin action, diseases of the exocrine pancreas, endocrinopathies, drug or chemical induced diabetes, infections, genetic syndromes and other forms of immune mediated diabetes (American Diabetes Association, 2010).

1.3.4 RISK FACTORS OF DIABETES

Risk factors associated with diabetes are categorized into the modifiable and non-modifiable risk factors (Chen *et al*., 2012).

The non- modifiable risk factors include; family history of diabetes, history of gestational diabetes, ethnicity, sex, age and polycystic ovarian syndrome while the modifiable risk factors are; sedentary life style, obesity, smoking, abnormal lipid levels, hypertension and inflammation.

1.3.5 DIAGNOSIS

Diabetes can be diagnosed using the following methods according to American Diabetes Association (2018);

1. Diabetic symptoms including random blood sugar level greater than 11.1 mmol/L (200 mg/dL)
2. Fasting blood glucose level greater than or equal to 7.0 mmol/L (126 mg/dL)
3. Oral glucose tolerance test (2 hours after 75 g glucose load) greater than or equal to 11.1 mmol/L (200 mg/dL)
4. Acetylated haemoglobin (HbA1c) level greater than or equal to 6.5 % (53 mmol/mol)

1.3.6 SIGNS AND SYMPTOMS

According to the American Diabetes Association (2011), some signs and symptoms of diabetes include; polyphaghia, polydipsia, polyuria, weight loss, blurred vision, growth impairment and susceptibility to infections.

1.3.7 COMPLICATIONS OF DIABETES

Complications of diabetes may be divided into two which are; the acute complications and the chronic complications. The chronic complications may be divided into vascular and non-

vascular complications. The vascular complications, further divided into microvascular and macrovascular complications which occurs as a result of prolong period of hyperglycemia. The management of these complications that arises as a result of diabetes costs more than the management of the diabetes (Tripathi and Srivastava, 2006).

1.3.7.1 ACUTE COMPLICATIONS

1. Diabetic ketoacidosis: which is mainly observed in type 1 diabetes, occurs when there is an imbalance between insulin and counter hormones such as; glucagon, growth hormones and catecholamines which enhances the release of ketone bodies. The main symptoms observed are; nausea and vomiting. More serious complications like; cerebral edema and coma have also been observed (Tripathi and Srivastava, 2006).
2. Non-diabetic ketoacidosis: this is mainly observed in type 2 diabetic patients who are elderly. It occurs as a result of insulin deficiency and insufficient fluid intake. The major symptoms observed in this case are; orthostatic hypotension and polyuria, various neurological symptoms that may lead to coma (Tripathi and Srivastava, 2006).

1.3.7.2 CHRONIC COMPLICATIONS

1. **Vascular complications**

a. Macrovascular: Examples of macrovascular complications that arises as a result of prolonged untreated hyperglycemia are; cerebrovascular diseases, peripheral vascular diseases and coronary artery diseases (Tripathi and Srivastava, 2006).

b. Microvascular: Examples of microvascular complications that may arise as a result of prolonged untreated hyperglycemia are; neuropathy, retinopathy and nephropathy (Tripathi and Srivastava, 2006).

2. **Non-vascular complications**

Non-vascular complications of diabetes do not affect any vessel in the body like the vascular complications instead, they may cause things like; skin defects or colour change, affect the gastrointestinal tract and also cause sexual dysfunction (Tripathi and Srivastava, 2006).

1.3.8 CO-MORBIDITIES OF DIABETES

People diagnosed with diabetes usually have associated co-morbidities which makes management of the disease difficult because the signs and symptoms of these co-morbidities have to be managed as well. The existence of co-morbidities encourages the use of poly-pharmacy in the management of the condition and this may lead to treatment failure as a result of non-compliance (Young *et al*., 2016). Co-morbidities associated with diabetes can be classified into the following headings according to Young *et al* (2016);

1. Respiratory system disorders e.g. lung infection and asthma
2. Musculoskeletal disorders e.g. osteoarthritis and rheumatoid arthritis
3. Cardiovascular diseases e.g. hypertension, heart failure, diabetic cardiomyopathy, stroke and myocardial infarction
4. Neuropathy e.g. cognitive dysfunction, dementia and epilepsy
5. Cancer
6. Gastrointestinal disorders
7. Infections
8. Mental disorder

1.3.9 MANAGEMENT OF DIABETES

The goals of diabetes management are to relive symptoms, to improve patient quality of life, to prevent complications, reduce mortality, morbidity and economic burden, also to improve the productivity of patients. (W.H.O, 1994). The management involves the use of both pharmacological and non-pharmacological approach. The non-pharmacological approach involves yearly laboratory tests to determine the lipid profile, serum creatinine level, glomerular filtration rate, liver function and other relevant tests, dietary management, exercise and self-glucose monitoring while pharmacologic approach to managing diabetes involves the use of insulin therapy and oral hypoglycemic agents. Medicinal plants are also used by many people in the prevention and management of diabetes (Ogbera and Ekpebegh., 2014).

1.3.9.1 Pharmacological approach to diabetes management

1. **Insulin therapy**

   Insulin therapy is used in the management of type 1 diabetes and type 2 diabetes that do not respond adequately to the available oral hypoglycemic agents. It is needed for the management of type 1 diabetes because of the absence of insulin as a result of the defective beta cells. Insulin is a protein with a molecular weight of about 6000 (Lorenzati *et al*., 2010). It is made up of two polypeptide chains which are; Chain A, containing 21 amino acids and chain B containing 30 amino acids. These two chains are linked together by a disulphide bridge. It is stored in the pancreas as a precursor molecule called pro-insulin which is cleaved by a proteolytic enzyme to obtain the active form of insulin when it is needed in the body. It is metabolized mainly in the liver and has a short half-life of 5-6 minutes (Lorenzati *et al*., 2010). Insulin facilitates the uptake of glucose into the adipose tissues, liver and muscles. When the tyrosine kinase at the insulin receptor sites are activated, it causes an increase in the

phosphorylation of the membrane which in turn increases the permeability of the membrane to glucose hence causing increased glucose uptake by target cells (Lorenzati *et al.,* 2010). Insulin Resistance to insulin therapy may develop during the management of patients with both type 1 and type 2 diabetes but it is mainly observed in patients with type 2 diabetes especially as a result of obesity (Ahmed, 2005).

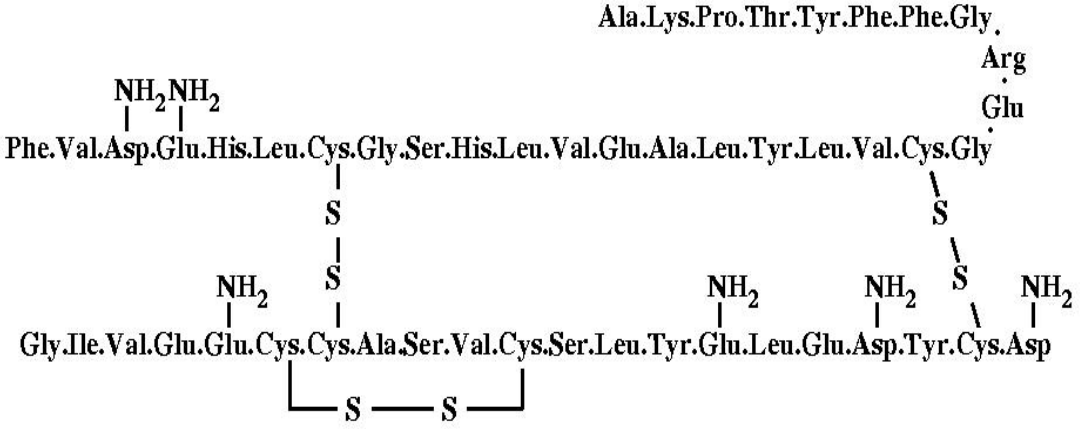


Figure 1.9: Structure of insulin

2. Oral hypoglycemic agents

Oral hypoglycemic agents are used mainly in the management of type 2 diabetes and they are divided into seven (7) major classes which are; the biguanides, sulfonylureas, meglitinides, thiazolidinediones, alpha (α) glucosidase inhibitors, incretin agonists and dipeptidyl peptidase 4 inhibitors (Lorenzati *et al.,* 2010).

Biguanides

Metformin has remained the first line drug for the management of diabetes mellitus in some countries (Bailey, 1992). It does not cause stimulation of insulin release from the pancreas and it is not laden with the side effect of overweight hence beneficial for the overweight patient managing diabetes. Metformin is an antihyperglycemic agent and does not take the blood glucose level below the euglycemic level. It is sometimes used in combination with sulfonylureas in cases were sulfonylureas alone cannot bring down the blood glucose level. Metformin action is independent of the insulin level, age and duration of hyperglycemia (Bailey, 1992). Metformin which is a dimethylbiguanide contains two linked guanidine rings and not only acts by reducing blood sugar level but also reduces hepatic gluconeogenesis. It is also useful in the treatment of polycystic ovary disease, gestational diabetes, nephrotoxicities and in cancer therapy (Foretz *et al*., 2014). Some side effects associated with the use of metformin are; lactic acidosis at a very high dose, nausea, vomiting, metallic taste in the mouth, diarrhea and weakness. It may also cause hypoglycemia when used in combination with certain hypoglycemic agents (Nasri and Mahmoud, 2014). Other drugs in this class are; buformin and phenformin.

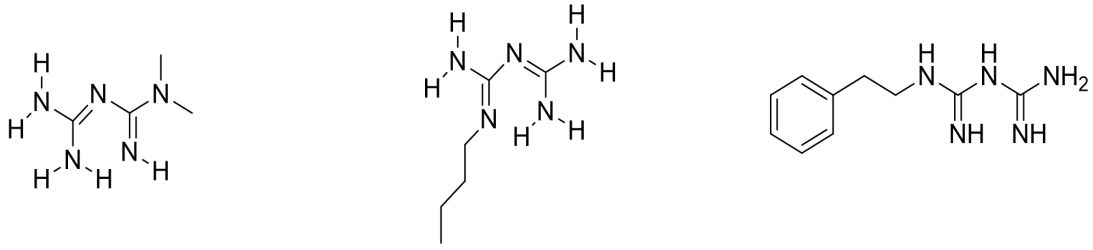
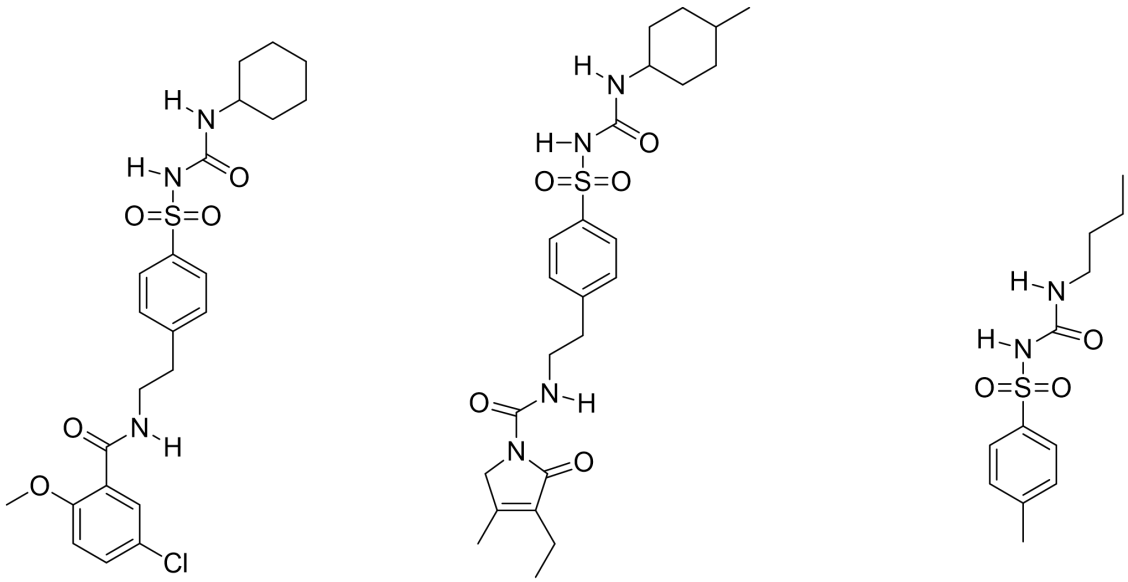


Figure 1.10: Examples of biguanides

Sulphonylureas

Sulfonylureas are drugs used in the control of blood glucose level by stimulating the release of insulin from the beta cells of the islet of Langerhans in the pancreas and they carry-out their action irrespective of the level of glucose in the blood (Kalra and Gupta, 2015). They also act by reducing hepatic clearance of insulin after it has been secreted from the pancreas (Sola *et al*., 2013). There are two generations of sulfonylureas. They are the first-generation sulfonylureas which includes; chlorpropamide and tolbutamide and the second-generation sulfonylureas which includes; glibenclamide, glimepiride, gliclazide and glipizide. The first-generation sulfonylureas are no longer in use because of their numerous side effects and low potency. The second-generation sulphonylureas are 100 times more potent and have lesser side effects (Sola *et al*., 2013). Some side effects associated with sulfonylureas are; hypoglycaemia, gastrointestinal disturbance, myocardial infarction, hematologic dyscrasias, weight gain, hepatotoxicity and allergic reactions (Confederat *et al*., 2016).

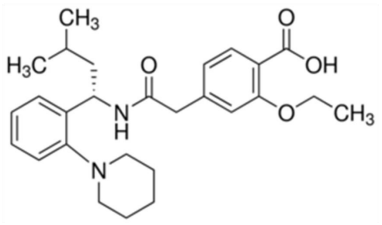


Glibenclamide Glimepiride Tolbutamide

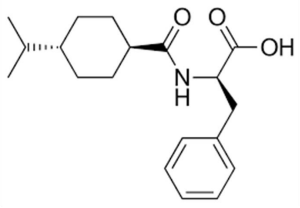
Figure 1.11: Examples of sulfonylureas

Meglitinides

Repaglinide is an example of meglitinides which is a short acting anti-diabetic agent and very useful in the regulation of post prandial hyperglycemia (Pei-chen *et al*., 2017). Repaglinide which is the S (+) enantiomer of 2-ethoxy4-(2-((3-methyl-1-(2-(1-piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid, was the first meglitinide to be approved for the management of diabetes. It stimulates the secretion of insulin by binding to the sulfonylurea receptor site on the beta islet cells in the pancreas. They block the adenosine triphosphate (ATP) dependent potassium channels which makes the beta islet cells become depolarized therefore causing the influx of calcium ions thereby increasing the level of intracellular calcium and hence causing secretion of the insulin containing granules. This action is similar to the activity of sulfonylureas (Mendoza *et al*., 2013). The most common side effects observed with the use of repaglinides are rhinitis and bronchitis (Landgraf, 2000). Nateglinide which is a phenylalanine derivative (N-[(trans-4-isopropylcyclohexyl)-carbonyl]-D-phenylalanine) is another example of meglitinides which share same mechanism of action with repaglinide (Rodolfo *et al*., 2012). The meglitinides in general stimulate insulin release only when blood glucose level is high which means that it should be taken during meals or immediately after meals hence they have lower incidence of hypoglycemic side effect compared to the sulfonylureas (Richard and Raskin, 2011).



Repaglinide



Nateglinide

Figure 1.12: Examples of meglitinides

Thiazolidinediones

Pioglitazone and rosiglitazone are examples of thiazolidinediones that are currently used in the management of diabetes. They carry out their hypoglycemic activity by stimulating glucose uptake in the skeletal muscles and to some extent, reduce hepatic gluconeogenesis (Krishnaswami *et al*., 2010). They also increase insulin sensitivity in adipose tissues thereby reducing the concentration of free fatty acids. Their major side effect is weight gain and increase in the mass of subcutaneous adipose tissues. (Hannele, 2004).

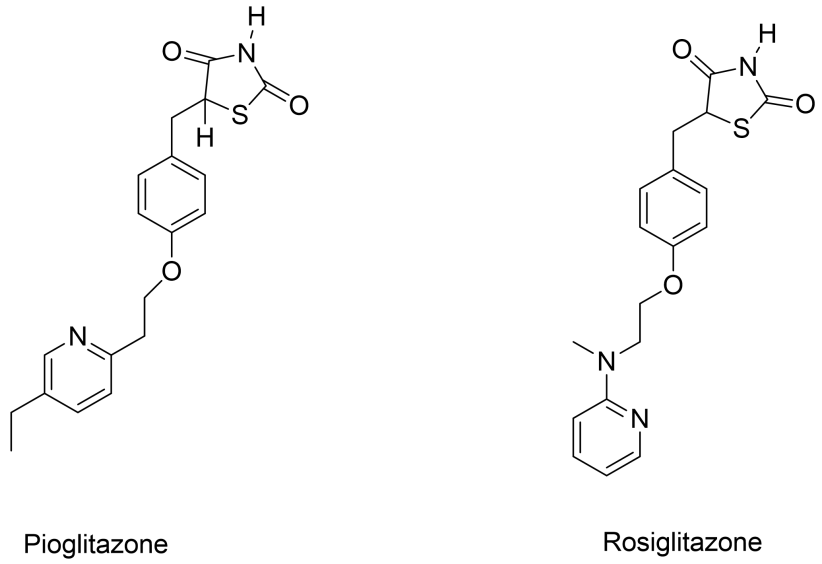
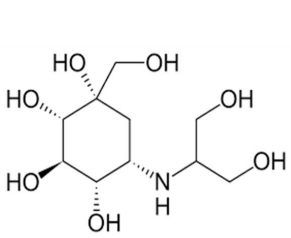


Figure 1.13: Examples of thiazolidinediones

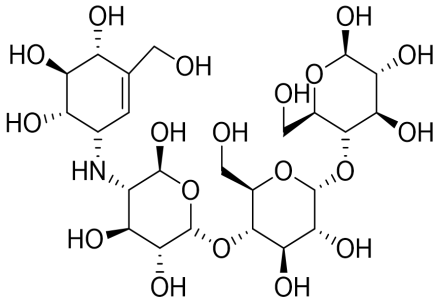
Alpha (α) glucosidase inhibitors

Postprandial blood glucose level increases after a carbohydrate rich meal when alpha glucosidase enzyme in the intestine hydrolysed the α-1,4-glycosidic linkages in carbohydrates to release glucose. α - glucosidase inhibitors inhibits the activity of the alpha glucosidase enzymes therefore reducing postprandial glucose level (Salehi *et al*., 2013). Some of the side effects of these drugs are; hypoglycemia which occurs when higher doses

are administered, lactic acidosis, gastrointestinal disturbances and liver problems (Tundis *et al*., 2010). Examples of alpha glucosidase inhibitors used for the management of diabetes are acarbose and voglibose. Acarbose is the most frequently used α-glucosidase inhibitors which have nitrogen in between the first and second glucose unit it is a pseudotetrasaccharide, it is obtained from the fermentation of *Actinoplanes utahensis* which is a microorganism. It can inhibit α-glucosidase enzymes as well as glucoamylase, dextranase, maltase, and sucrose but not β-glucosidase enzymes such as lactase. They are usually taken with the first bite of a meal since it acts by inhibiting the breakdown of complex carbohydrates (Derosa and Maffioli, 2012).



Voglibose



Acarbose

Figure 1.14: Alpha (α) glucosidase inhibitors

Incretin agonists/mimetics

Incretins are hormones in the body which stimulates the release of insulin when there is an increase in blood glucose level. They also give a satisfying feeling to prevent further intake of food (Maxwell *et al*., 2016). There are two types of incretin hormones they are; the glucose-dependent insulinotropic polypeptide (GIP) and the glucagon-like peptide 1 (GLP-1). Their level in the blood increases after a meal. They are removed from the system by the enzyme

dipeptidyl peptidase 4 or by renal clearance. Both incretin hormones have receptor sites on the β islet cells and once stimulated, they cause an influx of calcium into these cells leading to exocytosis of insulin. Side effects of these drugs are mainly gastrointestinal (Drucker and Nauck, 2006).

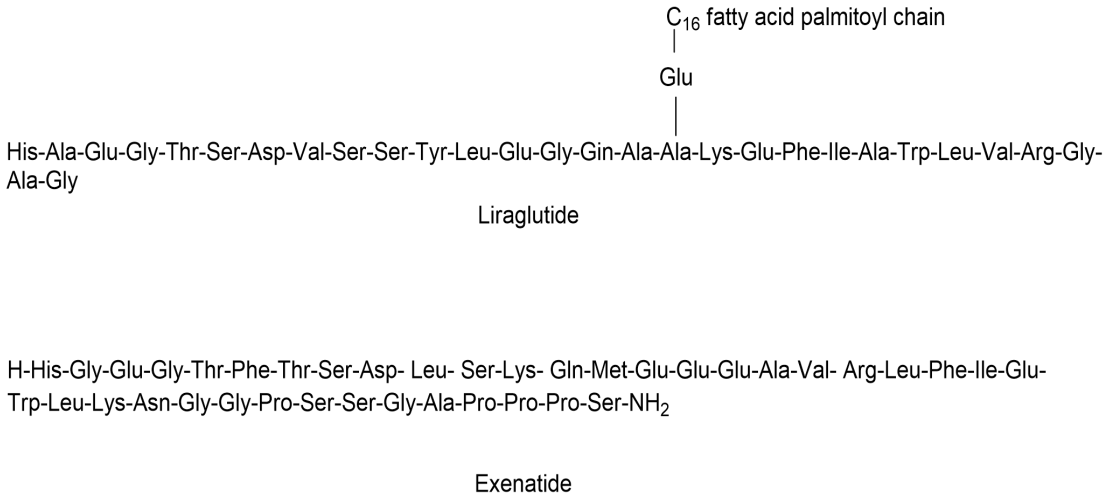


Figure 1.15: Examples of incretin agonists/mimetics

Dipeptidyl peptidase 4 inhibitors

Dipeptidyl peptidase 4 are enzymes which inactivate the glucagon-like polypeptide- 1 (GLP-1) hormone which is an incretin hormone that causes glucose-dependent secretion of insulin from the islet cells, decrease glucagon secretion, reduce the rate of gastric emptying and increase the feeling of reduce satiety in order to reduce food intake . The dipeptidyl peptidase 4 inhibitors inactivates the GLP-1 hormone by removing the N- terminal amino acids of the GLP-1 hormone this makes the hormones have a very short half-life of one minute (Vella, 2012). Examples of dipeptidyl peptidase 4 inhibitors are; vidagliptin, linagliptin, saxagliptin, azetidine based compounds (2-cyanoazetidines, 2-ketoazetidines and 2-fluoroazetidines), alogliptin, sitagliptin, pyrolidine and xanthine based dipeptidyl peptidase 4 inhibitors (Gaba

et al., 2009). Some common side effects associated with the use of dipeptidyl peptidase 4 inhibitors are; headaches, nasopharyngitis, pancreatitis, urinary tract infections, angioedema, exfoliative dermatological reactions and anaphylactic shock (Mkele, 2013).

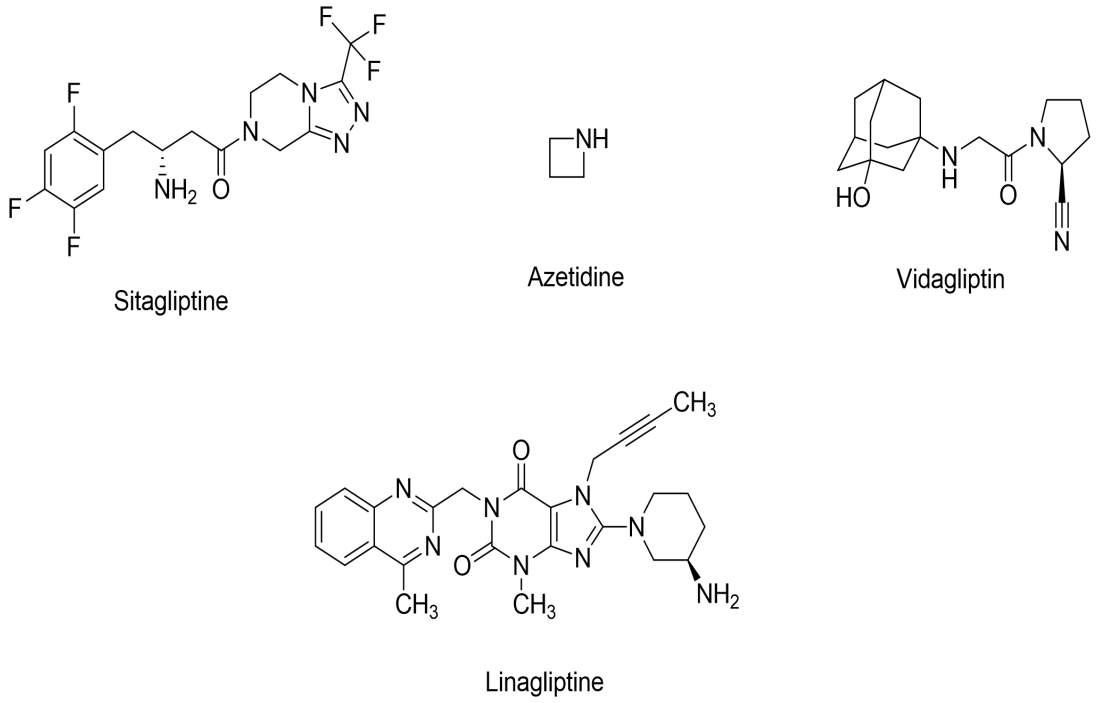


Figure 1.16: Dipeptidyl peptidase 4 inhibitors

1.3.9.2 Plants used in the management of diabetes

Although the currently available drugs have been used for the management of diabetes, issues still arise based on their efficacy, tolerability and side effects. Also, patients with diabetes have to take multiple drugs or engage in poly-pharmacy in order to manage the disease and its complications. This has greatly affected compliance to therapy and resulted to increased financial burden on diabetic patients. Many still rely on herbal medicine for the management of diabetes because it is readily available and associated with less side effects (Ezuruike and Prieto, 2014). According to WHO (2008) about 80% of Africans and Asians rely on

traditional medicine as primary health care. Traditional medicine has been used for a very long time in the management of diabetes. In 2002, the World Health Organization (WHO) encouraged the use of medicinal plants for the management of diabetes and also encouraged more scientific investigations to evaluate the hypoglycemic effect of different plants (WHO, 2002). Also, WHO (2013) describes traditional medicine as an available and affordable form of healthcare which helps to reduce the burden associated with the management of chronic non-communicable diseases. Some plants used in the management of diabetes are; *Citrus aurantifolia* (lime) (Soladoye *et al*., 2012), *Cassia alata* (Ringworm bush), *Picralima nitida* (pilcralima), *Syzygium aromaticuum* (clove), *Rauwolfia vomitoria* (swizzle stick) (Arowosegbe *et al*., 2015), and *Eleusine coracana* (Oseghale *et al*., 2017), *Momordica charantia* (bitter melon) (Olanipekun *et al.*, 2016), *Citrus limon* (Lemon) (Arowosegbe *et al*., 2015).

1.4 EFFECT OF PLANT ANTIOXIDANTS ON DIABETES MELLITUS

Antioxidants are believed to be useful in the management of diabetes since low level of antioxidants and free radical scavengers have been recorded in diabetic cases. Also, many of the complications of diabetes both microvascular and macrovascular complications have been linked to oxidative stress (Letitia and Timothy, 2002). Since hyperglycemia enhances the generation of free radicals the presence of antioxidants in the system will help to reduce the level of oxidative stress caused by these free radicals and protect the beta islet cells in the pancreas from oxidative damage (Rajendiran *et al*., 2018). Flavonoids have been shown to be relevant in the prevention of excess production of superoxide free radicals and also involved in the metal chelation process which is useful in the management of diabetes (Fuente and Manzanaro, 2003). There are many studies that have been carried out to show the usefulness of antioxidants in plants in the management of diabetes (Rahimi *et al*., 2005). Some of these

studies involve the use of animal models in screening plants with high antioxidant properties for hypoglycemic effect in order to discover newer, efficacious and affordable drugs with minimal side effects that can be used in the management of diabetes. Chemicals or drugs are used in this regard for the induction of diabetes in these animals. Some of the chemicals used are;

1. **Alloxan (2,4,5,6-tetraoxypyrimidine)** which causes damage to the beta islet cell is mainly used to induce type 1 diabetes.
2. **Streptozotocin** which is a derivative of nitrosourea and can be used to induce both type 1 and type 2 diabetes based on the doses administered.
3. **Dithizone** (8-(p-toluene-sulfonylamino)–quinoline) which is a zinc chelating agent that causes damage the beta cells.
4. **Gold thioglucose**
5. **Monosodium glutamate**
6. **Corticosteroids** such as prednisolone and dexamethasone
7. **Hormones** such as growth hormone
8. **Some viruses** such as the coxsackie virus and the encephalomyocarditis D virus (Tripathi and Verma, 2014).

1.5 OXIDATIVE STRESS

Oxidative stress refers to the disparity between free radicals and antioxidant enzymes in the body (Manisha *et al*., 2017). Oxidative stress plays either a main or secondary role in the initiation of several ailments such as; pancreatic diseases, cardiovascular diseases, joint disorders and the aging process (Taibur *et al*., 2012).

1.6 FREE RADICALS

Free radicals are molecules formed from endogenous or exogenous sources that have the ability to affect lipids, nucleic acids as well as proteins in the biological system leading to various redox reactions in the biological system hence causing oxidative stress. The oxidative stress caused by these radicals are associated with different disease conditions like; cardiovascular problems, respiratory problems, diabetes mellitus as well as atherosclerosis (Alugoju *et al*., 2015). Free radicals could be ions, atoms or molecules having unpaired electrons which makes them very unstable and highly reactive. These free radicals could either be reactive oxygen species (ROS), reactive sulphur species (RSS) or reactive nitrogen species (RNS) (Marcio and Isabel, 2013). The main target of these reactive species are; lipid, proteins, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and sugars (Marcio and Isabel, 2013). According to Saikat *et al*., (2010), free radicals are generated from different sources such as; pollutants from the atmosphere, radiations (such as UV radiations, X-rays, gamma rays and microwaves), electron transport reactions (which take place in the mitochondria), inflammatory processes (which stimulates the production of free radicals by neutrophils and macrophages), metal catalyzed reactions, chemicals (such as pesticides and herbicides), cigarette smoke, exhaust fumes, burning organic matter, xanthine oxidases, lipid peroxidation, mitochondrial cytochrome oxidases, neutrophils, macrophages, platelets, arachidonic acid peroxidation and from smooth muscle cells.

1.6.1 Reactive oxygen species (ROS)

They are formed in the peroxisomes, mitochondria and chloroplast due to normal processes of metabolism such as respiration as well as photosynthetic processes. They can cause destruction of DNA, other proteins and lipids in the body (Apel and Hirt, 2004). Reactive

oxygen species are of two types which are; the free oxygen radical ROS (alkoxyl radicals (RO•), hydroxyl radical (•OH),disulphides, nitric oxide (NO•), organic radicals (R•), peroxylradicals (ROO•), sulphonyl radicals, superoxide (O_2•–), thiyl radicals and thiyl peroxyl) radicals (RSOO•)) and the non-radical ROS (dinitrogen dioxide (N_2O_2),very reactive lipids or carbonyl compounds obtained from carbohydrates, hydrogen peroxide (H_2O_2), hypochloride (HOCl), nitrocarbonate anion (O_2NOCO_2–), nitronium (NO_2), nitrosoperoxycarbonate anion ($ONOOCO_2$–), organic hydroperoxides (ROOH), singlet oxygen (O_2), ozone/trioxygen (O_3), and peroxynitrite (ONO) (Boguslaw, 2011).

1.6.2 Reactive nitrogen species (RNS)

These are nitrogen derived free radicals such as; complexes derived from dinitrosyl iron (except NO_3^-), nitrosonium cation (NO^+), nitric oxide, nitroxy (HNO), certain nitrogen oxides, S-nitrosothiols (RSNOs) as well as peroxy nitrites ($ONOO^-$). These reactive nitrogen species causes nitroxylation of metals, nitroxylation of sulfhydryls and also causes tyrosine residue nitration. When the level of RNS production in biological system is high, destruction of body cells may occur (Martínez and Andriantsitohaina, 2009).

1.6.3 Reactive sulphur species (RSS)

Reactive sulphur species are molecules containing sulphur that are capable of oxidizing or reducing biological molecules under physiologic conditions (Martin and Alan, 2012). Non-radical examples of reacting sulphur species are; disulphide, sulphenic acid, thiol, thiosulphinate, and thiosulphonate while the thiyls are examples of radical reactive sulphur species (Martin and Alan, 2012). Initially, it was thought that reactive sulphur species were

formed only during pathological conditions but further investigations have shown that they take part in cellular signaling, metabolism and mitochondrial functions (Miriam *et al*., 2017).

1.7 ANTIOXIDANTS

These are substances that significantly delays or minimizes the oxidation of a susceptible substrate when present in very small concentrations compared to the concentration of the susceptible substrate (Apak *et al*., 2007). Antioxidants help to neutralize the activity of free radicals and hence minimizes the occurrence of oxidative stress caused by reactive species present in the body system (Ansari *et al*., 2013). These antioxidants interact with free radicals and prevent the damages caused by these free radicals to body cells and tissues. The antioxidants are reduced in the process by accepting electrons from the free radicals hence preventing the oxidative chain reactions initiated by free radicals (Hamid *et al*, 2010). Antioxidants are of two types namely; Primary or chain breaking antioxidants and secondary or preventive antioxidants (Madhavi *et al.,* 1996).

1.7.1 Primary or chain breaking antioxidants

These antioxidants inhibit the initiation steps as well as the propagation steps of the oxidative processes.

$$L^{\cdot} + AH \longrightarrow LH + A^{\cdot}$$

$$LO^{\cdot} + AH \longrightarrow LOH + A^{\cdot}$$

$$LOO^{\cdot} + AH \longrightarrow LOOH + A^{\cdot}$$

Where; $L^{\cdot}$ represents lipid radical (initiation)

$LO^{\cdot}$ represents alkoxyl radical (propagation)

$LOO^{\cdot}$ represents peroxyl radical (propagation)

1.7.2 Secondary or preventive antioxidants

These antioxidants reduces the degree of oxidation, examples are the metal chelators which reduces the occurrence of Fenton type reactions that produces hydroxyl radicals.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {\cdot}OH + OH^-$$

Fenton type reaction

Antioxidants can also be classified into two major groups which are;

1.7.3 Enzymatic antioxidants

The enzymatic antioxidants act by converting the reactive species in the presence of co-factors to hydrogen peroxide (H_2O_2) and then to water (H_2O) (Satish and Dilipkuma, 2015) .The enzymatic antioxidants are also categorized into; the primary enzymatic antioxidants (catalase (CAT), glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) and the secondary enzymatic antioxidants (glutathione reductase, glucose-6-phosphate dehydrogenase) (Marcio and Isabel, 2013).

1.7.4. Non-enzymatic antioxidants

The non-enzymatic antioxidants work by disrupting the chain reactions of free radicals (Satish and Dilipkuma, 2015). They include the flavonoids, the co-factors, the vitamins and their derivatives and also the phenolic acids. Numerous synthetic antioxidants have also been developed such as; propyl gallate which is used as a food antioxidant (Marcio and Isabel, 2013).

1.7.5 Antioxidant screening methods

Several methods have been developed by scientists for investigating the antioxidant activities of medicinal plants. These methods may be divided into the *in vitro* antioxidant screening method and the *in vivo* antioxidant screening method (Alam *et al*., 2013).

1.7.5.1 *Invitro* antioxidant screening methods

These methods include; 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, ferric reducing-antioxidant power (FRAP) assay, nitric oxide scavenging activity, peroxynitrite radical scavenging activity, trolox equivalent antioxidant capacity/ ABTS radical cation decolorization assay, total radical-trapping antioxidant parameter (TRAP), Superoxide radical scavenging activity (SOD), hydroxyl radical scavenging activity, hydroxyl radical averting capacity (HORAC) method, oxygen radical absorbance capacity (ORAC) method, reducing power method (RP), phosphomolybdenum method, ferric thiocyanate (FTC) method, thiobarbituric acid (TBA) method, DMPD (N,N-dimethyl-p-phenylene diamine dihydrochloride) method, β-carotene linoleic acid method/conjugated diene assay, xanthine oxidase method and metal chelating activity (Alam *et al*., 2013).

1.7.5.2 *In vivo* antioxidant screening methods

These methods of antioxidant assay include; ferric reducing ability of plasma, reduced glutathione (GSH) estimation, glutathione peroxidase (GSHPx) estimation, glutathione-S-transferase (GSt), catalase (CAT), γ-glutamyl transpeptidase activity (GGT) assay, glutathione reductase (GR) assay, lipid peroxidation (LPO) assay and low-density lipoprotein (LDL) assay (Alam *et al*., 2013).

Another way of classifying antioxidant screening methods is based on their mode of reaction which are; the hydrogen atom transfer (HAT) method and the electron transfer (ET) method.

1.7.5.3 Hydrogen atom transfer (HAT) method

This measures the ability of the antioxidant to donate a hydrogen atom to the free radical therefore quenching the activity of the free radical, examples of the HAT assay method are oxygen radical absorbance capacity (ORAC) assay which measures the inhibition of peroxyl radical induced oxidations (Apak et al., 2007). It is a chain-breaking antioxidant activity by H-atom transfer. Other examples of the HAT assay are total peroxyl radical-trapping antioxidant parameter (TRAP assay) and *β*-carotene bleaching assay. The HAT method is independent of the solvent system used as well as the pH of the medium. It also has an advantage of being a fast method of assay (Apak *et al*., 2007). The HAT mechanism can be summarized in the following equation. $ROO^{\cdot} + AH/ArOH \rightarrow ROOH + A^{\cdot} / ArO^{\cdot}$ (Apak *et al*., 2007).

1.7.5..4 Electron -transfer (ET) method.

Examples of this assay methods are the trolox equivalent antioxidant (TEAC) 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation decolorization assay, the DPPH free radical scavenging assay, Folin-Ciocalteu (FCR), ferric reducing-antioxidant power (FRAP) assay and cupric reducing antioxidant capacity (CUPRAC). The electron transfer method involves the use of a probe with redox potential such as a fluorescent or coloured probe which is an oxidizing agent to react with the antioxidant. They are affected by pH and the nature of solvent unlike the HAT method. ET assays that are based on

spectrophotometric analysis measure the ability of a substance with antioxidant properties to reduce an oxidant which changes to another colour when it has been reduced. The absorbance of the coloured oxidant either increases or decreases at a particular wavelength in the presence of the antioxidant. The rate of colour change correlates with the level of the antioxidant present in that substance. ABTS/TEAC and DPPH assays are decolorization assays but an increase in absorbance is observed in CUPRAC, Folin total phenol and FRAP assay (Moharram and Youssef, 2014), (Apak *et al*.,2007).

$$\mathbf{M\,(n) + e\ (from\ AH) \longrightarrow AH^{\cdot} + M\,(n-1)}$$

E.g.

$$\mathbf{ROO^{\cdot} + AH/ArOH \longrightarrow ROO^{-} + AH^{\cdot +}/ArOH^{\cdot +}}$$

$$\mathbf{AH^{\cdot +}/\ ArOH^{\cdot +} + H_2O \longleftrightarrow A^{\cdot}/ArO^{\cdot} + H_3O^{+}}$$

$$\mathbf{ROO^{-} + H_3O^{+} \longleftarrow ROOH + H_2O}$$

1.7.6 Equations for some assay methods

DPPH assay method

$$DPPH^{\cdot} + ArOH \rightarrow DPPH + ArO^{\cdot} + H^{+}$$

CUPRAC assay method

$$n\ Cu(Nc)_2^{2+} + Ar(OH)_n \rightarrow n\ Cu(Nc)^{2+} + Ar(=O)_n + n\ H^{+}$$

Total phenol assay

Folin: Mo(VI) (yellow) + e- (from AH) → Mo(V) (blue)

FRAP

FRAP: $Fe(TPTZ)_2^{3+} + ArOH \rightarrow Fe(TPTZ)_2^{2+} + ArO. + H$

ABTS

ABTS/TEAC: $ABTS + K_2S_2O_8 \rightarrow ABTS.^+$

$ABTS.^+ + ArOH \rightarrow ABTS + ArO. + H^+$

1.8 *Eleusine coracana Linn* (POACEAE)

Scientific classification

Domain: Eukaryotes

Kingdom: plantae (plant)

Subkingdom: Tracheobionta (Vascular Plant)

Superdivision: Spermatophyta (Seed Plant)

Division: Magnoliophyta (Flowering Plants)

Class: Liliopsida (Monocotyledons)

Subclass: Commelinidae

Order: Cyperales

Family: Poaceae/Graminae (Grass)

Genus: *Eleusine* Gaertn (Goosegrass)

Specie: *Eleusine Coracana* (L) Gaertn (Finger Millet)

Botanical name: *Eleusine coracana*

Synonyms: *Cynodon coracanus* Raspail, *Eleusine cerealis* Salisb

Common names: Finger millet

Local name: Tamba (Hausa)

1.8.1 Plant description

Eleusine coracana is an annual plant with erect stem of about 60-200 cm long and about 20 mm in diameter. It's sheath keeled leaves are mainly basal with hairy outer margin. The leaf blades are 30-60 cm long and 6-12 mm wide. The fruits are caryopsis of about 1.5- 2.5mm long which may be dark brown or rugose. They are allopolyploids with chromosome number 2n = 4x = 36 and are obtained from crossing *Eleusine indica* and *Eleusine floccifolia* or *Eleusine tristachya* which are diploid species (Chandra *et al*., 2016). Their cell walls are made up of a mixture of lignocellulosic complexes, arabinoxylans, (1-3),(1-4) β-D-glucans and glucomannans (Latha *et al*., 2007).

Figure1.17: *Eleusine Coracana* (L) Gaertn (Finger Millet) in its natural habitat

Figure1.18: Seeds of *Eleusine coracana* (L) Gaertn (Finger Millet)

1.8.2 Habitat and geographical distribution

Eleusine coiracana is found mainly in the arid regions of Asia and Africa where it is used as staple food (Devi *et al*., 2014). It is a cereal that reaches maturity 3-6 months after planting and generally found within 1000 to 2000 m of altitude in East Africa and South African regions and up to 3000 m altitude in the Hilmalayas. A well distributed annual rainfall of 500 – 1000 mm is suitable for its growth and it grows best at a temperature of 23^{o}C although it can survive higher and lower temperature (David *et al*., 2014).

1.8.3 Phytochemical composition

The seeds of *Eleusine coracana* is rich in proteins, carbohydrates, minerals, micro nutrients, calcium and dietary fibers (Erhabor *et al*., 2013). It also contains vitamins such as ascorbic acid, thiamine, tocopherols and riboflavin (Amir *et al*., 2016).

1.8.4 Ethnomedicinal uses

It is used locally in the management of diabetes, anaemia, ulcer and osteoporosis. It is also used locally to manage depression, anxiety and insomnia because of its anxiolytic effect. It is believed to enhance milk flow in mothers who have problems with lactation after birth. Finger millet is also used locally to prevent weight gain because of the high fiber content and the feeling of satiety it gives after consumption, it is used to repair damaged or worn out tissues, reduce cholesterol levels and slow down the aging process associated with hyperglycemia (Mathanghi and Sudha 2012; Mall and Tripathi, 2016).

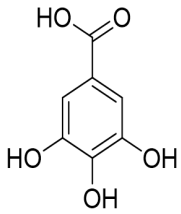
1.8.5 Previous scientific investigation

Investigations carried out on the seeds of *Eleusine coracana* have shown that it has good antioxidant properties (Sreeramalu *et al*., 2009;Mathanghi and Sudha, 2012), anticancer properties (Singh *et al*., 2015), antimicrobial activity (Shukla *et al.,* 2015), anti-aging activity (Hegde *et al*., 2002), wound healing activity (Rajasekarana, 2004), antilithiatic effect (Bahuguna *et al.,* 2009), aldose reductase inhibitory effect (Chethan *et al*., 2008), antidiabetic effect (Shukla and Srivastava, 2014), anticataract effect (Shobana *et al*, 2010), weight loss activity and prevention of obesity (Murtaza *et al*., 2014), hepatoprotective activity (Pingle *et al.,* 2011) and immunomodulatory activity (Prashanth *et al*., 2014).

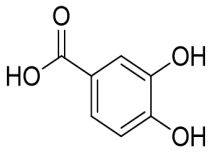
1.8.6 Compounds isolated from *Eleusine coracana*

Compounds isolated from the seeds of *Eleusine coracana* are mainly polyphenols. Singh *et al* (2008) identified the presence of caffeic acid, ferulic acid, gallic acid, tannic acid, vanillic acid, and chlorogenic acid. Chethan *et al* (2008) used High Performance Liquid Chromatography (HPLC) to identify the presence of ferulic acid, trans-cinnnamic acid, p-coumaric acid, gallic acid, vanillic acid, syringic acid, proto-cathechuic acid, quercetin and P-hydroxyl benzoic acid from the HCl-methanol seed extract of *Eleusine coracana*. Shobana *et al*, (2009) were able to identify naringenin, phloroglucinol, luteolin glycoside (orientin), diadzein, 4-O-Methyl gallic acid, trans feruloyl-malic acid, kaempferol, apigenin, (+)-catechin –epicatechin, dimer of prodelphinidin, catechin gallates, trimmers of catechin and tetramers of catechin in the seed coat of *Eleusine coracana*. Banerjee *et al*, (2012) also used reverse phase HPLC to identify sinapic acid, gentisic acid and salicylic acid in finger millets. Gas chromatography-mass spectrometric (GC-MS) method was used by Singh *et al*, (2015) to identify the presence of I,2-benzenedicarboxylic acid contained in the ethylacetate fraction

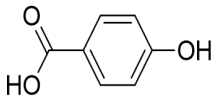
of the millet. Arabinoxylans have also been identified in *Eleusine coracana* by Prashanth *et al*., (2014) using Carbon-13 and proton NMR. The polyphenols present in finger millet are said to be highly concentrated in the seed coat which is also edible (Chethan *et al*., 2008). *Eleusine coracana al*so contains; palmitic acid, oleic acid and linoleic acid (Poonia *et al*., 2012). Amino acids such as tyrosine, methionine, tryptophan and cysteine have also been identified in finger millet seeds (Ambavane *et al*., 2014). They are said to contain alkaloids, terpenoids and tannins (Shukla *et al*., 2015). Nirmala and Muralikrishn, (2003) purified three α-amylases from the seeds and Saxena *et al*., 2010 also purified a bifunctional amylase/protease inhibitor from the seeds of *Eleusine coracana*.



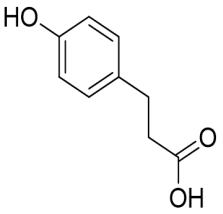
Gallic acid
3,4,5-Trihydroxylbenzoicacid



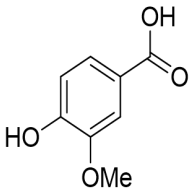
protocatechuic acid
3,4-dihydroxybenzoic acid



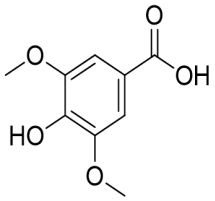
P-hydroxylbenzoic acid
4-hydroxylbenzoic acid



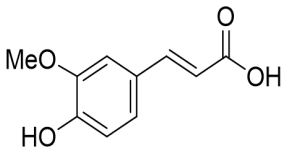
p-coumaric acid
Trans-4-hydroxycinnamic acid



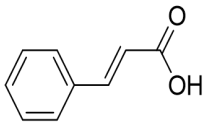
Vanillic acid
4-Hydroxy-3-methoxy benzoic acid



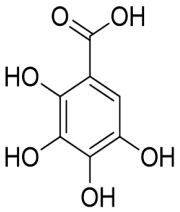
Syringic acid
3,5 dimethoxy-4-hydroxybenzoic acid



Ferulic acid
4-hydroxy-3-methoxycinnamic acid



Trans-cinnamic acid
3-phenylacrylicacid



Gentisic acid

Figure 1.19: Compounds isolated from *Eleusine coracana* seed

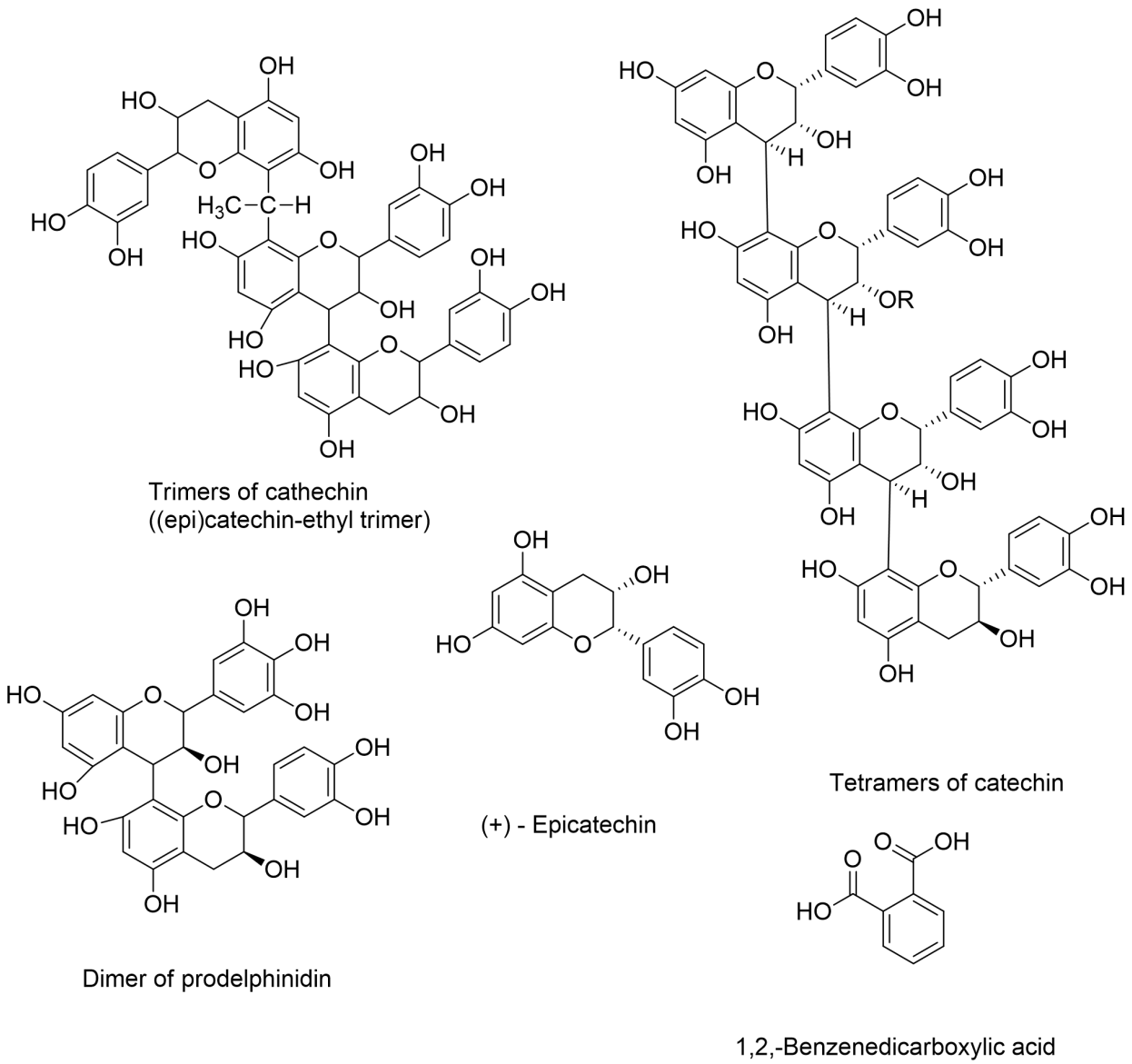


Figure 1.19: Compounds isolated from *Eleusine coracana* seed

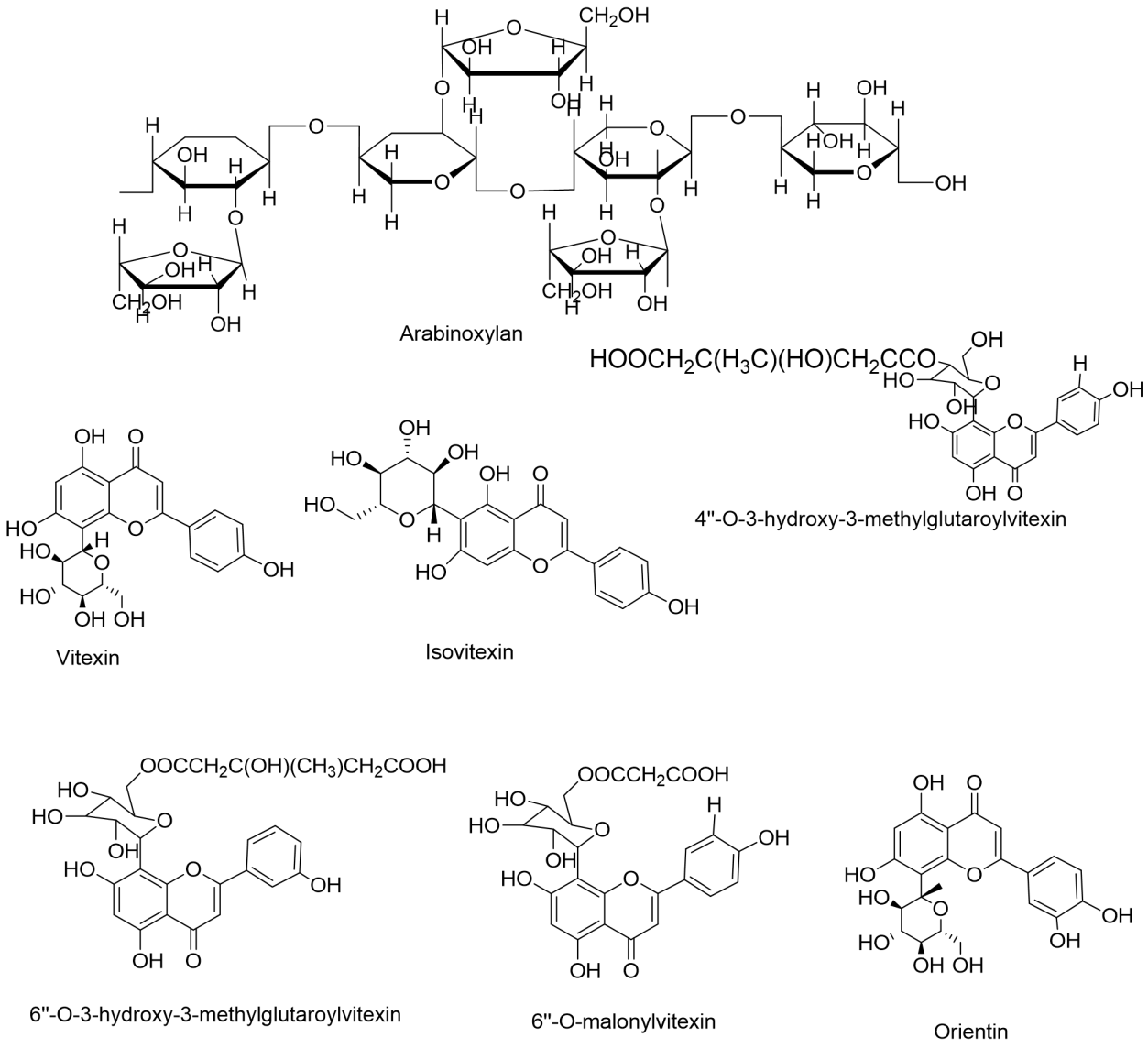


Fig 1.19: Compounds isolated from *Eleusine coracana* seed

1.9 JUSTIFICATION FOR THE STUDY

Diabetes is a serious public health problem and there is currently no known cure. Drugs used for its management are not only expensive but also not readily available to people living in the rural areas. Numerous side effects are associated with the available orthodox drugs which may encourage non-compliance and hence treatment failure. WHO recognizes traditional medicine as an accessible and affordable form of health care which could help to reduce financial burden on patients (WHO, 2013). As a result, there is a need for the search for alternative agents that will be able to address the problems associated with the currently available drugs.

1.10 AIMS AND OBJECTIVES OF THE STUDY

The aim of this study is to determine the antidiabetic effect of different fractions of *Eleusine coracana* seeds and identify chemical compounds present in active fractions. To achieve this aim, the following objectives were considered;

1. To establish the proximate parameters of the powdered seeds of *Eleusine coracana*.
2. To determine the phytochemical constituents in the powdered seeds of *Eleusine coracana*.
3. To determine the mineral composition of the seeds of *Eleusine coracana*.
4. To determine the antioxidant properties of crude extracts and fractions of the seeds of *Eleusine coracana*.
5. To determine the antidiabetic effect of the crude extract and fractions of *Eleusine coracana* seeds.
6. To identify compound(s) present in the active fractions.

CHAPTER TWO

MATERIALS AND METHODS

2.1. MATERIALS

2.1.1 Solvents and Reagents

Acetic anhydride (Sigma-Aldrich, Germany)

Acetone (Sigma-Aldrich, Germany)

Alkaloidal reagents (Drangedorff, Mayer, picric acid and Wagner's reagent)

Alpha-naphthol (Sigma-Aldrich, Germany)

Aluminium chloride hydrate (BDH, England)

Ammonium hydroxide (Sigma-Aldrich, Germany)

Antimony potassium tartarate (Kermel, China)

Calcium chloride (BDH, England)

Chloroform (Sigma-Aldrich, Germany)

Citric acid (Qualikems, India)

Dimethylsulfoxide (Sigma-Aldrich, Germany)

Ethanol (Sigma-Aldrich, Germany)

Ethylacetate (Sigma-Aldrich, Germany)

Ferric chloride (Qualikems, India)

Folin ciocalteu phenol reagents (Sigma-Aldrich, Germany)

Gallic acid (Sigma-Aldrich, Germany)

Gelatin (Qualikems, India)

Glacial acetic acid (Sigma-Aldrich, Germany)

Hydrochloric acid (BDH, England)

L-ascorbic acid (BDH, England)

Mass Hunter GC-MSD (Agilent 19091S-433UI)

Methanol (Sigma-Aldrich, Germany)

Ninhydrin (Qualikems, India)

Petroleum ether extra pure 60 - 80^{o}C (Sigma-Aldrich, Germany)

Perchloric acid (Sigma-Aldrich, Germany)

Potassium acetate (Sigma-Aldrich, Germany)

Quercetin (Sigma-Aldrich, Germany)

Silver nitrate (Merck)

Sodium carbonate (Qualikems, India)

Sodium Citrate (Qualikems, India)

Sodium hydroxide (Merck)

Tetraoxosulphate (vi) acid (BDH, England)

Trioxonitrate (v) acid (BDH, England)

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical (Sigma-Aldrich, Germany)

2.1.2 Glassware

Beakers, capillary tubes, chromatographic tanks, conical flasks, evaporating dish, glass funnels, glass columns 4cm x 90cm, 9cm x 120cm (Merck), glass jars, measuring cylinders, pipettes, reagent bottles, stirrer, test tubes. All glass wares were products of Fiolax (China), Pyrex (England), and Chengdu (China).

2.1.3. Equipment

Accu-check® active glucometer (Roche, USA)

Atomic absorption spectrophotometer (Buck scientific model VGP 210, USA)

Oven dryer (Gallen kamp, England)

Electronic weighing balance (Ohaus corporation Shangai)

Flame photometer (Sherwood model 410 flame photometer. England)

Heating mantle (Electrothermal engineering. London. England)

Infrared spectrometer (Agilent technologies Inc)

Julabo (Julabo F10, Germany)

Mass spectrometer (Bruker)

Mechanical grinder (Viking Exclusive, Joncod)

Muffle furnace (Gallen kamp, England)

Refrigerator (Express cool, LG, Nigeria)

Rotary evaporator (Bibby scientific limited. Standfordshire. UK)

Soxhlet apparatus (Pyrex Germany)

UV lamp (Camag)

UV-VIS Spectrophotometer (PG Instrument limited. Model T80)

Vacuum pump (Barloworld scientific limited. Staffordshire. ST 15 Osa. UK)

2.1.4 Animals

The animals (170 – 250 g) used were adult Swiss albino mice obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. They were fed with standard rodent pellets (Bendel feeds and Flour Mill Ltd, Ewu, Nigeria) and given water *ad libitum*. The animals were exposed to lighting conditions and handled according to protocol approved by Faculty of Pharmacy ethical committee. Ethical approval (EC/FP/018/26) was obtained from the Animal Ethics Committee, Faculty of Pharmacy, University of Benin, Benin City, Nigeria before the study was carried out.

2.1.5 Drug

Streptozotocin (Santa Cruz Biotechnology)

Glibenclamide (Daonil®) Sanofi –Aventis, Swiss Pharma Nigeria. Ltd.

2.1.6 Other materials

Cotton wool

Filter papers

Syringes (1 mL, 2 mL, 5 mL and 10 mL)

Silica gel 200-400 mesh (Merck)

TLC plates –F254 (Merck)

2.2 METHODS

2.2.1 Collection of plant materials

The plant sample was obtained from a local market (Jengre market under Bassa local government area) in Jos Plateau, Nigeria in June, 2016. The plant was then identified at the Forestry Research Institute of Nigeria by Mr. Ugbogu O. A. A herbarium specimen with voucher number (FHI 59920) was deposited in the herbarium of the Institute. The seeds were air dried and pulverized.

2.2.2 Proximate analysis of powdered seeds of *Eleusine coracana*

Standard methods were used to carry out the following proximate analysis (quantitative parameters) (AOAC, 1984; African Pharmacopoeia 1986).

2.2.2.1 Moisture content/Water loss on drying

The powdered seed (2 g) of *Eleusine coracana* was weighed into a previously weighed clean and dry crucible. The crucible containing the powdered seed was oven dried at a temperature of 105ºC until a constant weight was achieved. The average moisture content (percentage weight loss) was determined using six replicates with reference to the air-dried powdered drug.

$$Moisture\ content\ (\%) = \frac{(weight\ loss)}{(Initial\ Weight\ of\ Sample)} \times\ 100\%$$

2.2.2.2 Total ash

To determine the moisture content, twelve crucibles which were properly washed were placed in a hot oven set at 100°C. They were marked 1-12 while they were still warm. After

which, they were placed in a desiccator where they were allowed to cool and subsequently weighed. The powdered seeds (2 g) were weighed into each of the twelve crucibles. The crucibles containing the powdered seeds were heated in the furnace at a temperature of 600^{o}C for 6 hours. The furnace was switched off after 6 hours and the temperature was allowed to drop. The crucibles were removed from the furnace, allowed to cool in a desiccator and reweighed. The percentage ash was determined for six replicates.

$$Percentage\ Total\ Ash\ (\%) = \frac{(W - Z)}{N} \times 100\%$$

Where; W = Weight of the crucible and ash,

Z = Weight of empty crucible,

N = Weight of sample

2.2.2.3 Acid insoluble ash

The ash contained in the crucible from the above experiment (total ash) was transferred into a beaker containing 25 mL of dilute hydrochloric acid and boiled for 5 minutes after which, it was filtered using an ash less filter paper. The filter paper containing the residue was folded into a small cone and placed into the crucible and transferred to a furnace where it was heated until the filter paper was observed to be completely ashed. The crucible was allowed to cool, and the weight of the residue determined. The percentage acid insoluble ash was calculated based on the initial weight of the air-dried powdered drug. The determination was carried out in triplicates

$$Acid\ Insoluble\ Ash\ Value = \frac{Weight\ of\ Residue}{Initial\ Weight\ of\ the\ sample} \times 100\%$$

2.2.2.4 Water soluble ash

The remaining six crucibles with the total ash from the total ash experiment were transferred into six beakers each containing 25 mL of distilled water. The content of the beaker was boiled for 5 minutes and filtered through an ashless filter paper. The filter paper with the residue were folded into a small cone and returned to their respective crucibles. The crucibles were heated in a furnace until a temperature of 600°C was attained. This temperature was maintained for 6 hours. The crucible and its content were cooled in a dessicator before weighing. The weight of the residue was subtracted from the weight of the total ash and the difference was expressed as a percentage of the initial weight of the sample.

$$Water\ Soluble\ Ash\ Value = \frac{Weight\ of\ Ash - Weight\ of\ Residue}{Initial\ Weight\ of\ the\ sample} \times 100\%$$

2.2.2.5 Alcohol soluble extractive value

The powdered seed of *Eleusine coracana* (5 g) was weighed into a 250 mL conical flask and macerated with 100 mL of 98% ethanol for 24 hours during which it was shaken intermittently for the first 6 hours after which, it was allowed to stand for the remaining 18 hours. A Buckner funnel was used to filter the extract using suction filtration method. The filtrate (20 mL) was taken into a clean and dried previously weighed crucible and allowed to evaporate to dryness. The residue was allowed to dry until constant weight was achieved, and the final weight was noted. Based on the initial weight of the powdered seed, the alcohol extractive value was calculated and expressed as percentage.

2.2.2.6 Water soluble extractive value

The powdered seed of *Eleusine coracana* (5 g) was weighed into a 250 mL conical flask and macerated with 100 mL of a mixture of chloroform and water in the ratio 1:4 for 24 hours during which it was shaken intermittently for the first 6 hours after which, it was allowed to stand for the remaining 18 hours. A Buckner funnel was used to filter the extract using suction filtration method. The filtrate (20 mL) was taken into a clean and dried previously weighed crucible and allowed to evaporate to dryness. The residue was allowed to dry until constant weight was achieved, and the final weight was noted. Based on the initial weight of the powdered seed, the water soluble extractive value was calculated and expressed as percentage.

2.2.3 Phytochemical Screening of the powdered seed of *Eleusine coracana*

Standard methods were used to determine the presence of phytochemicals such as; carbohydrates, saponins, alkaloids, anthraquinones, tannins and other phenolics by simple chemical tests (Sofowora, 1982; Trease and Evans, 2002).

The crude powdered sample (5 g) was boiled in 75 mL of distilled water for 30 minutes. The solution was filtered while still hot using a filter paper and allowed to cool. The filtrate obtained was used to carry out the following tests.

2.2.3.1 General Tests for Alkaloids

2.2.3.1.1 Dragendorff's reagent (2 drops) was added to 2 mL of the filtrate.

Expected observation for a positive result: formation of a reddish brown precipitate.

2.2.3.1.2 Wagner's reagent (2 drops) was added to 2 mL of filtrate.

Expected observation for a positive result: formation of a brown precipitate.

2.2.3.1.3 Hager's reagent (2 drops) was added to 2 mL of filtrate.

Expected observation for a positive result: formation of a yellow precipitate.

2.2.3.1.3 Mayer's reagent (2 drops) was added to 2 mL of filtrate.

Expected observation for a positive result: formation of a milky precipitate.

2.2.3.2 Tests for Carbohydrates

2.2.3.2.1 **Molisch's Test**

Two drops of 1% alcoholic naphthol followed by 2 mL of concentrated sulphuric acid were added to 2 mL of the filtrate in a slanty position.

Expected observation for a positive result: formation of a violet ring at the interface of two liquid layers.

2.2.3.2.2 Tests for Reducing Sugars

Fehling's test

2 drops of Benedict's reagent (a mixture of equal volumes of Fehling's solution A and B) was added to 2 mL of the filtrate. The resulting solution was heated over a boiling water bath for 3 minutes.

Expected observation for a positive result: formation of orange or brick red precipitate.

Tollen's test

To silver nitrate solution containing a few drops of 10% sodium hydroxide, dilute ammonium hydroxide solution was added in drops until the precipitate of silver oxide almost completely dissolved. Few drops of the seed extract were then added to the mixture.

Expected observation for a positive result: formation of precipitate of silver mirror along the side of the test tube.

2.2.3.2.3 Keller Kiliani's test for deoxysugars

Few drops of dilute acetic acid containing a trace of 5% ferric chloride was added to 2 mL of filtrate. The resulting mixture was transferred to the surface of concentrated sulphuric acid.

Expected observation for a positive result: formation of a violet ring at the interface of two liquid layers.

2.2.3.3 Test for saponins

2.2.3.3.1 Frothing Test

Distilled water (10 mL) was used to dilute 1 mL of the filtrate and shaken vigorously for one minute.

Expected observation for a positive result: formation of a persistent frothing.

2.2.3.3.2 Fehling's test

Dilute H_2SO_4 (5 mL) was added to10 mL of the aqueous extract. The mixture was then boiled for 15 min in a water bath, filtered and cooled. 20% NaOH was added to 2.5 mL of the filtrate to make it alkaline and the mixture was boiled with 0.1 mL each of Fehling's solutions A and B for 2 minutes.

Expected observation for a positive result: colour change on heating.

2.2.3.4 Lieberman Burchard's test for steroidal saponins or phytosterols

Few drops of acetic anhydride and 1 mL of chloroform were mixed together and few drops from the mixture were added to 2 mL of the filtrate. 2 drops of concentrated sulphuric acid was then added to the final mixture.

Expected observation for a positive result: Gradual change of colour from violet to blue and to green.

2.2.3.5 Test for tannins

Gelatin test

To 2 mL of the aqueous filtrate was added 2 mL of 1% gelatin solution in 10% NaCl.

Expected observation for a positive result: formation of precipitate.

2.2.3.6 Test for terpenoids

Salkowski test

Exactly 5 mL of the filterate was mixed with 2 mL of chloroform. Concentrated sulphuric acid was then added in a dropwise manner to form a layer.

Expected observation of positive result: formation of reddish brown ring at the interface between the two layers.

2.2.3.6 Test for phenolic compounds

2.2.3.6.1 Ferric chloride test

Distilled water (5 mL) was added to 2 mL of filtrate then 2 drops of 5% ferric chloride solution was also added. A blank test was carried out by adding 2 drops of 5% ferric chloride solution to 5 mL of distilled water.

Expected observation for a positive result: formation of intense coloration in the test sample.

2.2.3.6.2 Folin Ciocalteu's test

To 5 mL of filtrate was added 10% folin ciocalteu's phenol reagent (0.5 mL) and 7% Na_2CO_3 (5 mL).

Expected observation for a positive result: formation of intense purple colour.

2.2.3.7 Test for flavonoids

2.2.3.7.1 Alkaline reagent test

Few drops of 20% sodium hydroxide solution were added to 2 mL of filtrate followed by few drops of dilute hydrochloric acid solution.

Expected observation for a positive result: formation of intense yellow precipitate which dissolves on addition of dilute acid.

2.2.3.7.2 Lead acetate test

Few drops of lead acetate solution were added to 2 mL of filtrate.

Expected observation for a positive result: formation of milky precipitate.

2.2.3.7.3 Aluminium chloride test

The filtrate (3 mL) was shaken with 0.1 mL of 1% $AlCl_3$ solution and 0.1 mL of 1M CH_3COOK solution. The mixture was allowed to stand for 30 min.

Expected observation for a positive result: formation of a yellow colour solution.

2.2.3.8 Test for anthraquinone derivatives

Bontreger's test

Petroleum ether (2 mL) was shaken with 2 mL of filtrate. The ether layer was then washed with 2 mL distilled water and then shaken with dilute ammonia solution.

Expected observation for a positive result: formation of pink colour on addition of ammonia solution.

2.2.3.9 Test for proteins

2.2.3.9.1 Xanthoprotenic test

Few drops of concentrated nitric acid were added to 2 mL of filtrate.

Expected observation for a positive result: formation of a yellow precipitate.

2.2.3.9.2 Ninhydrin test

Two drops of Ninhydrin solution was added to 2 mL of filtrate.

Expected observation for a positive result: formation of dark blue, violet or purple colour.

2.2.4 Extraction of plant material

The powdered plant material (5.5 kg) was extracted with 12.5 L of methanol using a soxhlet apparatus. The extract obtained was concentrated to dryness using a rotary evaporator at reduced temperature and subsequently refered to as crude methanol extract. The dried extract was weighed, and the percentage yield was calculated based on the initial weight of the crude powdered sample. The extract was stored in an air-tight container and kept in the refrigerator at 4^{o}C until further experiment.

2.2.5 Fractionation

The crude methanol extract (10 g) was weighed and triturated with 40 g of 200-400 mesh size silica gel. The resultant powder was filtered to obtain very fine powder which was then fractionated withVacuum Liquid Chromatography using 300 g of 200 – 400 mesh size silica gel and different solvent system/ solvent mixtures in the order of increasing polarity (n-hexane), dichloromehane, ethylacetate, 50% ethylacetate/methanol, methanol, 20% methanol/water, 50% methanol/ water, and 100% water). Fractions obtained were concentrated *in vacuo* using a rotary evaporator and freeze drier.

2.2.6 Determination of Antioxidant Activity

2.2.6.1 DPPH Radical Scavenging Assay

The free radical scavenging activity of the crude methanol extract and it's fractions of *Eleusine coracana* seeds on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was assessed using methods described by Jain *et al*., 2008. 1.0 mL of a solution of 0.1 mM DPPH in methanol was mixed with 3.0 mL of extract in methanol containing a concentration of 0.01 - 0.2 mg/mL of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured using a spectrophotometer set at 517 nm wavelength. The reference standard used was ascorbic acid. The ability to scavenge DPPH radical was calculated using the equation below:

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = \frac{[A0 - A1]}{A0} \times 100$$

Where; A0 represents the absorbance of the mixture DPPH radical and methanol

A1 represents the absorbance of DPPH radical plus sample extract /standard (Elmastase, 2007).

The 50% inhibitory concentration (IC_{50}) value was calculated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radical (Jain *et al*., 2008).

2.2.6.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out using methods described by Benzie and Strain (1996) with slight modification.

Preparation of FRAP reagent

STEP 1

300 mM Acetate buffer: sodium acetate trihydrate (0.62 g) was weighed accurately into a beaker and 3.2 mL of glacial acetic acid was added. The pH was adjusted to 3.6 and the solution was made up to 200 mL with deionized water.

40 mM Hydrochloric acid (HCl): Concentrated hydrochloric acid (0.34 mL) was accurately measured and added to 50 mL of deionized water. The solution was then made up to 100 mL volume.

10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine): TPTZ (0.15615 g) was accurately weighed and dissolved in 50 mL of 40 mM HCl. The mixture was then placed on a water bath set at 50^{o}C to enhance the dissolution of the TPTZ.

20 mM $FeCl_3.6H_2O$ (Ferric chloride hexahydrate): $FeCl_3.6H_2O$ (0.2703 g) was accurately weighed and dissolved in 50 mL of deionized water.

FRAP reagent was prepared by combining the 300 mM acetate buffer, 10 mM TPTZ and 20 mM $FeCl_3.6H_2O$ in the ratio 10:1:1 respectively.

STEP 2:

To get a standard calibration curve, ferrous sulphate heptahydrate ($FeSO_4.7H_2O$) was prepared in different concentrations (0, 5, 10, 20, 40, 60, 80 and 100 µM). The blank (0 µM) was prepared by adding 3 mL of FRAP reagent to 1.5 mL of distilled water while 3 mL of FRAP reagent was added to 1.5 mL of each of the other concentrations. The test tubes were incubated in the dark for 30 minutes after which the absorbance of the mixture was measured at 593 nm.

STEP 3:

Exactly 1.5 mL (0.1 mg/mL) of the crude extract and each fraction were measured into test tubes and 3 mL of FRAP reagent was added to each of the tubes. The mixture was allowed to incubate in the dark for 30 minutes and the absorbance was measured at 593 nm.

The results obtained were expressed as millimolar of ferrous sulphate equivalent per gram (mMFSE/g) of extract/fraction.

2.2.7 Determination of polyphenolic content

Total phenol

The total phenol content of the extract was determined using the method described by Kim *et al*., 2003. The solution of extract (0.5 mL) with a concentration of 1000 µg/mL was added to 4.5 mL of deionized distilled water and 0.5 mL of Folin Ciocalteu's reagent (previously diluted with water 1:10, v/v) was then added to the solution. After mixing, the mixture was maintained at room temperature for 5 minutes followed by the addition of 5 mL of 7% sodium carbonate and 2 mL of deionized distilled water. After mixing, the samples were incubated for 90 minutes at room temperature. The absorbance was measured using

spectrophotometer at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract). The standard curve was prepared by gallic acid in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

Total flavonoid

Total flavonoid contents were estimated using the method described by Ebrahimzadeh *et al*., 2008. Briefly, 0.5 mL of extract sample (1 mg/mL) was mixed with 1.5 mL of methanol and then, 0.1 mL of 10% aluminium chloride was added, followed by 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared using quercetin with six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

2.2.8 Mineral analysis

The powdered seed of *Eleusine coracana* (1 g) was weighed and transferred into a conical flask. 10 mL each of nitric acid (HNO_3) and perchloric acid ($HClO_4$) were mixed in the ratio 3:1. The preparation was added to the powdered seed in the flask and heated until colour fumes disappeared. The content of the flask was filtered using whatman No 1 filter paper into a 100 mL volumetric flask. Distilled water was used to make up to the 100 mL mark. A flame photometer was used to analyse for sodiuim and potassium metals and an atomic absorption spectrometer was used to analyse for the presence of other metals. (AOAC, 1984).

2.2.9 Acute Toxicity Study

2.2.9.1 Test Procedure

The acute toxicity study was carried out using method described by Lorke, 1983. The method involved two phases which are; phase 1 and phase 2.

Phase 1

This phase involved the use of nine (9) animals. The nine animals were divided into three groups with each group containing three animals. Each group of animals were administered different doses (10, 100 and 1000 mg/kg) of the crude extract through the oral route. The animals were placed under observation for 24 hours to monitor for behavioral changes as well as occurrence of mortality.

Phase 2

This phase involved the use of three animals (3) which were divided into three groups of one animal each. The animals were then administered higher doses (1600, 2900 and 5000 mg/kg) of the extract through the oral route and then observed for 24 hours for behavioral changes and mortality.

A control group was created using one mouse which was administered distilled water and monitored for 24 hours for behavioral changes and mortality.

$LD_{50} = \sqrt{(D0 \times D100)}$

D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality

2.2.10 Antidiabetic Screening of Crude Extract and Fractions of *Eleusine coracana*

2.2.10.1 Induction of diabetes in experimental animals

Male albino Wistars rats (170 – 250g) used for the experiment were fasted overnight and a single dose of 40 mg/kg body weight of streptozotocin in freshly prepared 0.1 M citrate buffer at pH 4.5 was administered intraperitoneally to each animal. Their blood glucose levels were determined after 48 hours of induction using accu-check® active glucometer (Roche, USA) and animals with blood glucose level of ≥ 200 mg/dL were recruited for the study (Rastogi *et al*.,1997; Chen *et al*., 2005).

2.2.10.2 Treatment groups

The animals were divided into 13 groups (containing 4 animals each) which included a positive control group (normal non-diabetic animals) and the negative control diabetic untreated animals (given distilled water). The other groups contained diabetic animals treated with; glibenclamide (5 mg/kg/day), crude extract (200 mg/kg/day), crude extract (400 mg/kg/day), methanol fraction (200 mg/kg/day), methanol fraction (400 mg/kg/day), 50% methanol/50% ethylacetate fraction (200 mg/kg/day), diabetic animals treated with 50% methanol/ethylacetate fraction (400 mg/kg/day), 100% ethylacetate fraction (200 mg/kg/day), 100% ethylacetate fraction (400 mg/kg/day), n-hexane fraction (200 mg/kg/day), n-hexane fraction (400 mg/kg/day). Treatments were administered orally to the animals using an oro-gastric tube and blood glucose level was monitored 0, 1, 2, 4, 8 hours and 7 days post administration. A sterile lancet was used on the tail tip of the animals to obtain blood samples for blood glucose determination.

2.2.10.3 Blood Glucose Measurement

An accu-check® active glucometer (Roche, USA) was used to measure the blood glucose level of animals which were obtained from the tail tip of the rats with a sterile lancet. The percentage glycemic change at any point in time was calculated using the formula below;

$$Glycemic\ change = \frac{GC - FBG}{FBG}$$

Where GC = Glucose concentration at any point in time

FBG = base line value of the fasting blood glucose level

2.2.10.4 Change in body weights

The changes in body weight of the animals were monitored using a weighing balance and the percentage changes in body weight for seven days were calculated as follows;

$$Percentage\ change\ in\ body\ weight\ (\%) = \frac{Initial\ weight\ - weight\ at\ time\ T}{initial\ weight} \text{X } 100$$

2.2.10.5 Determination of haematological parameters, lipid profile, renal function and histopathology of the pancreas

Blood samples were collected from the animals by cardiac puncture under chloroform anaesthesia after seven days treatment in order to determine haematological parameters, renal function and lipid profile of the animals. The blood samples were kept in an EDTA (Ethylenediamine tetraacetate) bottle and a plain blood sample bottle for analysis (Mahmoud,

2013). Also, the pancreas of animals used for the study were excised and preserved in 10% formosaline for histopathological analysis (Al-Qudah *et al*., 2016).

2.2.11 Chromatographic Techniques

2.2.11a Column Chromatography

Gravity column was prepared by adding slurry of silica gel (200-400 mesh size) to a glass column (60 x 3.5 cm). The excess solvent (Petroleum ether) was allowed to flow out.

The ethylacetate fraction (10.98 g) was adsorbed onto silica gel of 200 – 400 mesh size and the fine powder obtained was carefully placed in the column as concentrated band. The column was eluted using combination of solvents (petroleum ether and ethylacetate) in a stepwise manner of increasing polarity. Ninety eluates (100 mL each) were collected as shown in table 2.1..

2.2.11b Thin layer chromatography of fractions obtained from column

Thin layer chromatographic plates (TLC Silica gel 60 F_{254}) were used to analyze the eluates from the column using suitable solvent combination of petroleum ether: ethylacetate (9:1, 9.5:0.5). The developed plates were viewed under UV light at 254 nm and 366 nm before spaying with vanillin sulphuric acid (mixture of 5g vanillin in 100 mL ethanol and 10 mL sulphuric acid in 100 mL ethanol). Fractions observed to have similar TLC profiles were combined and labeled as; CO1, CO2, CO3, CO4, CO5 to CO16. Fractions CO3 and CO4 were combined based on the similarity of their R_f values to form CO3,4 fraction after which, all the fractions were concentrated to dryness *in vacuo* using a rotary evaporator.

2.2.11c Purification of fraction CO2

Pooled fraction CO2 (1.3333g) was subjected to further silica gel column chromatography. The column was eluted using petroleum ether: ethhylacetate (98:2). Twenty three fractions coded 1B – 23 B were collected.

Table 2.1: Column chromatography of ethylacetate fraction of *Eleusine coracana* seed.

Combination solvents used for elution (%)			
pet. ether	ethylacetate	vol of solvent used (ml)	eluate tube no.
98	2	400	1-3 (A-C)
96	4	900	4-11 (D-K)
94	6	400	12- 15 (L-O)
92	8	100	16 (P)
90	10	1700	17-31 (Q-31)
85	15	2100	32-49
80	20	800	50-59
75	25	800	60-80
70	30	1000	81-90

Table 2.2: Purification of CO3 and CO4

Combination solvents used for elution (%)			
pet. ether	ethylacetate	vol of solvent used (mL)	eluate tube no.
98	2	100	1-8
96	4	300	9-30

Table 2.3: Purification of CO2

Combination of solvents used for elution (%)			
Pet.ether	ethylacetate	vol of solvent used (mL)	eluent tube no.
98	2	300	1B-23B

2.2.11d Purification of CO3,4 fraction

A column of size 63 cm x 1.5 cm was used for the fractionation of 0.80 g of CO3,4 and different ratios of petroleum ether: ethylacetate was used for elution in a stepwise manner. Fractions obtained were collected in vials labeled 1- 30 and analyzed after concentration using TLC plates developed with different ratios of petroleum ether: ethylacetate. Fraction 9 obtained from the column was subjected to further purification based on its promising TLC profile.

2.2.11e. Purification of fraction 9 obtained from CO 3, 4

Fraction 9 (0.1354 g) was subjected to further silica gel column chromatography eluted isocratically with petroleum ether: ethylacetate (98:2). Fractions collected were labeled 9A1-9A39. The fractions were analyzed using TLC plates developed with different ratios of petroleum ether: ethylacetate.

2.2.12 Infrared (IR) spectrometric analysis of ethylacetate and n- hexane fractions of *Eleusine coracana.*

The ethylacetate fraction and n- hexane fraction were subjected to infrared spectrometric analysis in order to determine the fuctional groups present using the transmittance method with agilent carry 630 a universal sampler manufactured by agilent technologies . The samples were scanned 30 times with a resolution set at 8. The results were scanned between the ranges of 650- 4000 cm^{-1}.

2.2.13 Gas chromatography-mass spectrometric (GC-MS) analysis of ethylacetate fraction and n- hexane fraction of *Eleusine coracana.*

GC-MS analysis was carried out on the ethylacetate and n- hexane fraction to determine the components present in each of the fractions. Mass Hunter GC-MSD (Agilent 19091S-433UI) equipment by Agilent technologies Inc., equipped with HP-5ms Ultra Inert capillary column (30 m x 250 µm x 0.25 µm). The injection volume of each sample was 3 µL. Helium was used as the carrier gas at a flow-rate of 0.73677 mL/min. The temperature of the injection port was 250°C, and the column temperature program was as follows: 50°C for 1 min, followed by an increase to 200°C at a rate of 3°C/min, an increase to 300°C at a rate of 3 °C/min, and maintained at 325°C for 15 min. The MS conditions included an EI ion source temperature of 230°C, an ionization energy of 1276.51 MEV and a mass scan range of 46–600amu.

2.2.14 Statistical Analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA), and differences between means were determined by Duncan's multiple range tests using the Statistical Analysis System (SPSS Statistics 17.0). Correlations between polyphenol contents and antioxidant activity were established by regression analysis at 95% confidence level. P values ≤ 0.05 were regarded as significant.

CHAPTER THREE

RESULTS

3.1 Proximate analysis of the powdered seeds of *Eleusine coracana*

The values of the proximate analysis of the powdered seeds of *Eleusine coracana* is shown in the table 3.1. The result shows that the moisture content of *Eleusine coracana* seed is 0.38 ± 0.14%. The total ash content of the powdered seeds was 2.23 ± 0.13% and the water/chloroform extractive value (1.20 ± 0.08%) was seen to be higher than the alcohol extractive value (0.21 ± 0.03%).

3.2 Phytochemical analysis

The powdered seeds of *Eleusine coracana* contained carbohydrates, proteins, saponins, tannins, alkaloids, phenols, flavonoids, terpenoids and reducing sugars as shown in table 3.2.

3.2.1 Test for alkaloids

Formation of precipitates and different colour changes were observed when 2 mL of the aqueous extract of the powdered seed reacted with the different alkaloid reagents which indicated the presence of alkaloids in the powdered seed of *Eleusine coracana.*

3.2.2 Test for carbohydrates

The formation of a violet ring at the interface between the solution of 1% alcoholic naphthol, 2 mL of the aqueous extract and concentrated sulphuric acid confirmed the presence of carbohydrate in the powdered seed of *Eleusine coracana*.

3.2.3 Test for reducing sugars

The presence of reducing sugar in the aqueous extract of the powdered seed of *Eleusine coracana* was confirmed by the formation of an orange precipitate with Fehling's reagent and formation of silver mirror at the side of the reaction test tube with Tollen's reagent.

Table 3.1: Proximate parameters of the seeds of *Eleusine coracana*

Parameter	**Value ± SEM (%)**
Moisture content	0.38 ± 0.14
Total ash	2.23 ± 0.13
Acid insoluble ash value	0.76 ± 0.05
Water soluble ash value	0.01 ± 0.00
Alcohol extractive value	0.21 ± 0.03
Water/ chloroform extractive value	1.20 ± 0.08

3.2.4 Test for deoxy sugars

Deoxy sugars were found to be present in the aqueous extract of *Eleusine coracana* with the observation of a violet ring which was formed at the interface in the Keller Kiliani's test for deoxy sugars.

3.2.5 Test for saponins

Saponins were seen to be present in the aqueous extract of *Eleusine coracana* with the observation of frothing which persisted for upto 60 seconds after shaking vigorously as well as the formation of deep brown colouration when heated up with Fehling's solution A and B.

3.2.6 Test for steroidal saponins or phytosterols

Steroidal saponins or phytosterols were not present in the aqueous extract of *Eleusine coracana*. No colour change was observed after Lieberman Burchard's test was carried out.

3.2.7 Test for tannins

Formation of a precipitate on addition of 1% gelatin solution in 10% sodium chloride to the aqueous filtrate revealed the presence of tannins in the powdered seed of *Eleusine coracana*.

3.2.8 Test for terpenoids

Formation of reddish brown ring at the interface between the two layers confirmed the presence of terpenoids in the seeds of *Eleusine coracana*.

3.2.9 Test for phenolic compounds

Phenolic compounds were observed in the powdered seeds of *Eleusine coracana* due to the formation of an intense yellow coloration after the ferric chloride test and an intense violet coloration after the Folin Ciocalteu's test was carried out.

3.2.9 Test for flavonoids

The various tests for flavonoids carried out on the aqueous extract of the powdered seed of *Eleusine coracana* revealed the presence of flavonoids. An intense yellow precipitate which dissolved on addition of dilute hydrochloric acid was observed with the alkaline reagent test

for flavonoid. A milky precipitate was observed after the lead acetate test for flavonoid was carried out and a yellow colour was observed with the aluminium chloride test for flavonoids.

3.2.10 Test for anthraquinone derivatives

No colour change was observed indicating that anthraquinone derivatives were not present in the aqueous extract of *Eleusine coracana*.

3.2.11. Test for proteins

Xanthoproteic test for proteins carried out on the aqueous seed extract showed the presence of protein with the observation of a yellow precipitate on addition of few drops of concentrated nitric acid.

3.3 Percentage yield of the methanol extract and fractions

The percentage yield of the total methanol crude extract obtained from soxhlet extraction (as shown in table 3.3) was 2.72%, while the percentage yield of the n-hexane, dichloromethane, 100% ethylacetate, 50% ethylacetate:methanol, 100% methanol, 20% water/methanol and 50% water/methanol fractions were 0.13%, 0.25%, 10.98%, 38.92%, 10.40%, 3.82% and 0.58%, respectively.

3.4 Elemental constituents of *Eleusine coracana* using flame photometer and atomic absorbtion spectrometer

The elemental analysis carried out on the powdered seeds of *Eleusine coracana* showed that it contains essential elements such as; sodium, potassium, calcium and magnesium. It also contains trace elements like Zinc, iron and copper. The analysis also revealed that cadmium and nickel were absent in the powdered seed. The elements analyzed and their concentrations are shown in table 3.4.

Table 3.2: Phytochemical composition of the powdered seed of *Eleusine coracana*

Phytochemicals	**Inference**
Carbohydrates	+
Alkaloids	+
Proteins	+
Reducing sugars	+
Saponins	+
Tanins	+
Phenols	+
Flavonoids	+
Terpenoids	+
Deoxysugars	+
Anthraquinones	-

+ represents presence of components
- represents absence of components

Table 3.3: Percentage yield of total extract and fractions

Fractions	**Yield %**
Total extract	2.72
N- hexane	0.13
Dichloromethane	0.25
100% ethylacetate	10.98
50% ethylacetate : methanol	38.92
100% methanol	10.40
20% H_2O/ methanol	3.82
50% H_2O/ methanol	0.58

Table 3.4: Elemental constituents of *Eleusine coracana* seed

Metals	Concentration (PPM)	WHO limit (PPM)
Sodium (Na)	11.66±1.17	-
Potassium (K)	19.72±0.39	-
Calcium (Ca)	362.20±9.01	-
Magnesium (Mg)	1.06±0.00	-
Zinc (Zn)	0.35±0.01	27.40
Cadmium (Cd)	0.00±0.00	0.21
Lead (Pb)	0.01±0.00	10.00
Iron (Fe)	2.84±0.10	20.00
Copper (Cu)	0.19±0.01	3.00
Nickel (Ni)	0.00±0.00	1.63
Chromium (Cr)	1.23±0.07	-

3.5 Antioxidant activity

3.5.1 DPPH free radical scavenging activity

DPPH free radical scavenging activity study of the crude total extract and fractions of the seeds of *Eleusine coracana* showed that the 50% ethylacetate: methanol fraction had the least IC_{50} value (29.65 µg/mL), followed by the crude methanol extract with an IC_{50} value of 40.50 µg/mL while the 100% methanol fraction, 50% methanol: distilled water fraction, 20% methanol: distilled water fraction, 100 % ethylacetate fraction, dichloromethane fraction and n-hexane fraction had IC_{50} values of 42.49 µg/mL, 81.82 µg/mL, 116.90 µg/mL, 1825.41 µg/mL, 23364.27 µg/mL and 7.50 x 10^{19}, respectively. See figure 3.1 and table 3.5 below.

3.5.2 Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) of the crude extract and fraction of *Eleusine coracana* was reported as mM ferrous sulphate equivalent/g in figure 3.7 with reference to the FRAP calibration plot in figure 3.6 (y = 0.0063x - 0.0188; R2 = 0.9916).

The FRAP assay (figure 3.2) carried out on the crude extract and fractions of *Eleusine coracana* seeds showed that the 50% ethylacetate/methanol fraction (79.02±1.59 mMFSE/g) had the highest ferric reducing antioxidant power, followed by 100% methanol fraction (71.87±0.32 mMFSE/g), the crude extract (63.25±0.05 mMFSE/g), 50% methanol fraction (57.57±1.45 mMFSE/g), 20% methanol fraction (50.07±0.19 mMFSE/g), DCM fraction (26.48±0.56 mMFSE/g), ethylacetate fraction (23.04±0.10 mMFSE/g) and the n-hexane fraction (17.53±0.37 mMFSE/g) which had the lowest ferric reducing antioxidant power.

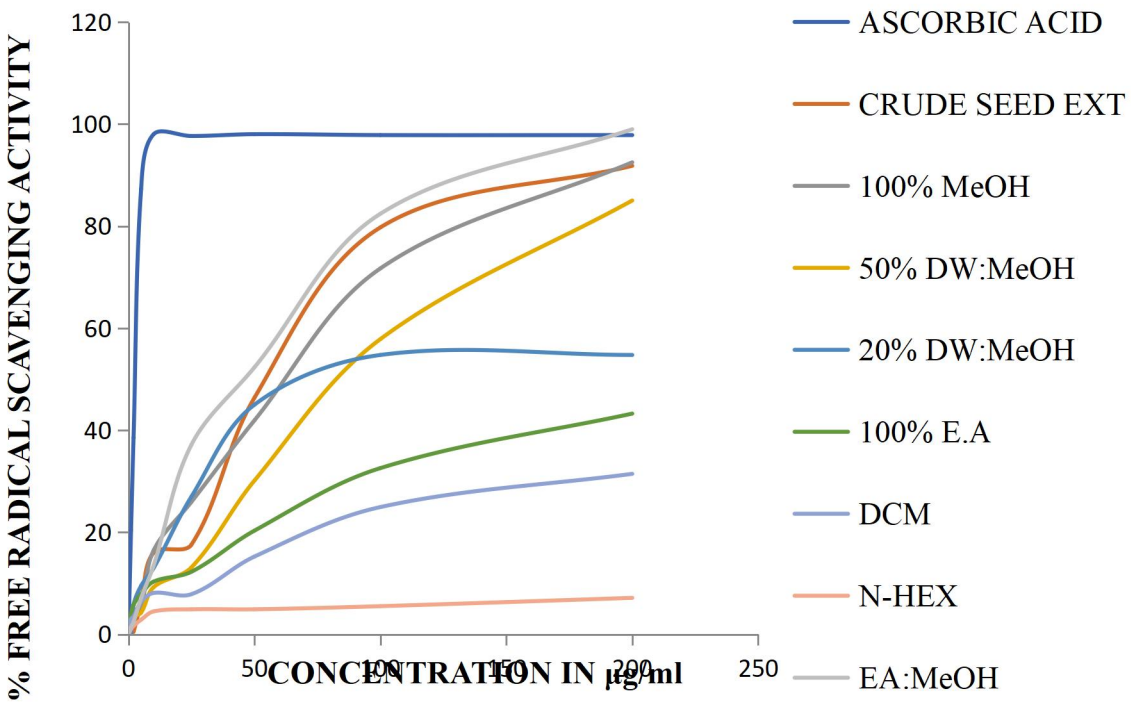


Figure 3.1: DPPH free radical scavenging activity of the crude extract and fractions of *Eleusine coracana* seed. (DW: distilled water, MeOH: methanol, Ea: MeOH ethylacetate: methanol, EA: ethylacetate, DCM: Dichloromethane, n-HEX: n- hexane).

Table 3.5: IC_{50} values for crude methanol extract and fractions of *Eleusine coracana* seed.

Extracts/fractions	**IC_{50} value (µg/ml)**
Ascorbic acid	1.75
Crude methanol extract	40.50
100% methanol	42.49
100% ethylacetate	1825.41
50% methanol/water	81.82
20% water/methanol	116.90
50% methanol: ethylacetate	29.65
Dichloromethane	23364.27
N-hexane	7.50×10^{19}

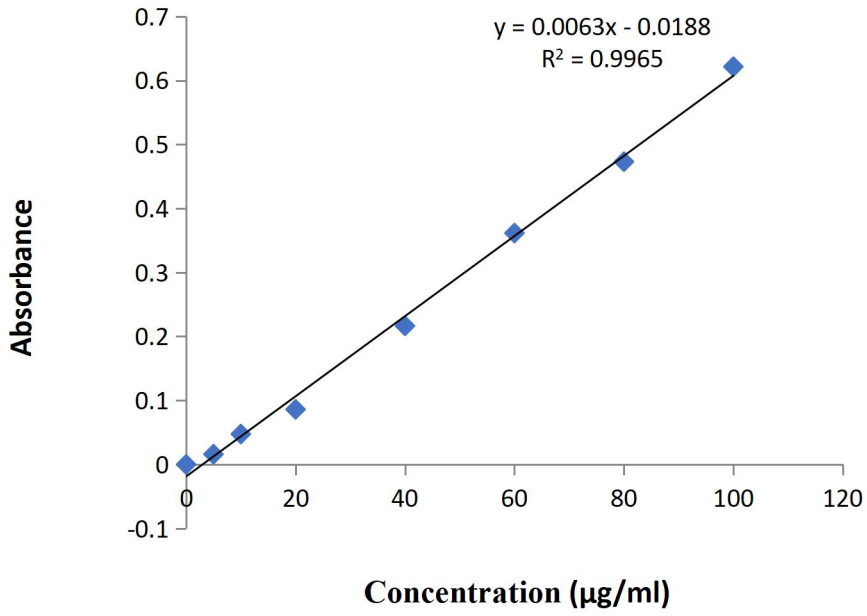


Figure 3.2: Ferric reducing antioxidant power (FRAP) calibration plot

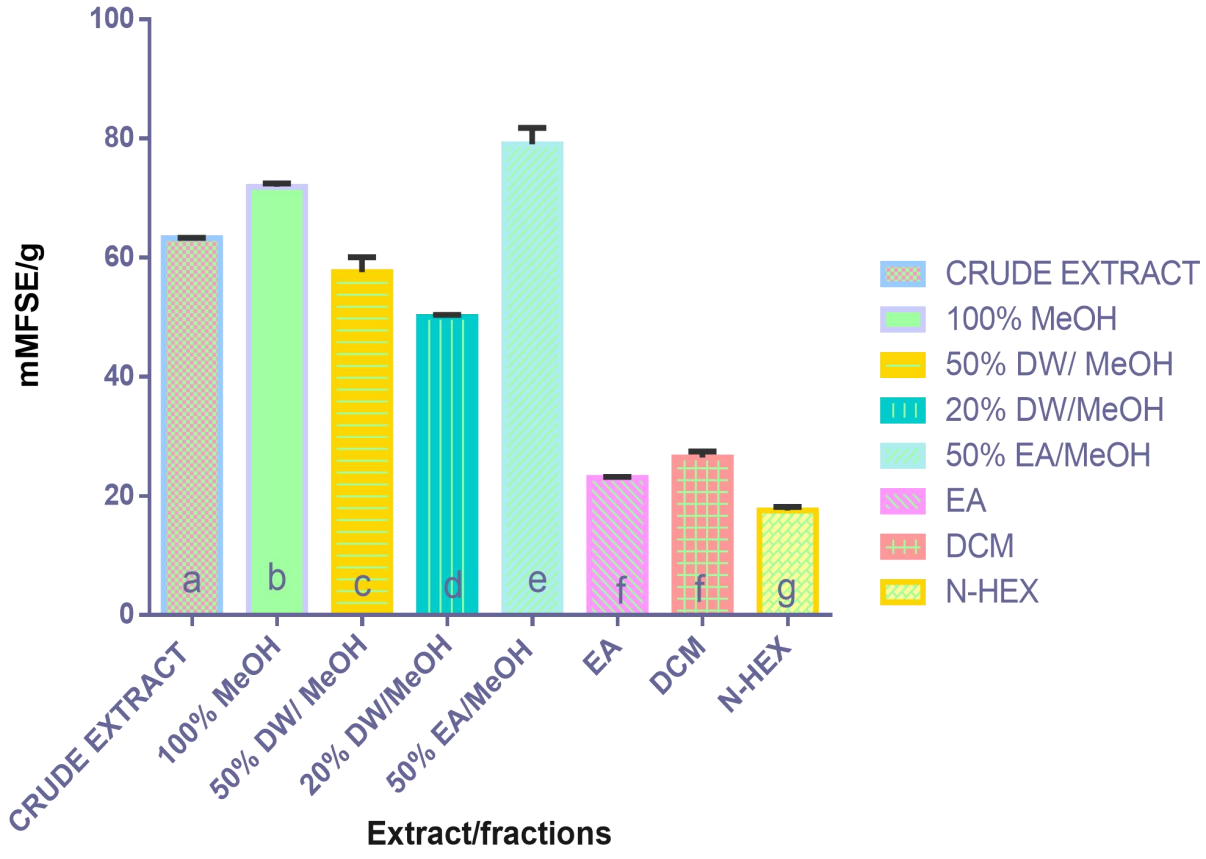


Figure 3.3: Ferric reducing antioxidant power of the crude extract and fractions of *Eleusine coracana* seed (100% MeOH; 50 % DW: MeOH; 20% DW: MeOH; 50% EA:MeOH;100% EA and DCM, n- hex).Where MeOH represents methanol, DW represents distilled water, EA represents ethylacetate, DCM represents dichloromethane and n-hex represents n- hexane.

3.6 Total phenol and flavonoid content

The total phenolic content was reported as mg gallic acid equivalent/g of extract in figure 3.3 with reference to gallic acid standard curve in figure 3.2 ($y = 0.0033x + 0.0103$; $R^2 = 0.9916$) while the total flavonoid content was reported as mg quercetin equivalent/g of extract in figure 3.5 with reference to quercetin standard curve in figure 3.4 ($y = 0.0063x - 0.0188$; $R2 = 0.9965$).

The result obtained from total phenol assay (figure 3.5) showed that 50% ethylacetate/ methanol fraction had the highest total phenol content (132.4 mgGAE/g) followed by the 20% methanol/water fraction (114.2 mgGAE/g), 100 % methanol fraction (110.9 mgGAE/g), crude extract (104.9 mgGAE/g), 50% methanol/water fraction (86.94 mgGAE/g), ethylacetate fraction (46.68 mgGAE/g), dichloromethane (DCM) fraction (29.50 mgGAE/g) then the n- hexane fraction (13.11 mgGAE/g) which had the least total phenol content while the results from the flavonoid assay (figure 3.7) showed that the 20% methanol/water fraction (36.62±1.04 mgQAE/g) had the highest flavonoid content followed by DCM fraction (27.36±0.28 mgQAE/g), n-hexane fraction (25.18±1.29 mgQAE/g), 100% ethylacetate fraction (24.70±1.59 mgQAE/g), 100 % methanol fraction (22.56±1.33 mgQAE/g), 50% methanol/water fraction (21.23±0.15 mgQAE/g), crude extract (12.74±0.75) and the 50% ethylacetate/ methanol fraction (9.27±0.29 mgQAE/g) which had the least flavonoid content.

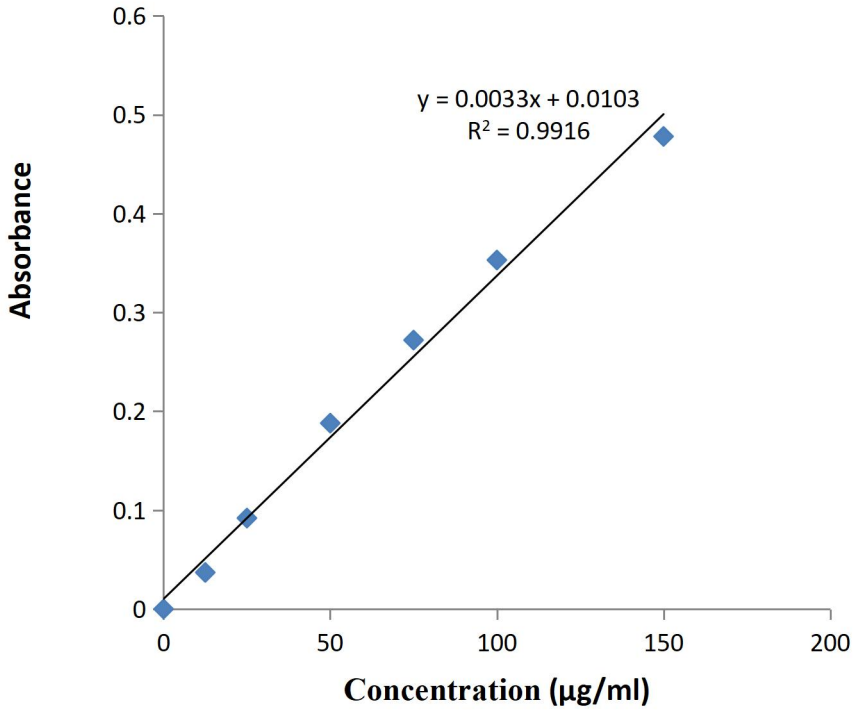


Figure 3.4: Gallic acid calibration plot

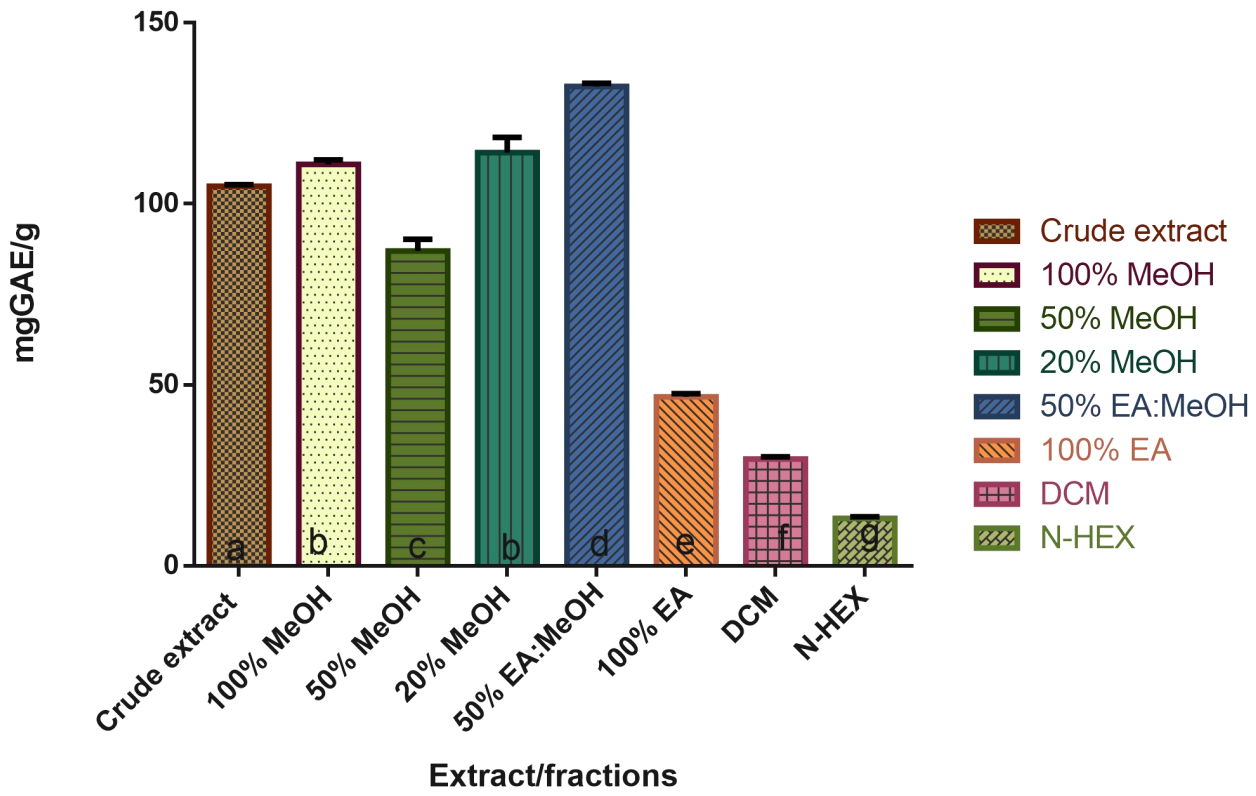


Figure 3.5: Total phenol content of the crude extract and fractions of *Eleusine coracana* seed (100% MeOH; 50 % MeOH/DW; 20% MeOH: DW; 50% EA:MeOH;100% EA, DCM and n-hex).Where MeOH represents methanol, DW represents distilled water, EA represents ethylacetate, DCM represents dichloromethane and n-hex represents n- hexane.

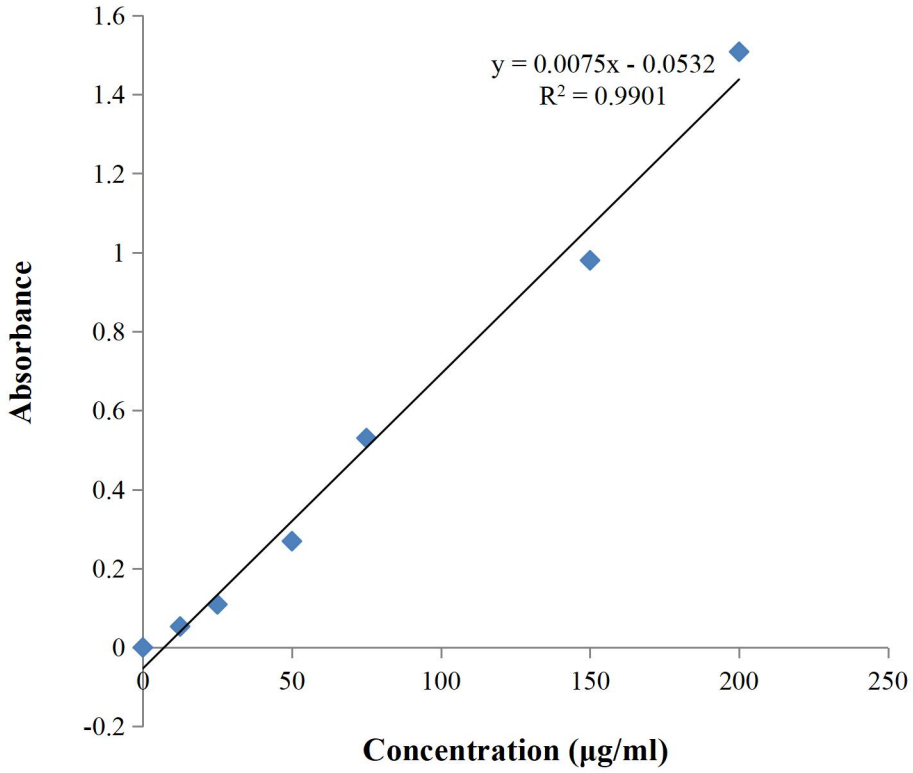


Figure 3.6: Quercetin calibration plot

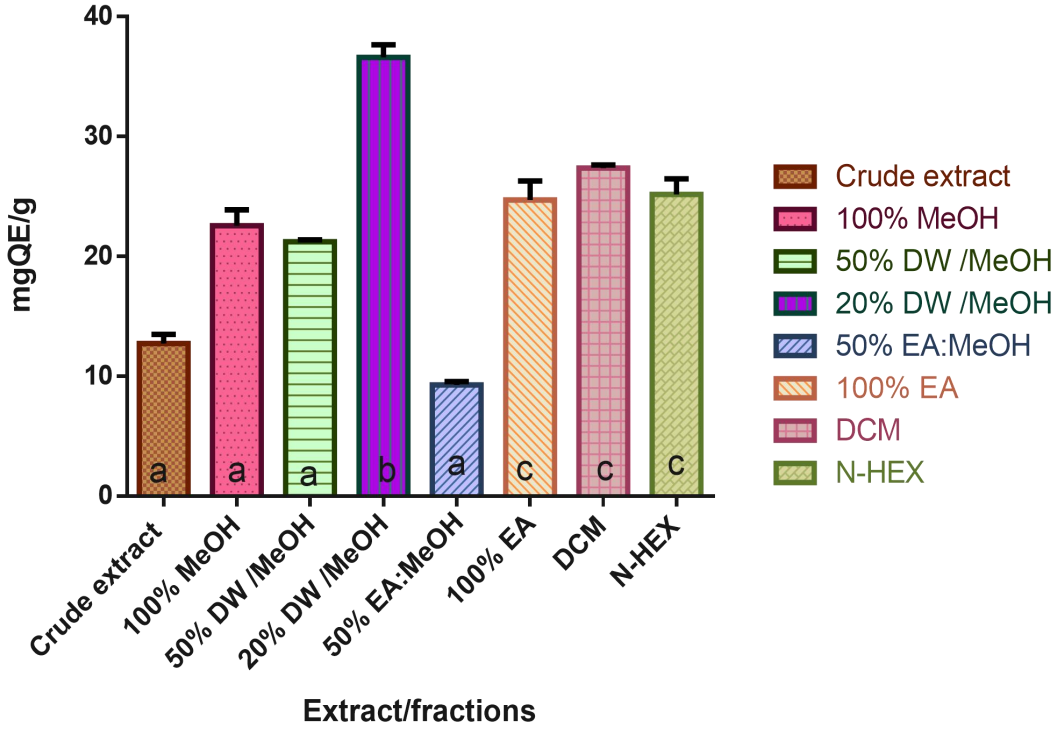


Figure 3.7: Total flavonoid content of the crude extract and fractions of *Eleusine coracana* seed (100% MeOH; 50 % DW/MeOH; 20% DW/MeOH; 50% EA:MeOH; 100% EA and DCM, n- hex). Where MeOH represents methanol, DW represents distilled water, EA represents ethylacetate DCM represents dichloromethane and n-hex represents n- hexane.

3.7 Acute toxicity study

The acute toxicity studies carried out on the crude methanol extract showed that there was no adverse effect or mortality in mice 24 hours and 14 days after oral administration of each dose of the extract up to 5000 mg/kg. This indicates that the LD_{50} (median lethal dose) is greater than 5000 mg/kg.

3.8 Effect of the crude extract and fractions of *Eleusine coracana* on body weights

The result (table 3.6) showed that there was no significant difference between the body weight of control animals and animals treated with crude extract and fractions of *Eleusine coracana* as well as the glibenclamide treated animals through out the period of study.

3.9 Effect of the crude extract and fractions of *Eleusine coracana* on blood glucose level.

The result (table 3.7) shows a significant reduction in blood glucose level of diabetic animals treated with 5 mg/kg/day of glibenclamide, 200 mg/kg/day and 400 mg/kg/day of the crude extract and fractions of *Eleusine coracana* compared to the diabetic untreated animals.

3.10 Effect of the crude extract and fractions of *Eleusine coracana* on renal function of diabetic animals.

Result obtained (table 3.8) showed that there was significant difference between the urea and potassium levels of treated animals (animals treated with crude extract, methanol and n-hexane fraction) and the diabetic untreated animals.

Table 3.6: Effects of crude extract and fractions of *Eleusine coracana* on the body weight of diabetic animals.

		Percentage change in body weight						
Treatments	**Dose (mg/kg)**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Control		11.84 ± 2.59	12.10 ± 1.69	8.85 ± 2.14	8.07 ±2.19	10.47 ± 2.70	11.54 ± 2.66	7.77 ± 2.20
Crude ext	200	9.34 ± 2.67	13.61 ± 2.40	13.78 ± 0.23	13.78 ± 0.23	15.58 ± 0.78	7.27 ± 9.14	11.71 ± 4.70
	400	10.19 ± 2.67	13.89 ±2.53	12.27 ± 2.62	11.57 ± 3.43	18.09 ± 3.07	19.79 ± 2.78	16.35 ± 3.36
MeOH fract	200	7.90 ± 0.82	5.58 ± 0.70	15.71 ± 17.42	17.42 ± 0.42	17.32 ± 0.96	17.32 ± 0.96	12.54 ± 2.24
	400	13.29 ± 1.51	19.19 ± 1.64	16.17 ± 1.32	19.33 ± 0.72	20.36 ± 1.25*	21.99 ± 1.45	15.44 ± 1.01
Me:EA fract	200	5.51 ± 2.18	3.40 ± 2.34	9.74 ± 1.57	11.36 ± 1.29	14.50 ± 1.35	14.86 ± 1.05	13.36 ± 2.29
	400	13.48 ± 2.44	13.17 ± 5.44	12.37 ± 5.71	13.28 ± 6.27	15.84 ± 7.52	11.69 ± 5.98	8.57 ± 4.28
EA fract	200	7.89 ± 1.27	2.96 ± 2.40	11.94 ± 1.90	9.48 ± 2.27	14.25 ± 1.13	15.82 ± 2.09	12.25 ± 2.57
	400	11.20 ± 8.34	14.34 ± 4.81	14.33 ± 4.91	17.28 ± 5.30	21.26 ± 5.05	20.21 ± 5.49	14.82 ± 5.57
n-HEX fract	200	0.99 ± 1.42	4.88 ± 0.59	2.53 ± 0.49	1.84 ± 0.67	2.44 ± 1.51	0.06 ± 1.56	-1.15 ± 2.24
	400	-0.16 ± 1.87*	-1.55 ± 4.33	-2.09 ± 3.20	-4.53 ± 8.42	3.55 ± 5.90*	-3.99 ± 9.54	1.15 ± 9.97
Glibenclamide	5	9.43 ± 4.01	12.01 ± 1.43	10.28 ± 2.35	7.73 ± 4.06	15.61 ± 1.97	9.97 ± 3.12	8.85 ± 2.18

n = 4

*:P< 0.05, - = percentage increase in weight

(Control: distilled water, Crude: crude extract, MeOH: methanol, Me: EA: methanol: ethylacetate, EA: ethylacetate, n-HEX: n- hexane, fract: fraction).

Table 3.7: Effect of the crude extract and fractions of *Eleusine coracana* seeds on blood glucose level of diabetic rats in mg/dL

		Mean blood glucose level (mg/dL)					
Treatments	**Dose (mg/kg)**	**0 hr**	**1 hr**	**2 hrs**	**4 hrs**	**8 hrs**	**Day 7**
Control		488.00 ± 73.70	512.30 ± 69.09	511.70 ± 73.84	397.50 ± 22.40	521.00± 79.00	600.00 ± 0.00
Crude ext	**200**	357.80 ± 64.90	322.20 ± 10.68**	314.60 ±16.39**	323.30 ± 19.65	226.40 ± 39.79**	156.50 ± 24.50**
	400	293.00 ± 19.33	341.80 ± 14.44**	308.50 ± 15.74**	301.00 ± 14.39	249.80 ± 11.52**	288.50 ± 49.75**
MeOH fract	**200**	313.80 ± 24.80	292.80 ± 23.66**	315.80 ± 10.99**	292.00 ± 37.06	215.30 ± 8.39**	118.30 ± 35.99**
	400	285.00 ± 18.94	358.30 ± 16.54*	337.00 ± 3.85**	301.00 ± 14.39	251.80 ± 15.16**	420.50 ± 90.32**
Me:EA fract	**200**	245.30 ± 17.10	332.80 ± 16.54**	329.30 ± 23.43**	213.30 ± 24.15*	231.50 ± 11.08**	166.00 ± 24.79**
	400	343.80 ± 87.13	370.50 ± 37.92*	359.30 ±3 7.27**	281.50 ± 44.93	222.50 ± 38.89**	184.50 ± 23.95**
EA fract	**200**	334.30 ± 34.76	368.50 ± 25.72*	339.00 ± 22.61**	267.30 ± 12.89**	193.50 ± 33.48**	240.30 ± 12.03**
	400	343.80 ± 12.15	384.30 ± 31.13	355.00 ± 10.70**	287.50 ± 21.74	355.80 ± 18.02	370.80 ± 52.88*
n-HEX fract	**200**	324.00 ± 13.79	321.30 ± 16.25**	332.50 ± 20.63**	298.00 ± 12.85	159.30 ± 20.16**	227.80 ± 49.65**
	400	413.80 ± 69.20	368.50 ± 61.02*	406.50 ± 33.58	281.50 ± 44.93	251.70 ± 99.74**	342.30 ± 35.96**
Glibenclamide	**5**	230.50 ± 32.66	337.80 ± 9.013**	295.00 ± 31.00**	281.50 ± 44.93	228.30 ± 63.77**	257.30 ± 19.60**

n = 4

*:P< 0.05, **: P<0.01, *** represents P<0.001

(Control: distilled water, Crude ext: crude extract, MeOH: methanol, Me: EA: methanol:ethylacetate, EA: ethylacetate, n-HEX: n- hexane, fract: fraction).

Table 3.8: Effect of the crude extract and fractions of *Eleusine coracana* seeds on renal function parameters

Treatments	Doses (mg/kg)	Urea (mg/dL)	Creatinine (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Control		47.40±3.33	0.77±0.09	128.7±0.67	7.05±0.35	100.00±1.00
Crude ext	**200**		0.75±0.05	139.50±0.50**	5.85±0.35	104.5±1.50
	400	51.00±6.47	0.75±0.05	131.80±2.10	5.00±0.49**	99.00±1.53
MeOH fract	**200**	57.50±3.88	0.68±0.05	138.50±1.84**	7.10±0.70	103.50±2.50
	400	48.00±2.89	0.73±0.03	131.30±0.88	5.87±0.17	97.50±0.50
Me:EA fract	**200**	58.50±2.90	0.78±0.03	137.30±1.25**	6.13±0.13	101.00±0.01
	400	53.75±2.50	0.63±0.08	136.00±1.35*	6.60±0.35	103.00±1.00
EA fract	**200**	59.00±6.56	0.67±0.07	139.70±1.20**	6.95±0.45	101.00±1.00
	400	43.25±4.13	0.63±0.05	138.50±1.44**	5.40±0.31*	99.00±0.58
n-HEX fract	**200**	60.75±6.42	0.65±0.06	139.50±1.56***	5.37±0.39	102.50±2.50
	400		0.77±0.03	137.70±1.33**	6.75±0.15	100.50±0.88
Glibenclamide	**5**	46.00±4.37	0.73±0.03	133.7±2.33	5.90±0.34	99.00±1.00

n = 4

*:P<0.05, **: P<0.01, *** represents P<0.001

(Control: distilled water, Crude ext: crude extract, MeOH: methanol, Me:EA: methanol: ethylacetate, EA: ethylacetate, n-HEX: n- hexane, fract: fraction).

3.11 Effect of the crude extract and fractions of *Eleusine coracana* seeds on the liver function of diabetic animals.

Result obtained (table 3.9) showed that there was no significant difference between the total CO_2, ALP (alkaline phosphatase), ALT (alanine aminotransferase), ASP (Aspartate aminotransferase) and TP (total protein) levels of treated animals (animals treated with crude extract, methanol and n-hexane fraction) and the diabetic untreated animals.

3.12 Effect of the crude extract and fractions of *Eleusine coracana* on serum proteins of diabetic animals.

The result (table 3.10) showed that there was no significant difference between the level of proteins in the serum of treated diabetic animals (5 mg/kg bw dose of glibenclamide, 200 and 400 mg/kg bw dose of the crude extract and fractions of *Eleusine coracana*) and control diabetic untreated animals.

3.13 Effect of crude extract and fractions of *Eleusine coracana* seeds on lipid level of diabetic animals.

The result in table 3.11 revealed that there was significant difference between the levels of total cholesterol (TC) and triglyceride levels of diabetic animals treated with different doses of the extract and fractions of *Eleusine coracana* and the untreated diabetic animals.

Table 3.9: Effect of the crude extract and fractions of *Eleusine coracana* seed on liver function.

Treatments	Doses (mg/kg)	TCO_2 (mmol/L)	ALT (U/L)	TP (mg/dL)
Control		19.40 ± 1.03	111.00 ± 6.81	5.94 ± 0.17
Crude ext	**200**	22.00 ± 2.00	116.00 ± 20.00	6.15 ± 0.45
	400	18.25 ± 0.63	138.50 ± 1.50	5.63 ± 0.09
MeOH fract	**200**	20.25 ± 1.32	77.33 ± 15.38	6.18 ± 0.24
	400	21.67 ± 0.88	102.00 ± 8.00	5.87 ± 0.15
Me:EA fract	**200**	22.00 ± 1.35	93.33 ± 15.38	6.15 ± 0.22
	400	20.25 ± 0.48	99.67 ± 13.86	6.28 ± 0.10
EA fract	**200**	23.33 ± 0.67	92.00 ± 6.11	5.83 ± 0.07
	400	23.00 ± 0.75	100.30 ± 12.12	5.95 ± 0.18
n-HEX fract	**200**	22.50 ± 1.19	92.67 ± 12.02	6.22 ± 0.28
	400	19.67 ± 1.20	153.50 ± 21.50	6.00 ± 0.21
Glibenclamide	**5**	18.75 ± 0.63	129.00 ± 37.00	6.23 ± 0.27

n = 4

*:P< 0.05, **: P<0.01, *** represents P<0.001

(Control: distilled water, Crude ext: crude extract, MeOH: methanol, Me: EA: methanol: ethylacetate, EA: ethylacetate, n-HEX: n- hexane, fract:fraction).

Where; TCO_2 = total CO_2, ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase and TP = Total protein.

Table 3.10 Effect of the crude extract and fractions of *Eleusine coracana* seeds on serum proteins.

Treatments	Doses (mg/kg)	ALB (mg/dL)	GLO (mg/dL)	TB (mg/dL)	CB (mg/dL)
Control		3.14 ± 0.15	2.80 ± 0.11	0.38 ± 0.04	0.18 ± 0.04
Crude ext	**200**	3.40 ± 0.30	3.00 ± 0.10	0.55 ± 0.15	0.20 ± 0.10
	400	2.80 ± 0.06	2.83 ± 0.03	0.43 ± 0.03	0.20 ± 0.06
MeOH fract	**200**	3.17 ± 0.08	3.00 ± 0.17	0.40 ± 0.04	0.18 ± 0.05
	400	2.90 ± 0.25	2.83 ± 0.03	0.43 ± 0.03	0.20 ± 0.06
Me:EA fract	**200**	3.15 ± 0.16	3.00 ± 0.12	0.38 ± 0.05	0.18 ± 0.03
	400	3.25 ± 0.06	3.03 ± 0.09	0.33 ± 0.03	0.15 ± 0.03
EA fract	**200**	3.13 ± 0.09	2.70 ± 0.06	0.33 ± 0.07	0.17 ± 0.07
	400	3.08 ± 0.14	2.89 ± 0.08	0.53 ± 0.05	0.17 ± 0.07
n-HEX fract	**200**	3.05 ± 0.12	3.18 ± 0.17	0.38 ± 0.02	0.20 ± 0.04
	400	2.87 ± 0.19	3.13 ± 0.03	0.47 ± 0.09	0.27 ± 0.09
Glibenclamide	**5**	3.30 ± 0.07	2.93 ± 0.20	0.48 ± 0.05	0.18 ± 0.03

n = 4

*:P< 0.05, **: P<0.01, *** represents P<0.001

(Control:distilled water, Crude ext: crude extract, MeOH: methanol, Me: EA: methanol:ethylacetate, EA: ethylacetate, n-HEX: n- hexane, fract: fraction). Where; ALB = Albumin, GLO = Globulin, TB = Total bilirubin and CB = Conjugated bilirubin

(Control: distilled water, Crude ext: crude extract, MeOH: methanol, Me: EA: methanol: ethylacetate, EA: ethylacetate, n-HEX: n- hexane, fract: fraction). Where; HDL = High density lipoprotein, LDL = Low density lipoprotein, TC = Total cholesterol and TGL = Triglyceride.

TREATMENTS (mg/kg)	Dose (mg/kg)	HDL (mg/dL)	LDL (mg/dL)	TC (mg/dL)	TGL (mg/dL)
Control		31.00 ± 3.81	16.50 ± 2.72	75.33 ± 1.76	168.00 ± 26.35
Crude ext	**200**	33.00 ± 0.00		70.50 ± 8.50	49.50 ± 7.50***
	400	31.00 ± 4.08	16.33 ± 2.03	71.33 ± 3.33	80.00 ± 19.86*
MeOH fract	**200**	30.50 ± 4.65	17.65 ± 1.33	64.67 ± 0.67	61.33 ± 8.76***
	400	37.00 ± 2.08	18.50 ± 7.50	70.00 ± 0.01	98.67 ± 25.43
Me:EA fract	**200**	28.25 ± 3.82	12.33 ± 2.73	63.00 ± 1.16*	103.00 ± 24.59**
	400	26.75 ± 3.61	13.67 ± 5.78	69.00 ± 1.73	100.30 ± 10.40
EA fract	**200**	30.33 ± 5.24	19.00 ± 3.00	67.33 ± 3.38	79.00 ± 7.55**
	400	27.25 ± 3.20	14.00 ± 3.78	69.50 ± 2.53	109.30 ± 12.25
n-HEX fract	**200**	33.25 ± 1.25		78.50 ± 1.50	60.33 ± 6.12***
	400	35.33 ± 3.48		81.00 ± 2.00	59.67 ± 7.75**
Glibenclamide	**5**	30.25 ± 3.04	13.50 ± 5.33	68.00 ± 1.76	97.50 ± 22.50*

Table 3.11: Effect of crude extract and fractions of *Eleusine coracana* seeds on lipid profile of diabetic animals.
n = 4

*:P< 0.05, **: P<0.01, *** represents P<0.001

3.14: Effect of crude extract and fractions of *Eleusine coracana* seeds on haematological parameters of diabetic animals.

Tables 3.12a, 3.12b and 3.12c below shows significant difference in haematological parameters of animals treated with different doses of the crude extract and fractions of *Eleusine coracana* as well as animals treated with glibenclamide compared to the control diabetic untreated animals. Haematological parameters which these significant differences were observed are; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and haemoglobin (Hgb).

Table 3.12a: Effect of the crude extract and fractions of *Eleusine coracana* seeds on haematological parameters in diabetic rats.

TREATMENTS	Dose (mg/kg)	WBC X10^8/UL	RBC X 10^8/UL	PLT X10^8/UL	LY X10^8/UL	HCT%	RDW%
Control		8.05 ± 0.65	8.58 ± 0.47	386.30 ± 6.89	3.22 ± 0.98	44.73 ± 3.05	17.72 ± 0.32
Crude ext	**200**	13.30 ± 9.70	7.88 ± 0.97		2.50 ± 1.10	42.40 ± 8.20	18.35 ± 0.95
	400	4.40 ± 1.00	8.52 ± 1.03	259.30 ± 72.92	4.35 ± 0.98	56.30 ± 5.75	18.98 ± 0.93
MeOH fract	**200**	6.40 ± 1.20	8.80 ± 0.16	420.50 ± 9.50	5.48 ± 0.58	47.13 ± 1.15	18.18 ± 0.26
	400	4.25 ± 1.75	6.68 ± 1.89	262.00 ± 70.00	3.98 ± 1.24	44.10 ± 12.47	18.18 ± 0.38
Me:EA fract	**200**	5.13 ± 0.75	6.46 ± 0.52	233.00 ± 47.63	2.65 ± 0.60	37.25 ± 4.05	18.15 ± 0.52
	400	5.77 ± 0.82	7.71 ± 0.39	232.50 ± 10.50	4.43 ± 1.14	47.15 ± 1.40	18.00 ± 0.60
EA fract	**200**	8.10 ± 0.01	7.13 ± 0.55	268.00 ± 9.00	4.83 ± 1.98	39.50 ± 1.20	17.43 ± 0.33
	400	7.45 ± 0.25	9.84 ± 1.24	433.00 ± 63.00	4.13 ± 0.33	60.73 ± 6.83	19.00 ± 0.49
n-HEX fract	**200**	9.95 ± 0.05	7.86 ± 0.31	270.0 ± 77.00	6.27 ± 1.78	47.93 ± 2.00	18.78 ± 0.37
	400	8.90 ± 2.30	7.94 ± 0.03	237.00 ± 58.23	4.43 ± 0.63	46.10 ± 1.57	18.93 ± 0.90
Glibenclamide	**5**	3.37 ± 0.57	7.88 ± 0.60	336.50 ± 74.50	2.43 ± 0.64	50.60 ± 3.30	18.63 ± 0.64

n = 4

*:P< 0.05, **: P<0.01, *** represents P<0.001

(Control: distilled water, Crude ext: crude extract, MeOH: methanol, Me: EA: methanol: ethylacetate, EA: ethylacetate, n-HEX: n- hexane, fract: fraction). Where; WBC = White blood cell, RBC = Red blood cell, PLT = Platelet, LY = Lymphocyte, HCT = Haematocrit and RDW = Red cell distribution width.

Table 3.12b Effect of the crude extract and fractions of *Eleusine coracana* seeds on haematological parameters in diabetic rats.

Treatments	Dose (mg/kg)	PCT%	MPV (fl)	MCV (fl)	MCH (pg)	MCHC (g/dL)	Hgb (g/dL)
Conrtol		0.28 ± 0.30	7.86 ± 0.46	57.48 ± 2.32	18.06 ± 0.27	31.52 ± 1.01	15.48 ± 0.78
Crude ext	**200**	0.32 ± 0.10	7.05 ± 0.25	53.30 ± 3.90	17.60 ± 1.20	33.15 ± 0.15	14.05 ± 2.65
	400	0.26 ± 0.07	7.68 ± 0.39	61.80 ± 129.00	17.20 ± 0.18	28.68 ± 0.28	14.73 ± 1.87
MeOH fract	**200**	0.47 ± 0.10	8.00 ± 0.58	53.58 ± 0.54	17.45 ± 0.05	31.93 ± 0.20	15.08 ± 0.41
	400	0.24 ± 0.10	7.95 ± 0.48	64.68 ± 2.33	18.35 ± 0.51	18.35 ± 0.51	12.95 ± 3.77
Me:EA fract	**200**	0.21 ± 0.05	7.10 ± 0.30	52.73 ± 0.82	17.30 ± 0.10	32.45 ± 0.25	10.98 ± 1.02*
	400	0.37 ± 0.10	8.20 ± 0.38	61.63 ± 3.83	18.30 ± 0.30	30.08 ± 1.87	14.18 ± 0.80
EA fract	**200**	0.20 ± 0.02	7.37 ± 0.62	51.90 ± 1.60	16.75 ± 0.15	32.40 ± 0.75	11.73 ± 1.19
	400	0.45 ± 0.05	8.72 ± 0.22	67.03 ± 5.51*	16.38 ± 1.82	31.52 ± 1.01	15.50 ± 0.24
n-HEX fract	**200**	0.39 ± 0.12	7.45 ± 0.50	60.88 ± 0.45	22.00 ± 0.12***	36.20 ± 0.34***	17.35 ± 0.68
	400	0.17 ± 0.05	6.83 ± 0.30	57.97 ± 1.91	21.67 ± 0.37	37.37 ± 0.72**	17.23 ± 0.30
Glibenclamide	**5**	0.31 ± 0.06	8.33 ± 0.57	64.77 ± 0.90*	19.50 ± 0.31*	30.17 ± 0.64	15.27 ± 0.90

n = 4

*:P< 0.05, **: P<0.01, *** represents P<0.001

(Control: distilled water, Crude ext: crude extract, MeOH: methanol, Me: EA: methanol: ethylacetate, EA: ethylacetate, n-HEX: n- hexane, fract: fraction). Where; PCT = Plateletcrit, MPV = Mean platelet volume, MCV = Mean corpuscular volume, MCH =Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration and Hgb = Haemoglobin.

Table 3.12c

Treatments	Dose (mg/kg)	MO $X10^8$/UL	MO%	LY%	GR%
Control		0.64 ± 0.22	12.54 ± 2.96	57.68 ± 10.36	29.78 ± 7.49
Crude ext	**200**	0.90 ± 0.70	6.55 ± 0.15	61.95 ± 22.95	31.60 ± 23.20
	400	0.78 ± 0.28	9.55 ± 0.77	58.63 ± 6.41	31.83 ± 6.71
MeOH fract	**200**	0.37 ± 0.06	5.68 ± 0.56	81.05 ± 2.74	13.28 ± 2.20
	400	0.45 ± 0.03	8.90 ± 2.15	60.70 ± 14.96	30.40 ± 12.82
Me:EA fract	**200**	1.70 ± 0.52	12.70 ± 4.06	56.28 ± 14.56	31.53 ± 10.70
	400	0.63 ± 0.17	10.38 ± 3.87	65.68 ± 11.51	23.95 ± 7.78
EA fract	**200**	0.63 ± 0.20	7.77 ± 2.17	58.03 ± 23.47	34.27 ± 21.45
	400	0.90 ± 0.30	9.60 ± 1.77	50.15 ± 7.69	40.25 ± 6.06
n-HEX fract	**200**	1.27 ± 0.43	11.80 ± 3.88	67.73 ± 11.49	20.48 ± 7.83
	400	1.35 ± 0.35	15.83 ± 1.00	45.30 ± 6.84	38.87 ± 6.62
Glibenclamide	**5**	0.23 ± 0.09	8.20 ± 3.52	71.23 ± 11.35	20.57 ± 7.84

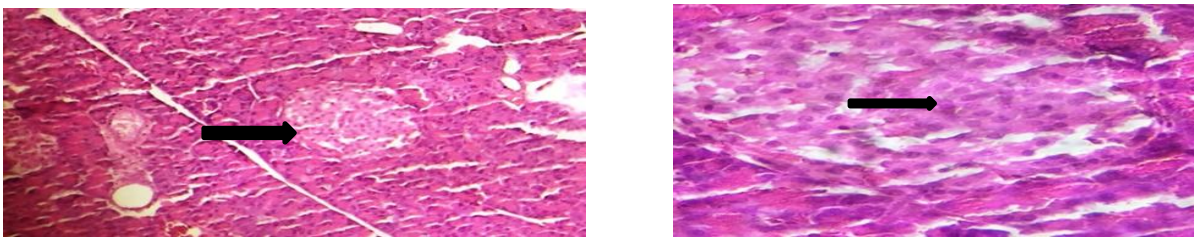
n = 4

*:P < 0.05, **: P<0.01, *** represents P<0.001

(Control: distilled water, Crude ext:crude extract, MeOH: methanol, Me:EA: methanol: ethylacetate, EA:ethylacetate, n-HEX: n- hexane, fract; fraction).Where; MO = Monocyte, LY = Lymphocyte and GR =Granulocyte.

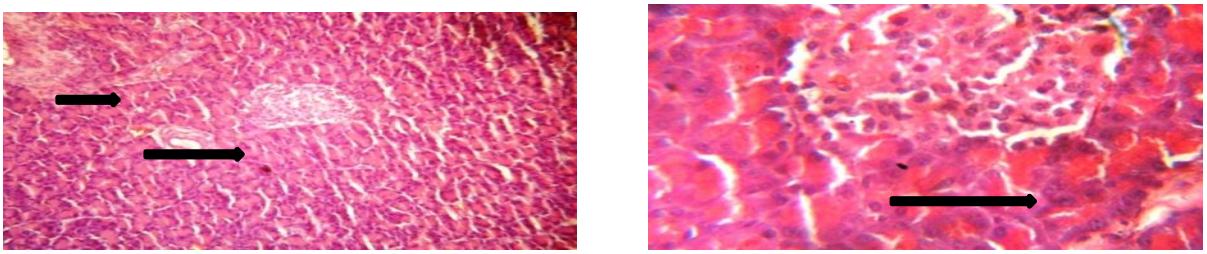
3.15 Effect of extract and fractions of *Eleusine coracana* seed on the pancreas of diabetic animals.

a1 a2



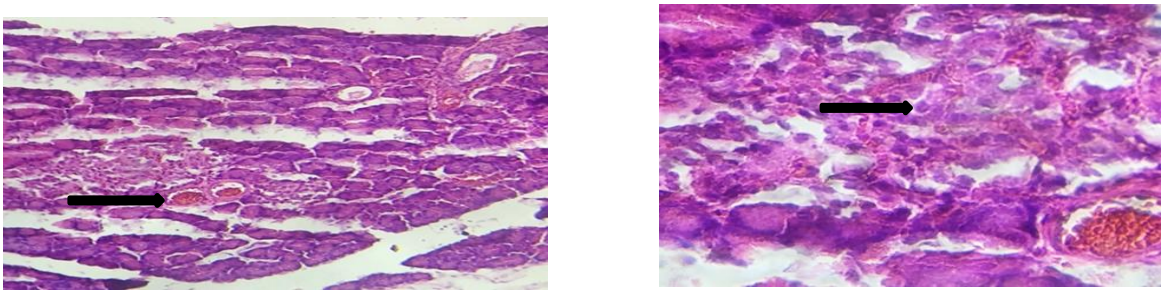
Arrow show normal islet cells in a normal rat without diabetes

b1 b2



Arrows show degenerating islet cells and clogged pancreatic blood vessels

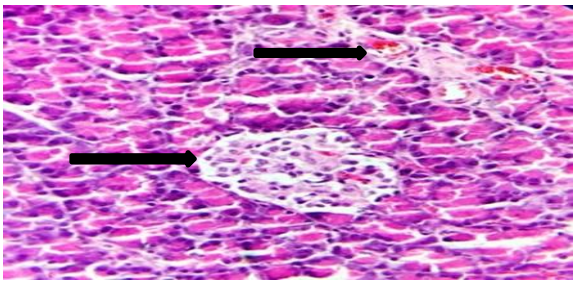
c1 c2



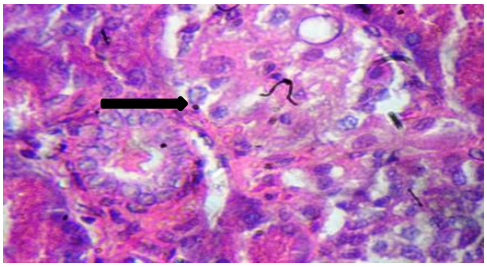
Arrows show clogged blood vessels and regenerating islet cells

Figure 3.8: Photomicrograph of pancreas (a: normal rat pancreas, b: pancreas of diabetic rats without treatment (control), c: pancreas of diabetic rat treated with 5 mg/kg body weight dose glibenclamide.(1: Mag x100, 2: Mag x400)

d1

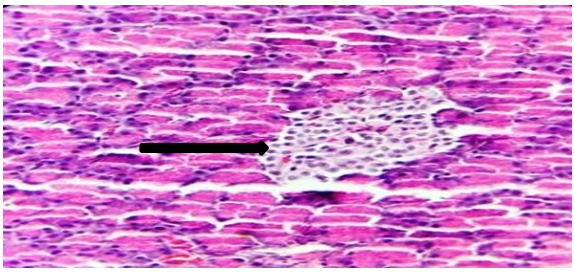


d2

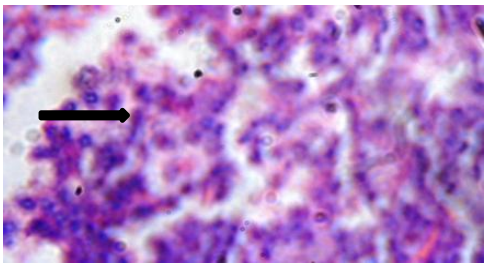


Arrows show distinct acinar cells and clogged vessels (d1) and regenerated islet cells (d2)

e1

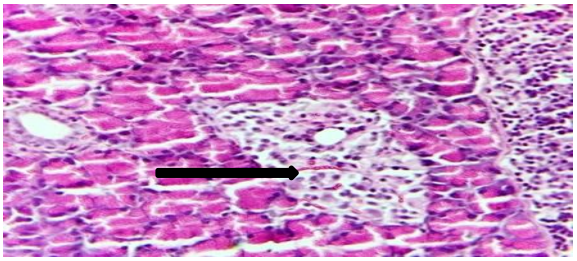


e2

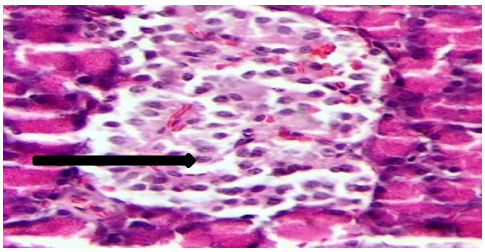


Arrows show regenerated islet cells

f1



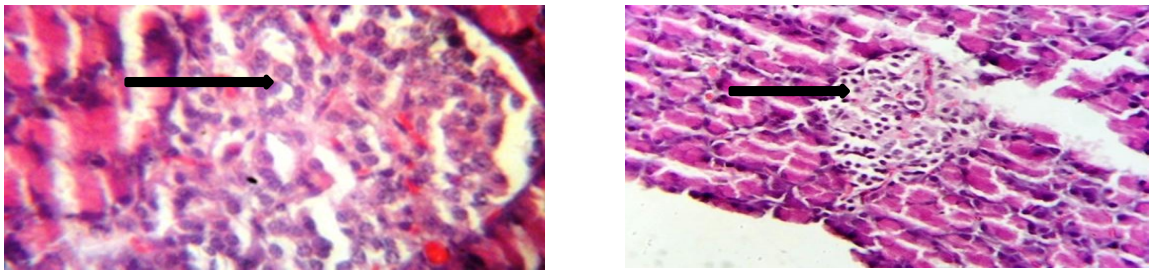
f2



Arrows show distinct acinar cells and regenerated islet cells

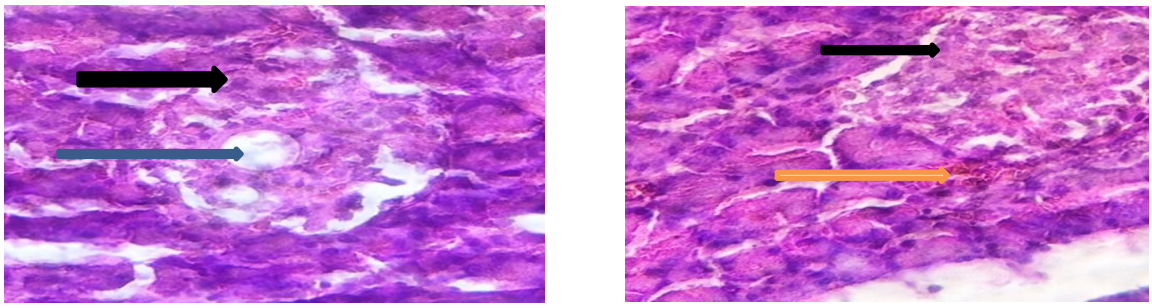
Figure 3.9: Photomicrograph of rat pancreas treated with polar fractions. (d: crude extract treated, e: methanol fraction treated, f: methanol: ethylacetate fraction treated. 1 and 2 : 200 mg and 400 mg body weight dose, respectively).

g1 g2



Arrows show regenerated islet cells

h1 h2



Black arrows show degenerated islet cells, blue arrow shows adipose cells and orange arrow shows clogged blood vessels.

Figure 3.10: Photomicrograph of rat pancreas treated with non- polar fractions. (g: ethylacetate fraction treated, h : n- hexane fraction treated. 1 and 2: 200 mg/kg and 400 mg/kg body weight

3.16 Column chromatography of ethylacetate fraction of *Eleusine coracana* seeds

Figures 3.11 and 3.12 below shows a TLC profile of different fractions eluted from the column chromatography of ethylacetate fraction of *Eleusine coracana*.

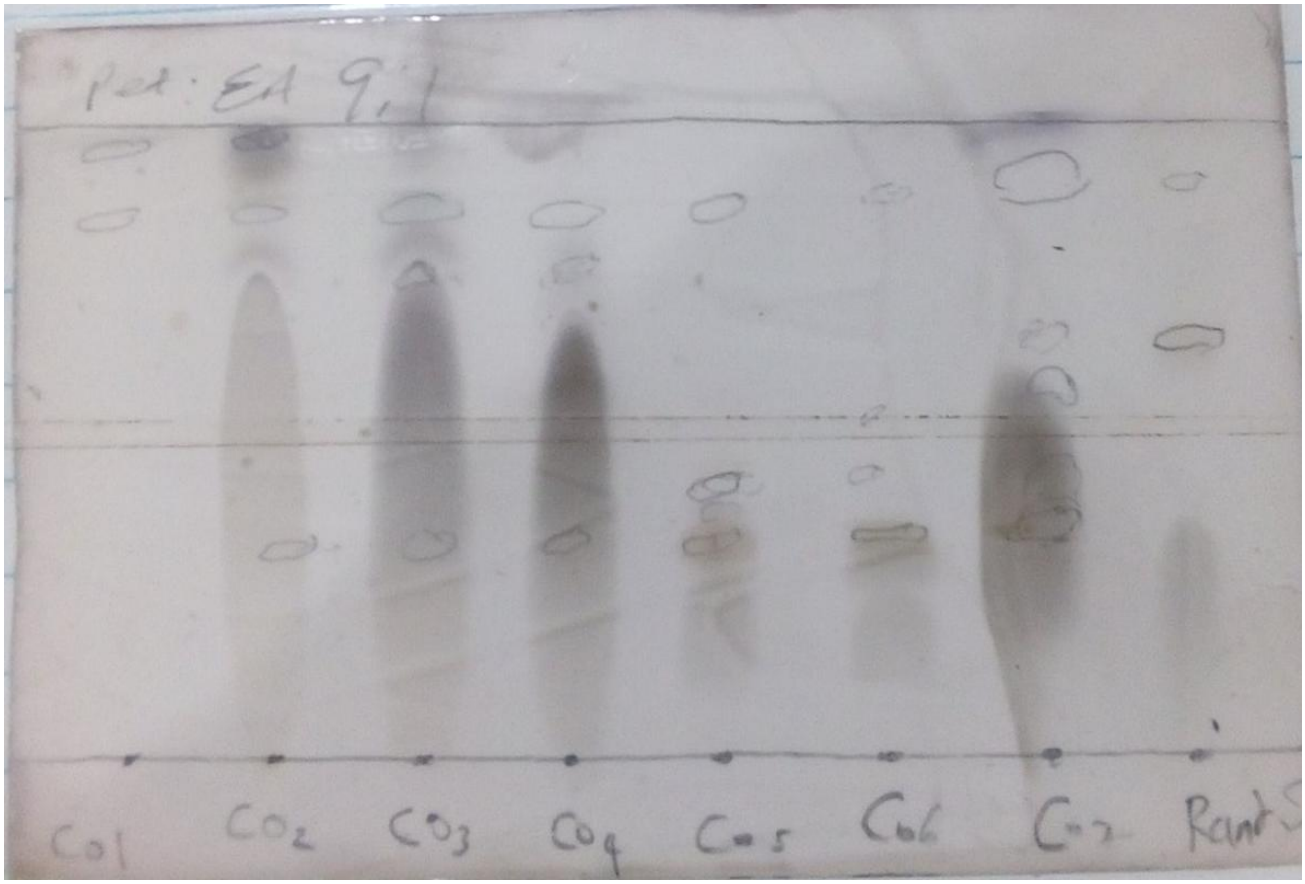
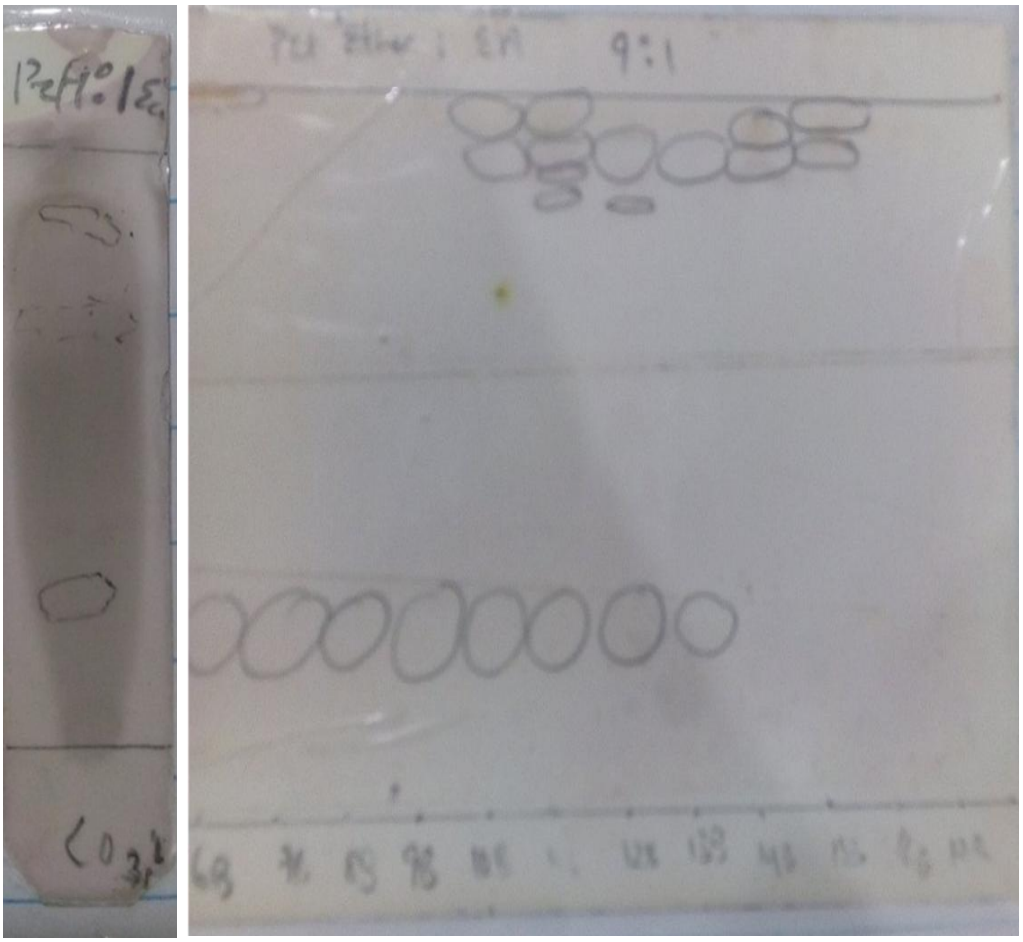


Figure 3.11: TLC chromatogram showing spots of combined fractions after development with petroleum ether: ethylacetate (9:1) and sprayed with vanillin sulphuric acid.



CO3,4 CO2

Figure 3.12: TLC chromatogram showing spots of CO3,4 and some fractions obtained from purification of CO2 after development with peroleumt ether: ethylacetate (9:1) and sprayed with vanillin sulphuric acid.

3.17 Infrared spectroscopy of ethylacetate fraction of *Eleusine coracana* seeds

Figure 3.13 shows the infrared spectrum of the ethylacetate fraction of *Eleusine coracana seeds* which indicates the presence of major functional groups present in the fraction.

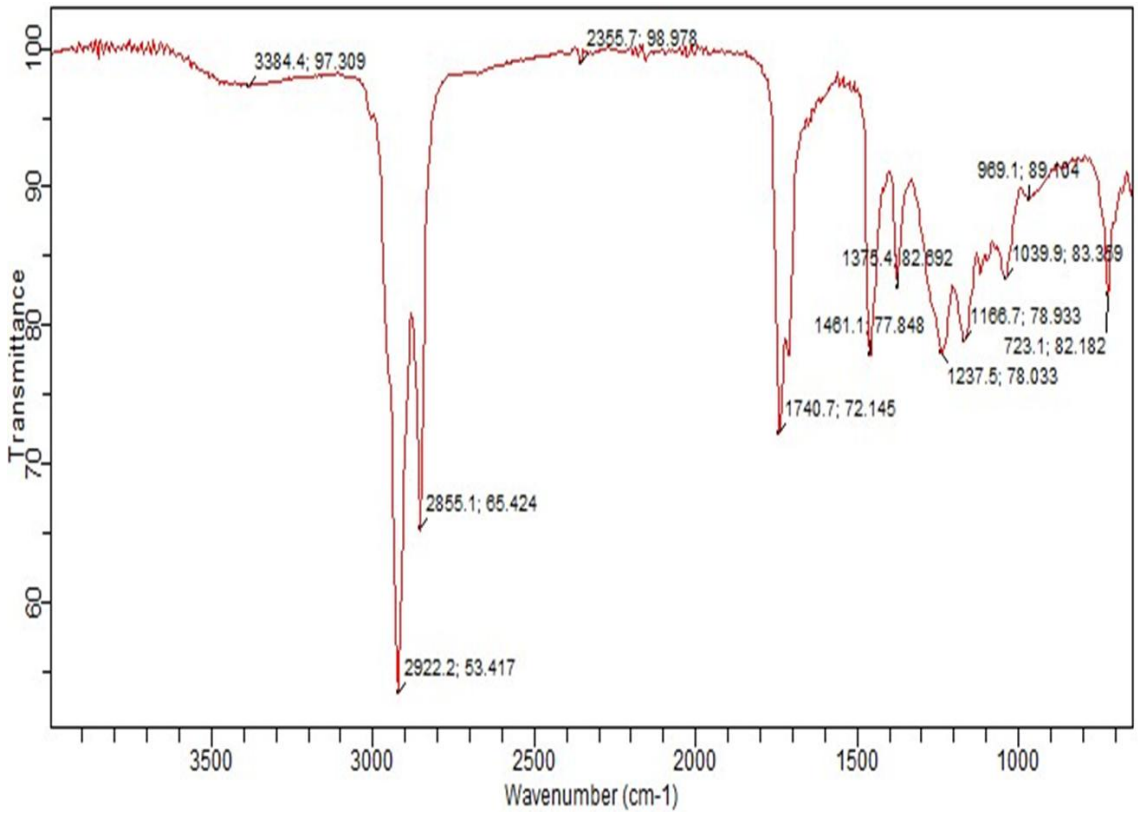


Figure 3.13: Infrared spectrum of ethylacetate fraction of *Eleusine coracana* seeds

3.18 Infrared spectroscopy of n- hexane fraction of *Eleusine coracana* seeds

Figure 3.14 below shows the infrared spectrum of the n-hexane fraction of *Eleusine coracana* seeds indicating the major functional groups present in the fraction.

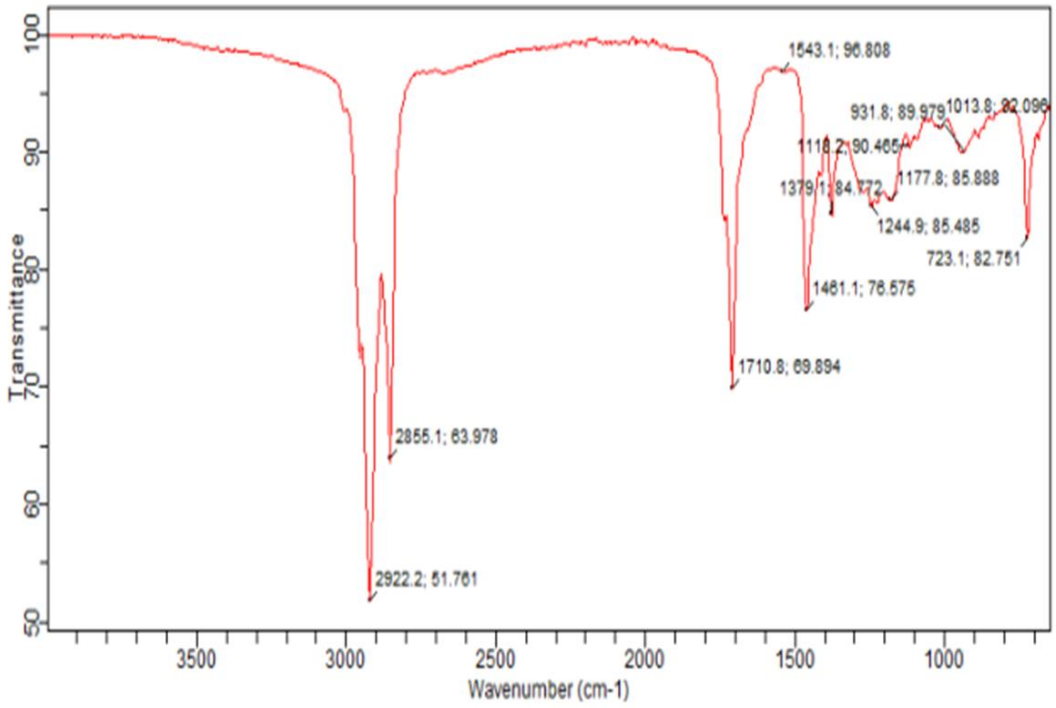


Figure 3.14: Infrared spectrum of n- hex fraction of *Eleusine coracana* seed

3.19 Gas chromatography mass spectroscopy of ethylacetate fraction of *Eleusine coracana* seeds

The figure below (Figure 3.15) shows the GC chromatogram of the ethylacetate fraction of *Eleusine coracana* indicating different peaks of compounds present in the fraction.

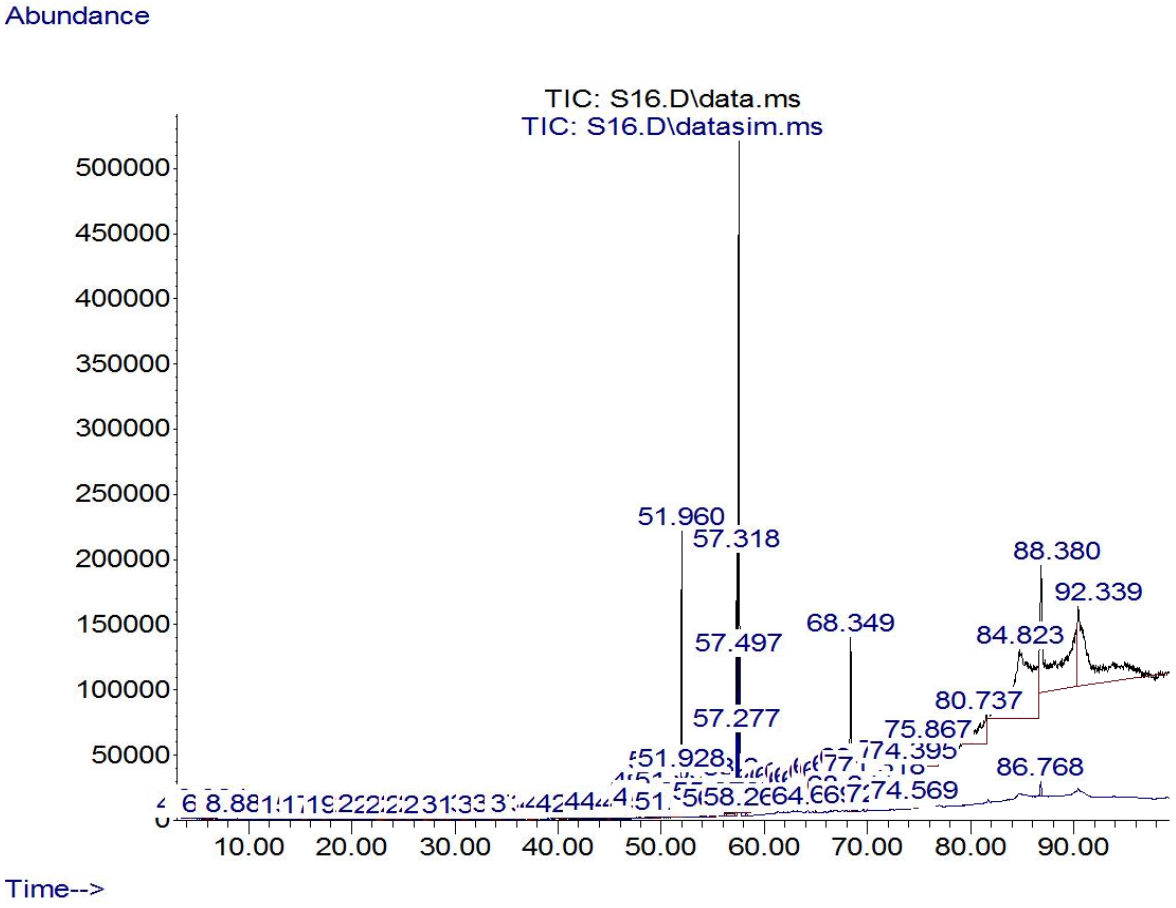


Figure 3.15: GC chromatogram of Ethylacetate fraction of *Eleusine coracana* seed extract

3.20 Gas chromatography mass spectroscopy of ethylacetate fraction of *Eleusine coracana* seeds

The figure below (Figure 3.15) shows the GC chromatogram of the n-hexane fraction of *Eleusine coracana* indicating different peaks of compounds present in the fraction.

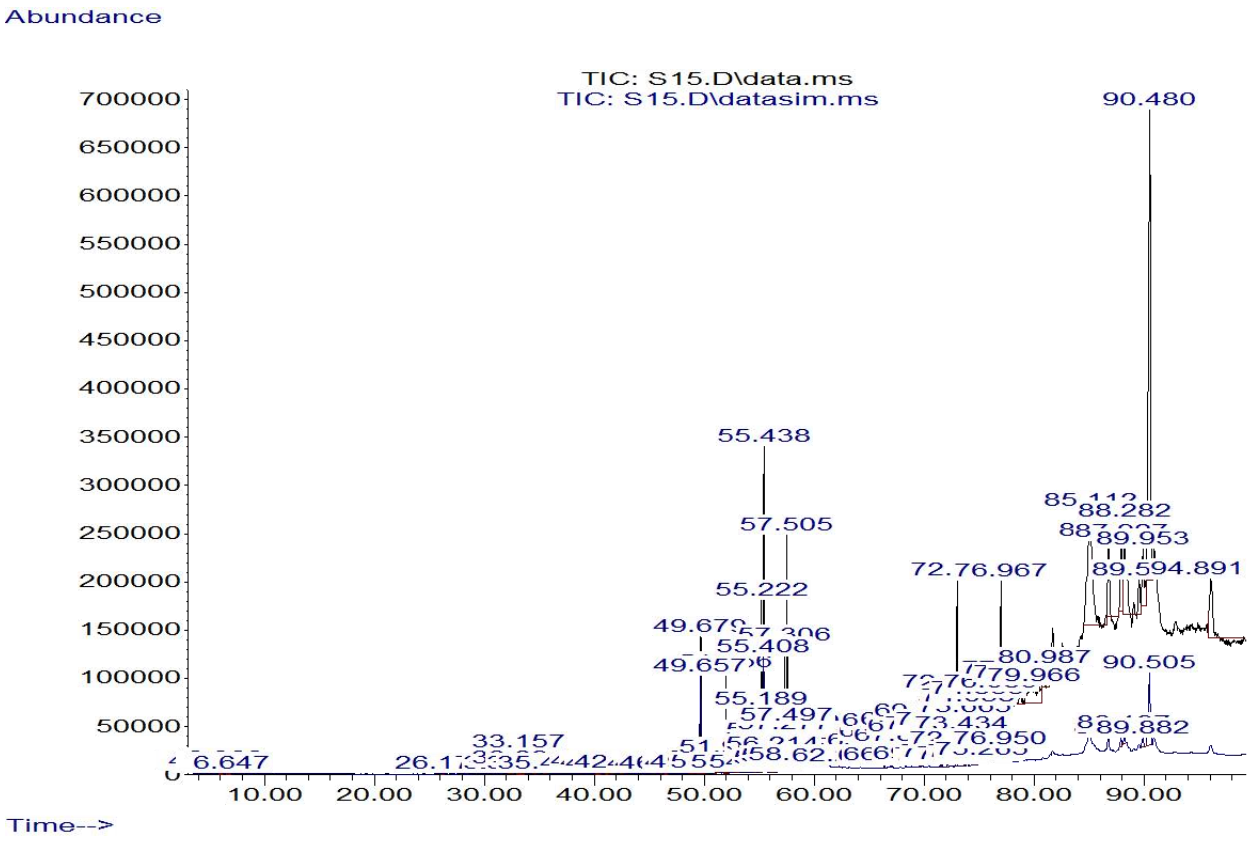


Figure 3.16: GC chromatogram of n-hexane fraction of *Eleusine coracana* seed extract

3.21 Compounds identified from GC-MS of ethylacetate fraction of *Eleusine coracana*

Table 3.13 below shows compounds identified in the ethylacetate fraction of Eleusine coracana seeds. Figure 3.17 shows the molecular structure of some of the compounds identified.

Table 3.13: Compounds Identified from the GC-MS of the ethylacetate fraction of *Eleusine coracana* seed.

S/N	Compound name	RT	Molecular formulae	Molecular Weight (g/mol)
1.	2-Propen-1-amine	4.3802	C_3H_7N	57.09
2.	p-Xylene	6.0243	C_8H_{10}	106.17
3.	Imidodicarbonic diamide	22.737	$C_2H_5N_3O_2$	103.08
4.	Triacetin	27.1002	$C_9H_{14}O_6$	218.20
5.	Cyclobut-1-enylmethanol	37.2503	C_5H_8O	84.12
6.	6-Heptenoic acid, ethyl ester	44.9517	$C_9H_{16}O_2$	156.22
7.	1-[.alpha.-(1-Adamantyl)benzylidene thiosemicarbazide	47.79	$C_{18}H_{23}N_3S$	313.46
8.	Dibutyl phthalate	49.3648	$C_{16}H_{22}O_4$	278.34
9.	Cyclopentaneundecanoic acid, methyl ester	49.6702	$C_{17}H_{32}O_2$	268.44
10.	2-Hydroxy-1-isoindolinone	50.0562	C_8H_7NO	149.15
11.	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	50.9971	$C_{20}H_{30}O_4$	334.45
12.	Paromomycin	51.2148	$C_{23}H_{45}N_5O_{14}$	615.63

13. Phthalic acid, hexyl propyl ester	51.6786	$C_{17}H_{24}O_4$	292.37
14. Hexadecanoic acid, ethyl ester	51.9601	$C_{18}H_{36}O_2$	284.48
15. 1-Undecanol	52.8981	$C_{11}H_{24}O$	172.31
16. Didodecyl phthalate	54.1448	$C_{32}H_{54}O_4$	502.77
17. 5-hexenyl- Oxirane	54.7929	$C_8H_{14}O$	126.19
18. 11-Hexadecyn-1-ol	55.1967	$C_{16}H_{30}O$	238.41
19. 4-Methyl-1,4-heptadiene	55.4311	C8H14	110.20
20. Methyl 10-oxo-8-decenoate	56.2179	$C_{11}H_{18}O_3$	198.26
21. 3-Tetradecyn-1-ol	56.7901	$C_{14}H_{26}O$	210.36
22. Ethyl 9.cis.,11.trans.-octadecadienoate	57.3178	$C_{20}H_{36}O_2$	308.49
23. Ethyl Oleate	57.5219	$C_{20}H_{38}O_2$	310.51
24. Dodecanoic acid, ethyl ester	58.3173	$C_{14}H_{28}O_2$	228.37
25. Sulfurous acid, dodecyl 2-propyl ester	60.1721	$C_{15}H_{32}O_3S$	292.48
26. Cyclopentaneundecanoic acid	61.072	$C_{16}H_{32}O_2$	254.41
27. Isocyanato- cyclohexane	61.3533	$C_7H_{11}NO$	125.17
28. Dodecyl acrylate	62.157	$C_{15}H_{28}O_2$	240.38
29. 1,15-Pentadecanediol	62.8354	$C_{15}H_{32}O_2$	244.41
30. Pentanoic acid, 10-undecenyl ester	63.3377	$C_{16}H_{30}O_2$	254.41
31. Cyclopentaneundecanoic acid	64.2296	$C_{16}H_{30}O_2$	254.41
32. Pentanoic acid, 10-undecenyl ester	64.6797	$C_{16}H_{30}O_2$	254.41
33. 6-Nitroundec-5-ene	65.183	$C_{11}H_{21}NO_2$	199.29
34. 17-Pentatriacontene	65.9166	$C_{35}H_{30}$	490.93
35. E,Z-2,13-Octadecadien-1-ol	66.5748	$C_{18}H_{34}O$	266.46
36. 4-Propionyloxytridecane	67.0232	$C_{16}H_{32}O_2$	256.42
37. trans-Traumatic acid	67.5968	$C_{12}H_{20}O_4$	228.29

38.	Cyclopentaneundecanoic acid	67.9316	$C_{16}H_{30}O_2$	254.41
39.	Bis(2-ethylhexyl) phthalate	68.3494	$C_{24}H_{38}O_4$	390.56
40.	E-2-Octadecadecen-1-ol	68.8318	$C_{18}H_{36}O$	268.48
41.	Dodecyl nonyl ether	69.6064	$C_{21}H_{44}O$	312.58
42.	Cyclohexanone, 2-(2-propenyl)-	70.0344	$C_9H_{14}O$	138.21
43.	Cyclopentaneundecanoic acid	71.3179	$C_{16}H_{30}O_2$	254.41
44.	6-Nitroundec-5-ene	73.3576	$C_{11}H_{21}NO_2$	199.29
45.	4-(4-ethylcyclohexyl)-1-pentyl-Cyclohexene	74.395	$C_{19}H_{34}$	262.47
46.	.alpha.-Thiodeoxyguanosine	75.8673	$C_{10}H_{13}N_5O_3S$	283.31
47.	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	80.7366	$C_{14}H_{44}O_6Si_7$	505.09
48.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	84.8231	$C_{16}H_{50}O_{17}Si_8$	579.25
49.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	88.3803	$C_{16}H_{50}O_7Si_8$	579.25
50.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	92.3388	$C_{16}H_{50}O_{17}Si_8$	579.25

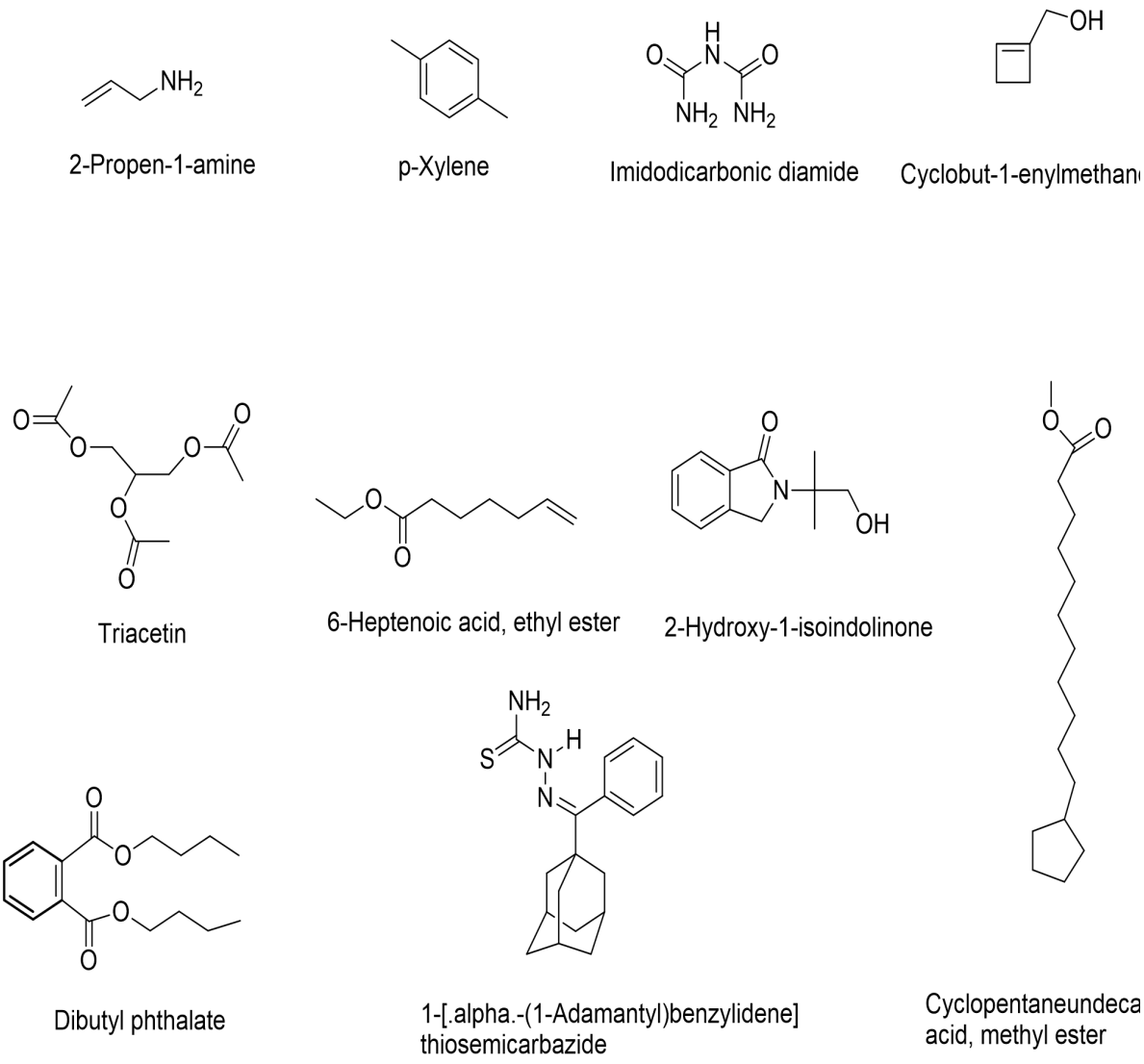
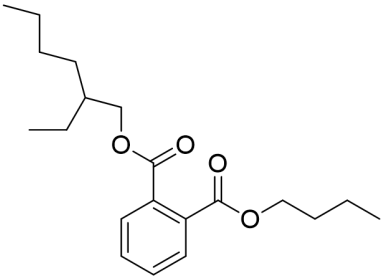
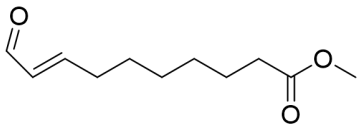


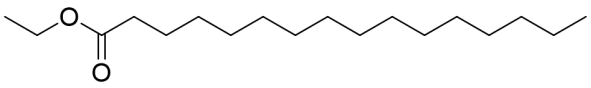
Figure 3.17: Molecular structure of some compounds identified in ethylacetate fraction of *Eleusine coracana*



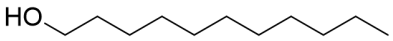
1,2-Benzenedicarboxylic acid,
butyl 2-ethylhexyl ester



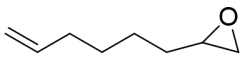
Methyl 10-oxo-8-decenoate



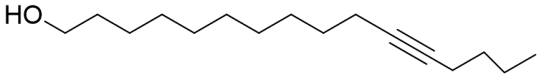
Hexadecanoic acid, ethyl ester



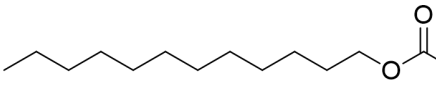
1-Undecanol



Oxirane, 5-hexenyl-

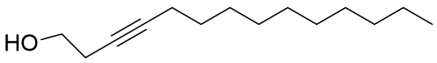


11-Hexadecyn-1-ol

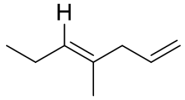


Didodecyl phthalate

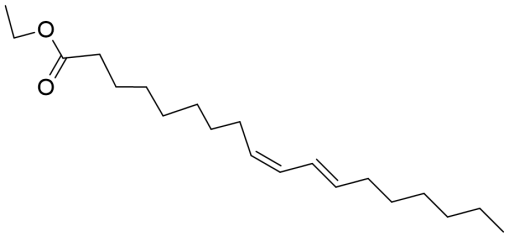
Figure 3.17 cont.



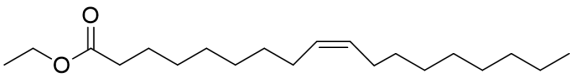
3-Tetradecyn-1-ol



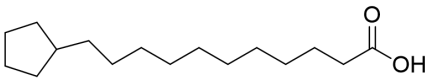
4-Methyl-1,4-heptadiene



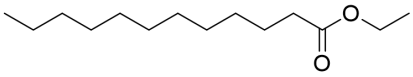
Ethyl 9.cis.,11.trans.-octadecadienoate



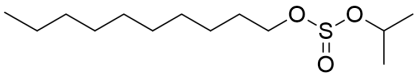
Ethyl Oleate



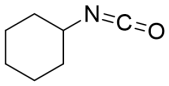
Cyclopentaneundecanoic acid



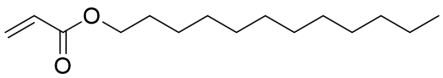
Dodecanoic acid, ethyl ester



Sulfurous acid, dodecyl 2-propyl ester



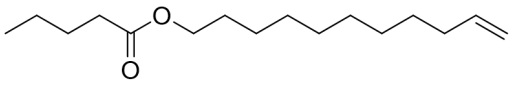
Isocyanato-cyclohexane



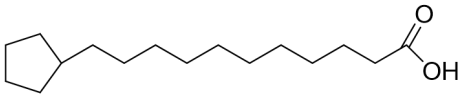
Dodecyl acrylate

Figure 3.17:

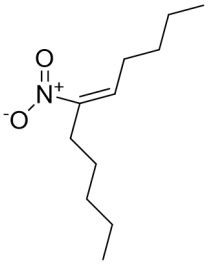
1,15-Pentadecanediol



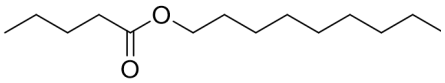
Cyclopentaneundecanoic acid



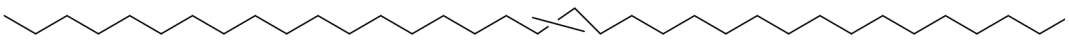
Cyclopentaneundecanoic acid



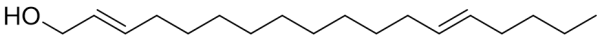
6-Nitroundec-5-ene



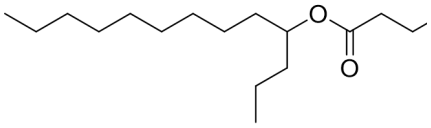
Pentanoic acid, 10-undecenyl ester



17-Pentatriacontene

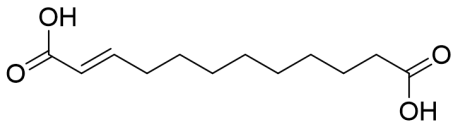


E,Z-2,13-Octadecadien-1-ol

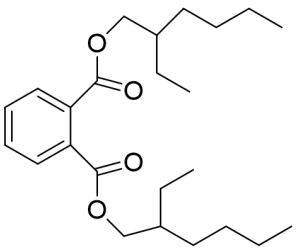


4-Propionyloxytridecane

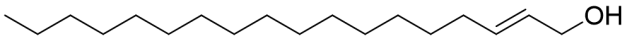
Figure 3.17:



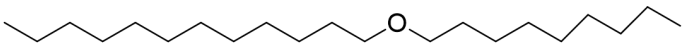
Trans-traumatic acid



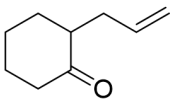
Bis(2-ethylhexyl) phthalate



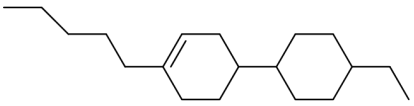
E-2-Octadecadecen-1-ol



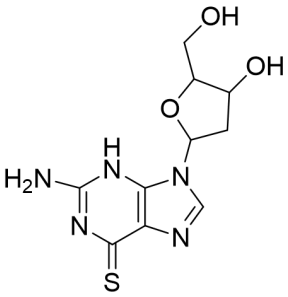
Dodecyl nonyl ether



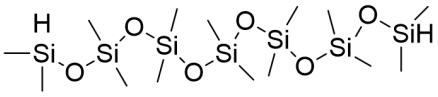
Cyclohexanone, 2-(2-propenyl)-



Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-

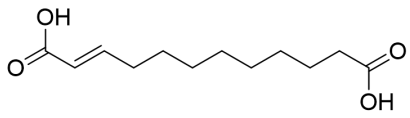


alpha-Thiodeoxyguanosine

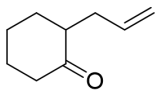


Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-

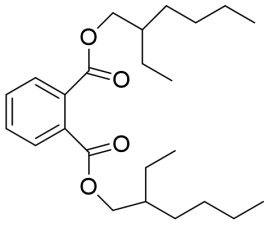
Figure 3.17 cont.



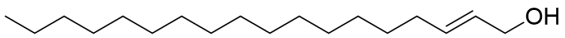
Trans-traumatic acid



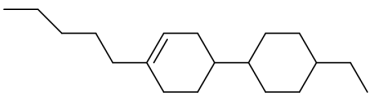
Cyclohexanone, 2-(2-propenyl)-



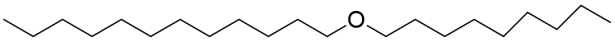
Bis(2-ethylhexyl) phthalate



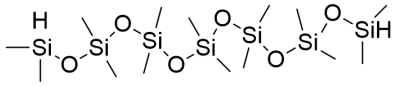
E-2-Octadecadecen-1-ol



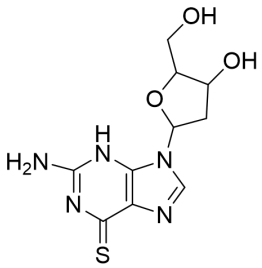
Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-



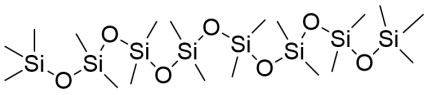
Dodecyl nonyl ether



Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-



alpha-Thiodeoxyguanosine



Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl

Figure 3.17:

3.22 Compounds identified from GC-MS of n-hexane fraction of *Eleusine coracana*

Table 3.14 below shows compounds identified in the n-hexane fraction of *Eleusine coracana* seeds. Figure 3.18 shows the molecular structure of some of the compounds identified in the fraction.

Table 3.14: Compounds Identified from the GC-MS of the n-hexane fraction of *Eleusine coracana* seed.

S/N	Name of compounds	RT	molecular formula	mol.wt (mg/mol)
1.	2-Butene, (Z)-	4.0376	C_4H_8	56.11
2.	n-Hexane	4.4515	C_6H_{14}	86.18
3.	1,1-Difluoro-trans-2,3-dimethyl-cyclopropane	6.0622	$C_5H_8F_2$	106.11
4.	1,1-Difluoro-2-vinylcyclopropane	6.7253	$C_5H_6F_2$	104.10
5.	Trichloroacetic acid, undecyl ester	33.1569	$C_{13}H_{23}Cl_3O_2$	317.68
6.	3-Heptyne	40.3339	C_7H_{12}	96.17
7.	Cetene	41.1885	$C_{16}H_{32}$	224.43
8.	Oxirane, (7-octenyl)-	46.5696	$C_{10}H_{18}O$	154.25
9.	Hexadecanoic acid, methyl ester	49.6789	$C_{17}H_{34}O_2$	270.45
10.	Piperidine, 3-(bromomethyl)-	50.9967	$C_6H_{12}BrN_2$	178.07
11.	Hexadecanoic acid, ethyl ester	51.9555	$C_{18}H_{36}O_2$	284.48
12.	D-erythro-Pentose, 2-deoxy-	53.0158	$C_5H_{10}O_4$	134.13
13.	9,11-Octadecadienoic acid, methyl ester, (E,E)-	55.222	$C_{19}H_{34}O_2$	294.47
14.	trans-13-Octadecenoic acid, methyl ester	55.4385	$C_{19}H_{36}O_2$	296.49
15.	Methyl stearate	56.2755	$C_{19}H_{38}O_2$	298.50
16.	17-Octadecynoic acid	56.9128	$C_{18}H_{32}O_2$	280.45
17.	Ethyl9.cis.,11.trans.-octadecadienoate	57.3062	$C_{20}H_{36}O_2$	308.50
18.	(E)-9-Octadecenoic acid ethyl ester	57.5049	$C_{20}H_{38}O_2$	310.51
19.	1-Eicosanol	59.5944	$C_{20}H_{42}O$	298.55

20.	9,12-Octadecadienoyl chloride, (Z,Z)-	62.1698	$C_{18}H_{31}ClO$	298.89
21.	Sulfurous acid, octadecyl 2-propyl ester	64.2444	$C_{21}H_{44}O_3S$	376.30
22.	Chloroacetic acid, 3-tetradecyl ester	65.4584	$C_{16}H_{31}ClO_2$	290.87
23.	2-Pentadecyn-1-ol	66.5657	$C_{15}H_{28}O$	224.38
24.	7,11-Hexadecadienal	66.8285	$C_{16}H_{28}O$	236.39
25.	Octadecane, 1-(ethenyloxy)-	67.8905	$C_{20}H_{40}O$	296.53
26.	D-Fructose, 1-O-methyl-	68.4214	$C_7H_{14}O_6$	194.18
27.	Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-11(1H)-one, 2,3,6a,9a-tetrahydro-1,3-dihydroxy-4-methoxy-	69.1633	$C_{17}H_{14}$	330.29
28.	1-Cyclohexylnonene	69.7572	$C_{15}H_{28}$	208.38
29.	n-Hexadecanoic acid	70.653	$C_{16}H_{32}O_2$	256.42
30.	Cyclopentaneundecanoic acid	71.617	$C_{16}H_{30}O_2$	254.41
31.	E-11-Tetradecenoic acid	72.2643	$C_{14}H_{26}O_2$	226.36
32.	Decane, 3,8-dimethyl-	72.9926	$C_{12}H_{26}$	170.34
33.	Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-11(1H)-one, 2,3,6a,9a-tetrahydro-1,3-dihydroxy-4-methoxy-	73.4338	$C_{17}H_{14}O_7$	330.29
34.	1-Octadecanesulphonyl chloride	73.6645	$C_{18}H_{37}ClO_2S$	353.00
35.	8-Hexadecenal, 14-methyl-, (Z)-	74.0383	$C_{17}H_{32}O$	252.44
36.	Oleic Acid	74.8328	$C_{18}H_{34}O_2$	282.46
37.	5,5-Dibutylnonane	76.0862	$C_{17}H_{36}$	240.47
38.	Heptacosane, 1-chloro-	76.9669	$C_{27}H_{55}Cl$	415.18
39.	Eicosane	77.7142	$C_{20}H_{42}$	282.55
40.	Phenytoin, 2TMS derivative	78.7824	$C_{21}H_{28}N_2O_2Si_2$	396.63
41.	Benzoic acid, 4-(cyclopentylthio)-3-nitro-, methyl ester	79.9659	$C_{13}H_{15}NO_4S$	281.33
42.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	80.9869	$C_{16}H_{48}O_7Si_8$	577.23

43.	2-Bromopropionic acid, cyclohexyl ester	85.1121	$C_3H_5BrO_2$	152.98
44.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	86.4741	$C_{16}H_{48}O_7Si_8$	577.233
45.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	87.9269	$C_{16}H_{48}O_7Si_8$	577.23
46.	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	88.2821	$C_{15}H_{26}O$	222.37
47.	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	89.5563	$C_{12}H_{36}O_5Si_6$	428.93
48.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	89.9529	$C_{16}H_{48}O_7Si_8$	577.23
49.	Testosterone cypionate	90.4798	$C_{27}H_{40}O_3$	412.60
50.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	94.8909	$C_{16}H_{50}O_7Si_8$	579.25

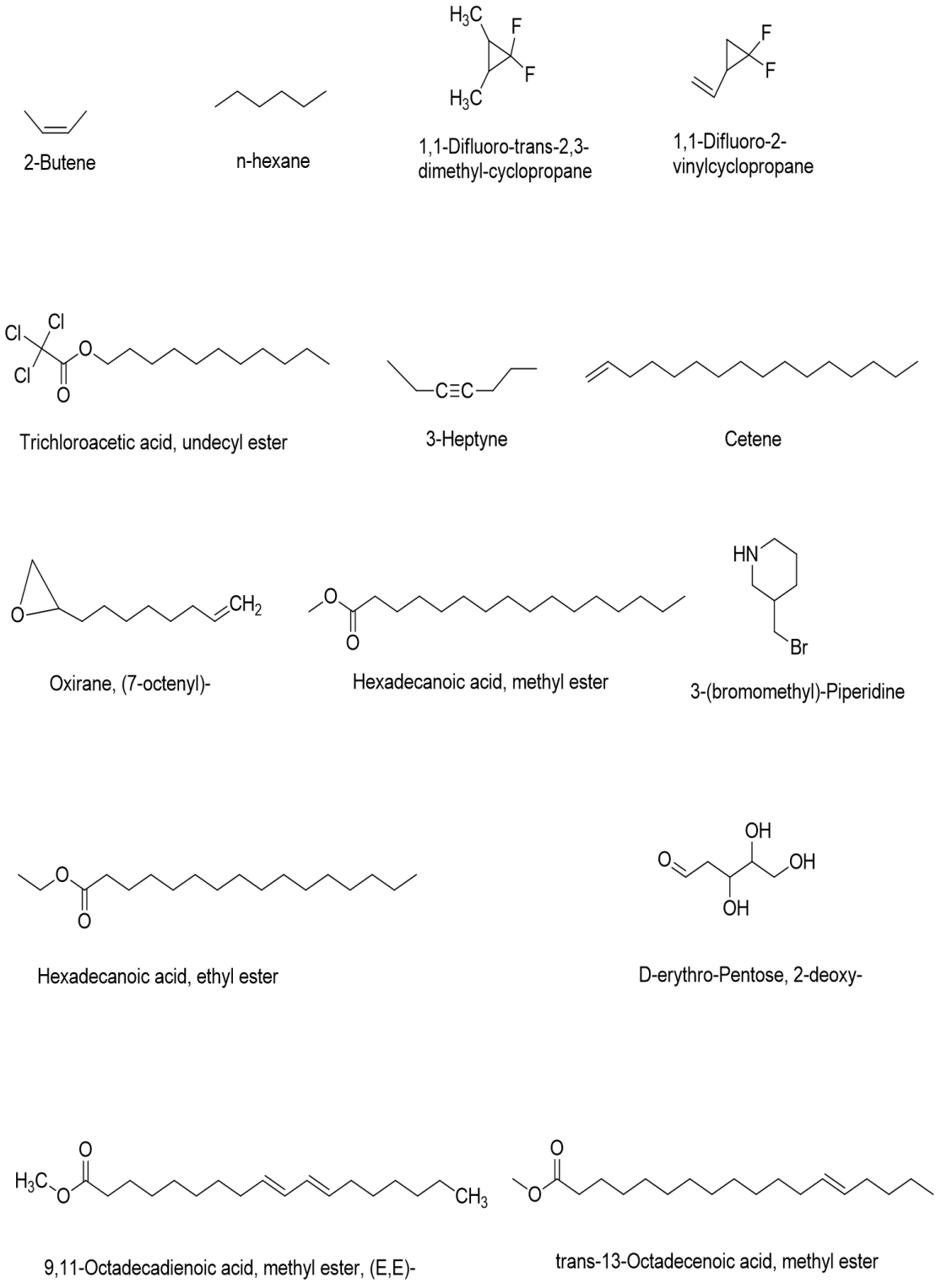
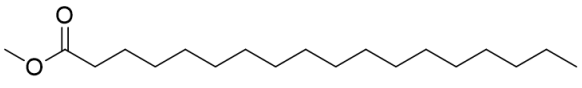
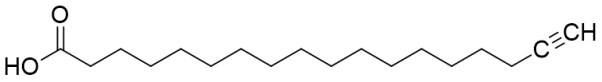


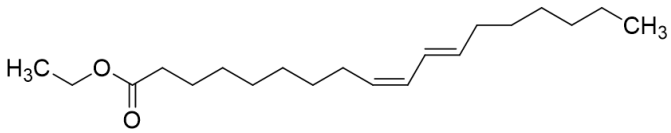
Figure 3.18: Molecular structure of some compounds identified in n-hexane fraction of *Eleusine coracana* seed



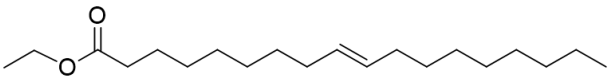
Methyl stearate



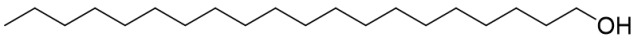
17-Octadecynoic acid



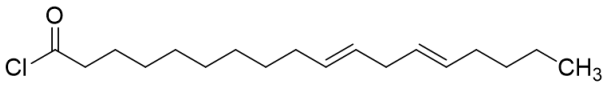
Ethyl 9.cis.,11.trans.-octadecadienoate



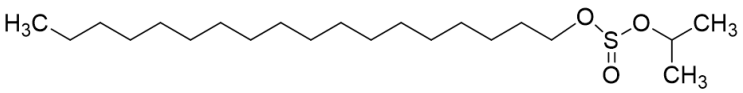
(E)-9-Octadecenoic acid ethyl ester



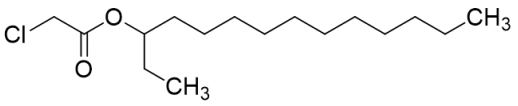
1-Eicosanol



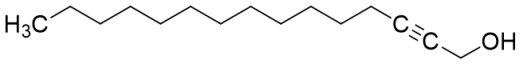
9,12-Octadecadienoyl chloride, (Z,Z)-



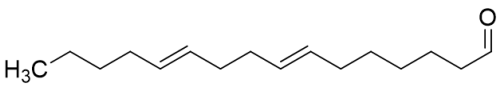
Sulfurous acid, octadecyl 2-propyl ester



Chloroacetic acid, 3-tetradecyl ester

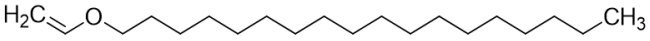


2-Pentadecyn-1-ol

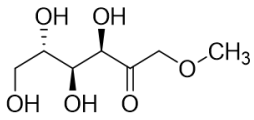


7,11-Hexadecadienal

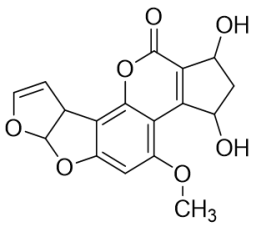
Figure 3.18



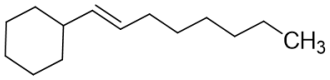
Octadecane, 1-(ethenyloxy)-



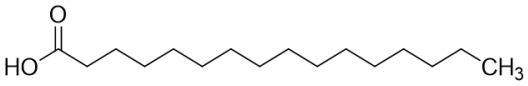
D-Fructose, 1-O-methyl-



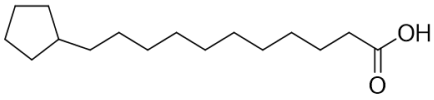
Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-11(1H)-one, 2,3,6a,9a-tetrahydro-1,3-dihydroxy-4-methoxy-



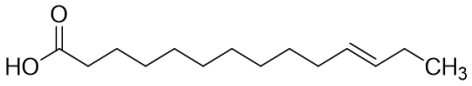
1-Cyclohexylnonene



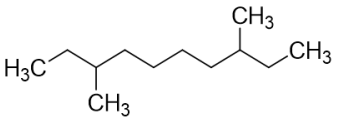
n-Hexadecanoic acid



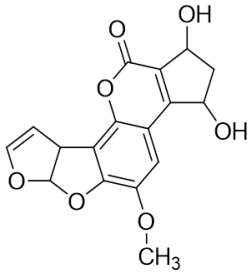
Cyclopentaneundecanoic acid



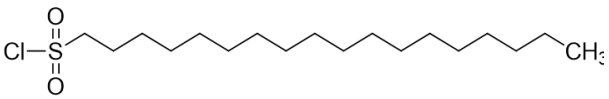
E-11-Tetradecenoic acid



Decane, 3,8-dimethyl-

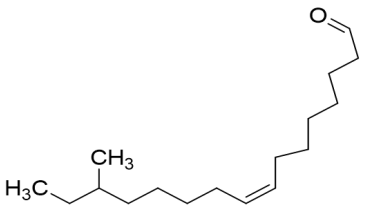


Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-11 (1H)-one, 2,3,6a,9a-tetrahydro-1,3-dihydroxy-4-methoxy-

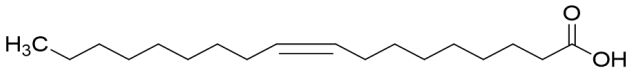


1-Octadecanesulphonyl chloride

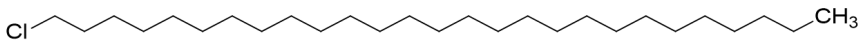
Figure 3.18.



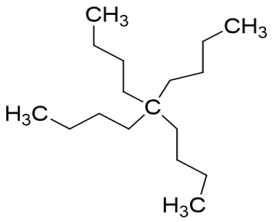
8-Hexadecenal, 14-methyl-, (Z)-



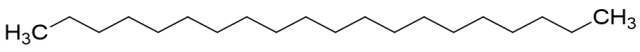
Oleic Acid



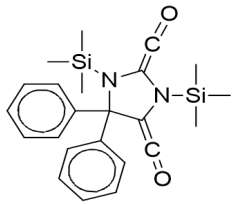
Heptacosane, 1-chloro-



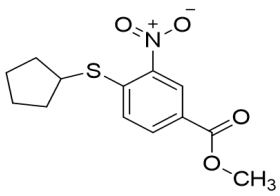
5,5-Dibutylnonane



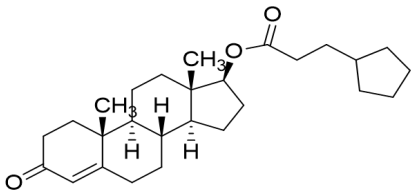
Eicosane



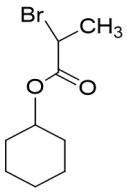
Phenytoin, 2TMS derivative



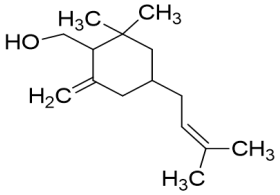
Benzoic acid, 4-(cyclopentylthio)-
3-nitro-, methyl ester



Testosterone cypionate

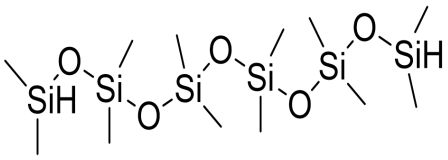


2-Bromopropionic acid, cyclohexyl ester

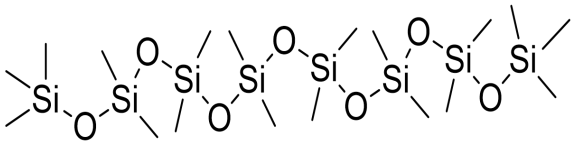


1-Methylene-2b-hydroxymethyl-3,3-dimethyl
-4b-(3-methylbut-2-enyl)-cyclohexane

Figure 3.18



Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl

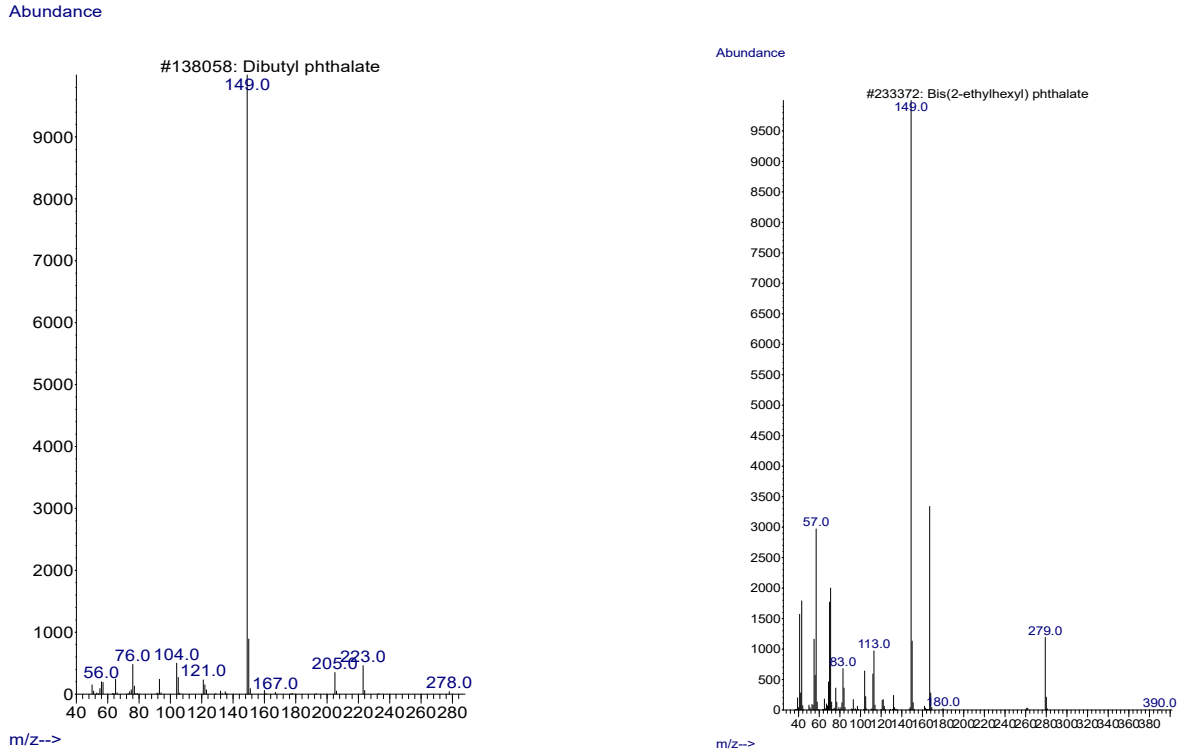


Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl

Figure 3.18

3.23: MS spectrum of compounds identified in the ethylacetate and n- hexane fraction of *Eleusine coracana* seeds

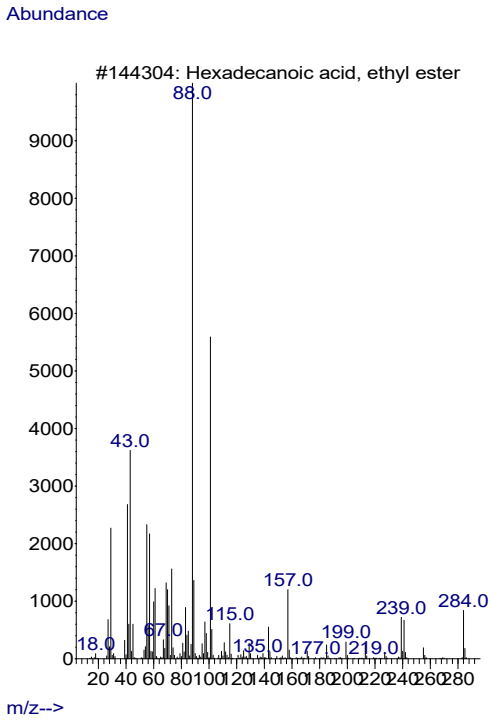
Figure 3.19 and 3.20 shows the MS spectrum of selected compounds present in the ethyl; acetate and n- hexane fraction of *Eleusine coracana seeds* respectively.



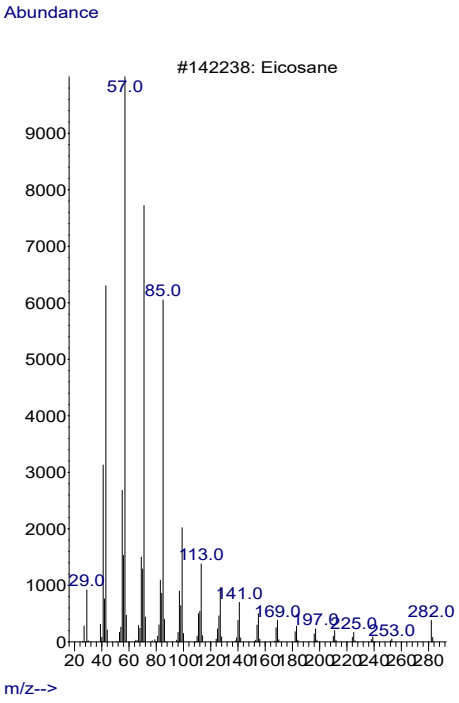
Dibutyl phthalate

Bis(2-ethylhexyl pthallate

Figure 3.19: MS spectrum of selected compounds identified in the ethylacetate fraction of *Eleusine coracana* seed extract



Hexadecanoic acid ethyl ester



Eicosane

Figure 3.20: MS spectrum of selected compounds identified in the N-hex fraction of *Eleusine coracana* seed extract.

CHAPTER FOUR

DISCUSSION

4.1 Proximate analysis

Proximate analysis was carried out on the powdered seeds of *Eleusine coracana* in order to ascertain the moisture content, total ash, acid insoluble ash value, water-soluble ash value, alcohol extractive value, and water/chloroform extractive value. The study revealed (table 3.1) the moisture content of the powdered seed of *Eleusine coracana* to be 0.38 ± 0.14%. This value is far below 6-8% (African Pharmacopoeia, 1986), the maximum permissible range for crude drugs. The implication of this finding is that the powdered seed of *Eleusine coracana* has very low moisture content which is unfavorable for microbial growth and degradation of active constituents in the powdered seed. It also shows that the powdered seeds of *Eleusine coracana* can be stored for a long time hence a long shelf life. Our value (0.38 ± 0.14%) is however not in agreement with that obtained by David *et al*., 2014 who reported the moisture content to be 6.99%. The ash value is an indication of the total amount of inorganic matter/mineral present in the crude powdered seeds. It is obtained after water and organic components have been removed from the sample by ashing leaving the mineral constituents (Bhargava *et al*., 2013). The total ash values usually vary within a wide margin between genuine samples. This may be due to the soil type, geographical location, and cultivation methods (Hannah and Krishnakumari, 2015). The presence of minerals in the plant is important because they may play important roles in the pharmacological effects exhibited by plants. The total ash value of *Eleusine coracana* seed was found to be 2.23 ± 0.13% (table 3.1) which represented the inorganic mineral content of the seed left after the organic component and moisture content has been removed by heating. This value is consistent with the 2.37% ash value of *Eleusine coracana* obtained by David *et al*, 2014. As shown in table 3.1, the acid insoluble ash and water-soluble values are 0.76 ± 0.05%, and 0.01 ± 0.01%, respectively. The

alcohol extractive value (0.21 ± 0.03%) is lower than the water/chloroform extractive value (1.20 ± 0.08%) as shown in table 3.1 which implies that a mixture of water/chloroform will be a better solvent of extraction of phytoconstituents from the powdered sample of *Eleusine coracana.*

4.2 Phytochemical screening

Phytochemical screening carried out on the powdered seed of *Eleusine coracana* revealed the presence of important phytochemicals such as alkaloids, carbohydrates, proteins, reducing sugars, saponins, tannins, phenols, flavonoids, and terpenoids (see table 3.2). The metabolites detected in the whole seed grain are similar to those detected in the leaf by Yaro *et al* (2018). Secondary metabolites present in a plant play a significant role in the pharmacological effect exhibited by that plant (Wink, 2015).

4.3 Mineral analysis

Mineral analysis carried out on the powdered seeds of *Eleusine coracana* (see table 3.4) revealed the presence of essential minerals such as; sodium (11.66±1.17 ppm), potassium (19.72±0.39 ppm), calcium (362.20±9.01ppm), iron (2.84±0.10 ppm) and magnesium (1.06±0.00 ppm) which are essential for normal physiological functions (Soetan *et al*.,2010) and also play a role in the pharmacological activity of the plant (Jasper *et al*., 2017). It also revealed the presence of trace elements such as; zinc (0.35±0.01 ppm), copper (0.19±0.01ppm) and chromium (1.23±0.07 ppm) which are components of enzymes in the body required for maintaining normal body homeostasis (Wada, 2004). The trace elements present were detected in amounts which did not exceed the WHO recommended limits. Heavy metals such as cadmium and nickel were not detected in the powdered seed. Lead was detected at very low concentration (0.01±0.00 ppm) in the powdered sample; this was

however below the WHO permissible limit for powdered seed samples. The presence of essential elements in the powdered seed of *Eleusine coracana* shows that the powdered formulation of the seed can be used to treat several ailments associated with deficiency of certain essential minerals. The trace elements present also shows that the seeds of *Eleusine coracana* can be used in managing ailments attributed to a deficiency of essential trace elements. The absence of heavy metals such as; cadmium and nickel and the negligible concentration of lead showed that the powdered seeds of *Eleusine coracana* is safe for consumption without the risk of heavy metal poisoning.

4.4 Percentage yield of extract and fractions

The percentage yield (2.72%) obtained from extracting the powdered seed of *Eleusine coracana* using methanol in a Soxhlet apparatus shows that the yield of the powdered seed of *Eleusine coracana* is very low. This information will be useful to researchers who intend to carry out further studies on the seeds. On fractionation with different organic solvents, the 50% ethylacetate/methanol fraction gave the highest yield (38.92%), followed by 100% ethylacetate fraction (10.98%), 100 % methanol (10.40%), 20% water/methanol (3.82%), 50% water/methanol (0.58%), Dichloromethane (0.25%) and n-hexane (0.13%). The result showed that most components fractionated into the semi-polar solvent system of 50% ethylacetate/ methanol fraction which shows that it is an efficient solvent system for extracting sufficient quantity of both polar and non-polar components from *Eleusine coracana* seeds. The percentage of components partitioned into n-hexane shows that the quantity of non-polar components in the seed is relatively small. The polar solvents generally extracted more components than the non-polar solvents (See table 3.3).

4.5 Acute toxicity

The result of the acute toxicity test showed that at the highest dose of 5000 mg/kg per body weight, there were no physical signs of toxicity and no mortality was recorded. This shows that the LD_{50} of the seeds of *Eleusine coracana* was greater than 5000 mg/kg in mice hence, the seed is relatively safe for consumption.

4.6 Antioxidant activity

4.6.1 DPPH free radical activity

Antioxidant activity of plants can be analyzed by 1,1, diphenyl-2-picryl hydrazine (DPPH) (Elmastas *et al*., 2007). DPPH is a stable free radical that changes colour from purple to yellow when reacted with a hydroxyl group of an antioxidant species which donates a proton to the free radical and this is measured spectrophotometrically at 517 nm or 519 nm wavelength. The result obtained (table 3.5) showed that the 50% ethylacetate/methanol fraction gave the lowest IC_{50} values (29.65 µg/mL) of all the fractions indicating that it has the highest free radicals scavenging potentials and may be effective in the management of disease conditions associated with oxidative stress. The results also showed that the free radical scavenging activity of the different fractions reduces with a decrease in solvents polarity with the ethylacetate, dichloromethane, and n-hexane having the least free radical scavenging activity. This shows that the polar fractions contained components that are capable of scavenging free radicals.

4.6.2 Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power assay (Benzie and Strain, 1996) was carried out on the extract and fractions of *Eleusine coracana* based on the principle that an antioxidant will be able to donate an electron to reduce iron III to iron II (Rajurkar and Hande, 2011) causing a

colour change from amber to blue which can be read at 595 nm using a spectrophotometer. The result of the ferric reducing antioxidant power (FRAP) assay revealed that the 50% methanol/ethylacetate fraction had the highest FRAP activity while the n-hexane fraction had the least activity. The FRAP activity of the total extract and fractions correlated with the result obtained from the IC_{50} value of the total extract and fractions.

4.7 Total Phenol

The determination of the total phenol content was carried out using the FolinCiocalteu reagent which contains molybdenum and tungsten (Kim *et al*., 2003). In the presence of a reducing agent, phosphomolybdate VI will be reduced to phosphomolybdate V with the observation of an intense blue colour which can be read at 750 nm using a spectrophotometer. The 50% ethylacetate/methanol fraction had the highest content of total phenol while the n-hexane fraction had the lowest total phenol content. These results show a correlation between the free radical scavenging activity of extracts (the crude extract and fractions) and their total phenol content at $P < 0.05$. It was observed that the higher the total phenol content, the lower the IC_{50} value which indicates that the total phenol content plays an important role in the free radical scavenging activity of the plant.

4.8 Total Flavonoid

The determination of flavonoid content was carried out using the method described by Ebrahimzadeh *et al* (2008). The method is based on the principle that flavonoids if present in the extract and fractions, will form a colour complex with aluminum chloride which can be read at 415 nm using a spectrophotometer. The total flavonoid assay result showed that the 20% methanol/water fraction had the highest flavonoid content while the least flavonoid content was found in the 50% ethylacetate fraction. The result showed no correlation between

the flavonoid content and IC_{50} values suggesting that the flavonoid content had little or no influence on the radical scavenging activity of the extract and fractions.

4.9 ANTIDIABETIC SCREENING

The present study evaluates the antidiabetic activity of the crude extract and fractions of *Eleusine coracana* using streptozotocin-induced diabetic rats. The results in table 3.6 showed that the n-hexane fraction of *Eleusine coracana* seed was able to significantly improve the weigt of diabetic animals compared to the diabetic control animals on day 5 of treatment. The results as shown in table 3.7 revealed that there was a significant decrease in blood glucose levels of diabetic animals treated with the crude methanol extract and fractions of *Eleusine coracana* compared with the untreated diabetic animals ($P<0.05$). The blood glucose levels of animals treated with 200 mg/kg/day of the crude extract (156.50 ± 24.50 mg/dL), 100% methanol (118.30 ± 35.99mg/dL), 50% methanol/ethylacetate (166.00 ± 24.79mg/dL), 100% ethylacetate (240.30 ± 12.03mg/dL) and n- hexane (227.80 ± 49.65mg/dL) fractions of *Eleusine coracana*, as well as animals treated with 400 mg/kg/day of the crude extract (288.50 ± 49.75mg/dL), 100% methanol (420.50 ± 90.32mg/dL), 50% methanol/ethylacetate (184.50 ± 23.95mg/dL), 100% ethylacetate (370.80 ± 52.88mg/dL), n-hexane (342.30 ± 35.96mg/dL) fractions and 5 mg/kg/day of glibenclamide (257.30 ± 19.60mg/dL) after seven days of treatment, were significantly lower than the diabetic untreated animals (600.00 ± 0.00mg/dL). This result is similar to the observed blood glucose lowering effects of the ethanol extract of *Eleusine coracana* leaves (Yaro *et al*., 2018). The blood glucose lowering effect induced by the extract and fractions was further buttressed from histological studies of the pancreas of the experimental animals. The photomicrographs (figures 3.8, 3.9 and 3.10) of the pancreas revealed that the crude extract and fractions of *Eleusine coracana* were able to protect the islet cells from rapid degeneration compared to the diabetic (untreated) animals

and the results obtained were comparable to that of the pancreas of animals treated with 5 mg/kg/day of glibenclamide. From the biochemical analysis (table 3.8), it was observed that animals treated with 200 mg/kg/day of the crude extract (139.50 ± 0.50mmol/L), 100% methanol (138.50 ± 1.84mmol/L), 50% methanol/ethylacetate (137.30 ± 1.25mmol/L), 100% ethylacetate (139.70±1.20mmol/L) and n-hexane (139.50±1.56mmol/L) fraction had higher levels of sodium in their blood compared to the diabetic untreated animals (128.7 ± 0.67mmol/L). Also, diabetic animals treated with 400 mg/kg/day of 50% methanol/ethylacetate (136.00 ±1.35mmol/L), 100% ethylacetate (138.50 ± 1.44mmol/L) and n-hexane fraction (137.70 ± 1.33mmol/L) had significantly higher sodium level than the diabetic untreated animals. This shows that the extract and fractions of *Eleusine coracana* may be effective in the prevention of hyponatremia associated with diabetes (Liamis *et al*., 2015). The diabetic animals treated with 400 mg/kg/day of the crude extract (5.00 ± 0.49mmol/L) and 400 mg/kg/day of the ethylacetate fraction (5.40 ± 0.31mmol/L) had potassium concentrations which were significantly lower than the diabetic untreated animals (7.05 ± 0.35mmol/L). This shows that the crude methanol extracts and fraction of *Eleusine coracana* may be capable of preventing hyperkalemia normally associated with diabetic ketoacidosis (Palmer and Clegg, 2015). No significant difference between the urea, creatinine and chloride levels of the treated and untreated diabetic animals, $P>0.05$. Although the ALT levels of the treated diabetic animals were observed to be lower compared to the diabetic untreated animals (table 3.9), this was not however significant ($P>0.05$). Higher levels of ALT are mostly due to liver injury in diabetic animals and several studies have reported that these levels are reduced when treated with medicinal plants with antidiabetic activity (Visweswara *et al*., 2013; AbdElwahab *et al*., 2017).

The result obtained from the lipid profile analysis showed that diabetic animals treated with 200 mg/kg/day of 50% methanol/ethylacetate (63.00 ± 1.16mg/dL) showed significantly

lower levels of total cholesterol than the diabetic untreated animals (75.33 ± 1.76mg/dL). No significant difference was recorded between other treated diabetic animals and the diabetic untreated animals at P< 0.05. Meanwhile, the triglyceride levels of diabetic animals treated with 200 mg/kg/day of the crude extract (49.50 ± 7.50mg/dL), methanol (61.33 ± 8.76mg/dL), 50% methanol/ethylacetate (103.00 ± 24.59mg/dL), ethylacetate (79.00 ± 7.55mg/dL) and n-hexane (60.33 ± 6.12mg/dL) fractions were significantly lower than the triglyceride level of diabetic untreated animals (168.00 ± 26.35mg/dL). Also, the triglyceride level of diabetic animals treated with 400 mg/kg/day of crude extract (80.00 ± 19.86mg/dL) and n-hexane fraction (59.67 ± 7.75mg/dL) of *Eleusine coracana* as well as 5 mg/kg/day of glibenclamide (97.50 ± 22.50mg/dL) were significantly lower than those of the diabetic untreated animals (168.00 ± 26.35 mg/dL). This shows that the extract and fractions of *Eleusine coracana* seeds have the ability to keep the lipid profile of diabetic animals under control. Yaro *et al* (2018) also observed this lipid-lowering effect with the leaf extract of *Eleusine coracana.*

The results obtained from the haematological analysis (table 3.12a) showed no significant difference between the level of white blood cell (WBC), red blood cell (RBC), platelet (PLT), lymphocyte (LY), haematocrit (HCT) and red cell distribution width (RDW) of diabetic treated animals and the diabetic untreated animals. However, table 3.12b showed that the mean corpuscular volume (MCV) of diabetic animals treated with 400 mg/kg/day of ethylacetate fraction (67.03 ± 5.51fl) and 5 mg/kg/day of glibenclamide (64.77 ± 0.90fl) were significantly higher than the MCV of diabetic untreated animals (57.48 ± 2.32fl). The mean corpuscular haemoglobin (MCH) level of animals treated with 200 mg/kg/day of n-hexane fraction (22.00 ± 0.12pg) and 5 mg/kg/day of glibenclamide (19.50 ± 0.31pg) were significantly higher than the levels observed in diabetic untreated animals (18.06 ± 0.27pg), while the mean corpuscular haemoglobin concentration (MCHC) of diabetic animals treated with 200 (36.20 ± 0.34g/dL) and 400 mg/kg/day (37.37 ± 0.72g/dL) of n-hexane fraction

where significantly higher than the MCHC values of the diabetic untreated animals (31.52 ± 1.01g/dL). Diabetes is usually associated with the occurrence of anaemia as a result of the non-enzymatic glycosylation of proteins in the membrane of the red blood cells which can initiate haemolysis (Oyedemi *et al*.,2011). Red blood cell parameters such as MCV, MCH, MCHC and haemoglobin concentrations were used to estimate the effect of the treatments on anaemia associated with diabetes. The n-hexane fraction showed significant ability to improve the MCH and MCHC concentrations while the ethylacetate fraction at 400 mg/kg/day was able to significantly improve the MCV in diabetic animals which implies that these fractions may be useful in preventing anaemia in diabetic rats. This result is similar to those obtained by Mahmoud (2013) where the RBC parameters were improved in diabetic rats treated with extracts. Table 3.12 c showed that there was no significant difference between the monocyte (MO), lymphocyte (LY) and granulocyte (GR) level of diabetic animals treated for 7 days and the diabetic untreated animals at $P<0.05$.

4.10 Infrared spectroscopic and mass spectrometric analysis

The Infrared spectra of the ethylacetate and n-hexane fractions were determined in order to determine the functional groups likely to be present in these fractions. These fractions were chosen despite their very low radical scavenging activity because they were also able to reduce the blood glucose level of the diabetic animals. The Infrared spectroscopic result of the ethylacetate fraction revealed that the fraction contained predominantly aliphatic compounds diagnosed by the absence of CH stretch vibrations above 3000 cm^{-1} wavenumber. Instead, the CH stretch occurred at 2922 cm^{-1} and 2855 cm^{-1} wave numbers which are peculiar to CH stretch of aliphatic compounds. The occurrence of CH_2 rocking vibration at the fingerprint region of 723 cm^{-1} wave number further substantiates the presence of long chain aliphatic compounds in the fraction. The presence of vibrations at the 2922 cm^{-1} and 2855 cm^{-1} also reveals the presence of a methylene (CH_2) stretch vibrations which further

confirms the presence of long chain aliphatic compounds. Vibrations at 1375 cm^{-1} confirm the presence of a methyl (CH_3) bend while 1461 nm represents the methylene (CH_2) bend. The presence of the vibrations between 1237 $cm^{-1}$and 1039 $cm^{-1}$are indicative of the presence of carbon to oxygen single bond (C-O) which is typical for groups such as acids, esters, ethers, amides, and even alcohols. Vibrations at 2855 cm^{-1} are common with SP hybridized carbons such as carbon to nitrogen triple bonds (C≡N). While the vibrations that occur at 969 cm^{-1} are common with CH_2 bends that are associated with carbon to carbon double bonds (C=C). In summary, the IR result for Ethylacetate fraction shows that it contains compounds with carbonyl functional group and long chain aliphatic compounds indicating that the constituents are mainly non-polar because no obvious vibrations were recorded for O-H and N-H stretch which occurs within the range of 3475- 3145cm^{-1}.

The infrared spectra of the n-hexane fraction were quite similar to the spectra obtained from the ethylacetate fraction. It showed prominent vibrations at 1710 cm^{-1} indicating the presence of a carbonyl functional group. The vibrations observed at 2922 $cm^{-1}$and 2855 cm^{-1} is common with aliphatic C-H stretches, the vibrations at the fingerprint region between 772 cm^{-1} and 723 cm^{-1} show the bending of clusters of CH_2 commonly observed with straight chain aliphatic compounds with four or more CH_2. The vibrations observed between 1013 cm^{-1} and 931 cm^{-1}shows CH_2 bends common with unsaturated double bonds. Vibrations observed at 1177 cm^{-1} and 1118 cm^{-1} is indicative of the carbon to oxygen single bond indicating the presence of alcohols, ethers, esters, and acids. Vibrations were also observed between 1461 $cm^{-1}$and 1379 cm^{-1}which indicated the presence of CH_3 and CH_2 stretches, respectively confirming the presence of long chain aliphatic compounds. The IR spectra show the presence of components with mainly long chain aliphatic components which may be ethers, esters, acids, ketones or even alcohols. But the absence of a pronounced vibration at 3600 cm^{-1} - 3200cm^{-1}region shows that polar components may not be present in large

quantities in this fraction. This is not surprising considering the fact that n-hexane is a non-polar solvent and more likely to extract more non-polar components from *Eleusine coracana* seeds (Pavia *et al*., 2001).

GC-MS was also used to analyze the ethylacetate and n-hexane fractions in order to identify specific components. From the results obtained, it was observed that the ethylacetate fraction contained; alkanes (16%), alkenes (12%), alcohols (16%), esters (32%), acids (10%), ketones (4%) and other compounds (10%) while the n-hexane fraction contained; alkanes (32%), alkenes (6%), alkynes (2%), aldehydes (4%) alcohols (4%), esters (26%), chlorides (4%), organic acids (10%), sugars (8%) and other compounds (4%). The GC-MS result supported to some extent the vibrations observed in the infrared spectra of both the ethylacetate and the n-hexane fractions which indicated the presence of mainly long chain aliphatic compounds which could be alcohols, esters, amides, ethers, organic acids, etc.

Semi-purified fractions were obtained from the column chromatography of the ethylacetate fraction as observed in the TLC chromatogram. The semi-purified fractions will be subjected to further analysis.

CHAPTER FIVE

CONCLUSION

From this study, it can be concluded that the seeds of *Eleusine coracana* contain bioactive constituents with antidiabetic and antioxidant

The seeds of *Eleusine coracana* are rich in phytochemical constituents and the crude extract and polar fractions have very good antioxidant properties with the 50% methanol/ethylacetate fraction showing the highest antioxidant activity. The crude extract and the polar fractions of the seeds are also rich in polyphenol content which may explain why they have good antioxidant properties since antioxidant activities usually correlate with high polyphenol contents in medicinal plants. The study revealed also that crude extract and fractions of EC were significant in controlling elevated blood glucose levels in STZ induced diabetes in experimental animals. This was evident in the histological study of the pancreas, which revealed a reduction in the rapid degeneration of the islet cells in the diseased pancreas. It can be concluded that the seeds of *Eleusine* coracana contain bioactive constituents with antidiabetic and antioxidant activities thus lending credence to the traditional claim. Further studies geared towards the isolation and characterization of the potential active principles responsible for the observed antidiabetic effects is suggested.

CONTRIBUTION TO KNOWLEDGE

1. The study revealed that 50% Ethylacetate: methanol, ethylacetate and n-hexane fractions obtained from the whole seed grain of *Eleusine coracana* have antidiabetic effect.
2. The study established a strong correlation between the antioxidant and antidiabetic activity of the 50% ethylacetate:methanol fraction.
3. The study revealed that fractions obtained from the whole seed grain of *Eleusine coracana* have protective effect on the β islet cells of the pancreas**.**
4. This is the first time the compound eicosane has been identified in the GC-MS analysis of n-hexane fraction of *Eleusine coracana*.

REFERENCES

Abdul QA, Syed AA, Waheed MA and Sayyed J. (2013). Extraction and determination of antioxidant activity of *Withania somnifera Dunal. European Journal of Experimental Biology* 3(5):502-507.

AbdElwahab AH, Yousuf AF, Ramadan BK and Elimam H. (2017). Comparative Effects of *Stevia rebaudiana* and Aspartame on hepato-renal function of diabetic rats: *Biochemical and Histological Approaches. Journal of Applied Pharmaceutical Science* 7 (8):34-42.

Adrian V. (2012). Mechanism of action of DPP-4 Inhibitors-new insights. *Journal of Clinical Endocrinology and Metabolism* 97(8):2626 -2628.

African Pharmacopoeia. (1986). Vol. 2, 1st ed., OAU/STRC publications: pp. 128-144.

Ahmed SM. (2005). Insulin and oral antidiabetic agents. *American Journal of Pharmaceutical Education* 69(5): 1-11.

Alam MN, Bristi NJ and Rafiquzzama M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*. 21.143-152.

Alugoju P, Dinesh BJ and Latha P. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry* 30(1):11–26.

Al-Qudah MMA, Haddad MA and EL-Qudah JMF. (2016). The effects of aqueous ginger extract on pancreas histology and on blood glucose in normal and alloxan monohydrate-induced diabetic rats. *Biomedical Research* 27(2): 350-356.

Ambavane AR, Sawardekar SV, Sawantdesai SA and Gokhale NB. (2014). Studies of mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet (*Eleusine coracana* L. Gaertn). *Journal of Radiation Research and Applied Sciences*. 8(1):120-125.

American Diabetes Association. (2010). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 33(1): 562-569.

American Diabetes Association. (2011). Diagnosis and Classification of Diabetes Mellitus. Diabetes care 34(1): 562-569.

American Diabetes Association. (2018). Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes. *Diabetes Care* 41(1): 13-27.

American Medical Association. (2009). Diabetes in Asia:Epidemiology, risk factors, and Pathophysiology. *Journal of American Medical Association* 30(20):2129-2140.

Amir G, Gulzar NA, Prasad K and Kumar P. (2016). Technological, processing and nutritional approach of finger millet (*Eleusine coracana*) - a mini review. *Journal of Food Processing and Technology* 7(6):1-4.

Ansari AQ, Ahmed SA, Waheed MA and Sayyed JA. (2013). Extraction and determination of antioxidant activity of *Withania somnifera* Dunal. *European Journal of Experimental Biology* 3(5):502-507.

Anurag K, Raghuveer I , Anumalik Y, Nitika G, Swadesh K, Nikhil G, Santosh K, Vinay YP and Himanshu G. (2014). Metabolites in plants and its classification. *World Journal of Pharmacy and Pharmaceutical Sciences* 4(1): 287-305.

Apak R, Guclu K, Demirata B, Ozyurek M, Saliha EC, Burcu B, Berker KI and Dilek O. (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules 12.* 1496-1547.

Apel K and Hirt H. (2004). Reactiveoxygen species: metabolism, Oxidative Stress, and Signal Transduction. *Annual Review of Plant Biology* 55.373–99.

Arowosegbe S, Olanipekun MK and Kayode J. (2015). Ethnobotanical survey of medicinal plants used for the treatment of diabetes mellitus in Ekiti south senatorial district, Nigeria. *European Journal of Botany, Plant Sciences and Phytology* 4(2):1-8.

Association of Official Analytical Chemists. (1984). Official method of analysis. Association of Official Analytical Chemists. Washinghton D.C: 1112-1114.

Ataroalsadat M, Abdodlah A, Seyed KG, Ramin P and Mohammad B. (2016). The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of type 1 diabetes mellitus and mortality rate in rats. *Laboratory Animal Research* 32(3): 160-165.

Bahuguna YM, Rawat MSM, Juyal V and Gnanarajan, G. (2009). Antilithiatic effect of grains of *Eleusine coracana*. *Saudi Pharmaceutical Journal* 17(2):182-188.

Bailey JC. (1992). Biguanides and NIDDM (non- insulin dependent diabetes mellitus. *Diabetes Care* 6(15):755-772.

Banerjee S, Sanjay KR, Chethan S and Malleshi NG. (2012). Finger millet (*Eleusine coracana*) polyphenols: Investigation of their antioxidant capacity and antimicrobial activity. *African Journal of Food Science* 6(13). 362-374.

Bartnik M and Facey PC. (2017). Pharmacognosy Fundamentals, Applications and Strategies. *Acaedemic Press*. 101- 161.

Benzie, IFF and Strain, JJ. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry* 239: 70-76.

Bhargava VV, Saluj AK and Dholwani K K. (2013). Detection of heavy metal contents and proximate analysis roots of *Anogeissus latifolia*. *Journal of Pharmacognosy and Phytochemistry* 1(6): 61-65.

Boguslaw L. (2011). Hydroxyl radical and its scavengers in health and disease. Oxidative *Medicine and Cellular Longevity* 1-9.

Borokini TI, Ighere DA, Clement M, Ajiboye TO, Alowonle AA. (2013). Ethnobiological survey of traditional medicine practice for the treatment of piles and diabetes mellitus in Oyo state. *Journal of Medicinal Plants Studies* 1(5):20-30.

Carmen MM and Ramaroson A. (2009). Reactive nitrogen species: molecular mechanisms and potential significance in health and disease. *Antioxidants and Redox Signaling* 11(3): 670-702.

Chandra D, Pallavi SC and Sharma AK. (2016). Review of finger millet (*Eleusine coracana* (L.) Gaertn): A power house of health benefiting nutrients. *Food Science and Human Wellness* 57. 1-7.

Chauhan A, Sharma PK, Srivastava P, Kumar N and Dudhe R. (2010). Plants having potential antidiabetic activity: *A review. Scholars Research Library* 2(3):369-387.

Chen H, Brahmbhatt S, Gupta A and Sharma AC. (2005). Duration of streptozotocin induced diabetes differentially affects p38 mitogen-activated protein kinase (MAPK) phosphorylation in renal vascular dysfunction. *Cardiovasc Diabetiol* 4:3-6.

Chen L, Magliano DJ and Zimmet PZ. (2012). The worldwide epidemiology of type 2 diabetes mellitus present and future perspectives. *Nature Review Journal of Endocrinology* 8 228-236.

Chethan S, Daharmesh SM, Nagappa G and Malleshi G. (2008). Inhibition of aldose redutase from cataracted eye lenses by finger millet (*Eleusine coracana*) polyphenols. *Bioorgarnic and Medicinal Chemistry*.16:10085-10090.

Chikezie PC, Ojiako OA and Nwufo KC. (2015). Overview of anti-diabetic medicinal plants: The Nigerian research experience. *Journal of Diabetes Metabolism* 6(6):1-7.

Confederat L, Roxana S, Lupaşcu F, Constantin S, Avram I, Doloca A and Profire L. (2016). Side effects induced by hypoglycaemic sulfonylureas to diabetic patients - a retrospective study. *Farmacia* 5(64): 674-679.

David BM, Afolayan M, Odukomaiya D, Ikokoh P and Orishadipe A. (2014). Proximate composition, mineral and phytochemical constituents of *Eleusine coracana* (finger millet). *International Journal of Advanced Chemistry* 2(2):171-174.

Derong L, Mengshi X, Jingjing Z, Zhuohao L, Baoshan X, Xindan L, Maozhu K, Liangyu L, Qing Z, Yaowen Li, Hong C, Wen Q, Hejun W and Saiyan C. (2016). An Overview of Plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. *Molecules* 21(1374): 1-19.

Derosa G and Maffioli P. (2012). α-Glucosidase inhibitors and their use in clinical practice. *Archives Medical Science* 8(5): 899-906.

Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG and Priyadarisini VB. (2014). Health benefits of finger millet (*Eleusine coracana L.)* polyphenols and dietary fiber: a review. *Journal of Food Science Technology* 51(6):1021–1040.

Dilfuza E. (2017). Phytochemical and Pharmacological Properties of Medicinal Plants from Uzbekistan: A Review. *Journal of Medicinally Active Plants* 5 (4): 59-60.

Drucker DJ and Nauck MA. (2006). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* (368): 1696–705.

Duduku K, Rosalam S and Rajesh N. (2011). A review of the antioxidant potential of medicinal plant species. *Food and bioproducts processing* 8(9): 217-218.

Ebrahimzadeh MA, Pourmorad F and Bekhradinan AR. (2008). Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. *African Journal of Biotechnology* 7(18):.3188-3192.

Edeoga HO, Okwu DE and Mbaebie BO. (2005). Phytochemica1 constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4(7): 685-688.

Elmastas M, Isildak O and Turkeku I., Temur N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis* 20:337-345.

Erhabor JO, Idu M and Udo FO. (2013). Ethnomedicinal survey of medicinal plants used in the treatment of male infertilty among the IFA Nkari People of Ini Local Government Area of Akwa Ibom State, Nigeria. *Research Journal of Recent Sciences* 2:5-11.

Ezuruike UF and Prieto JM. (2014). The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. *Journal of Ethnopharmacology* 155:857–924.

Foretz M, Guigas B, Bertrand L, Pollak M and Benoit V. (2014). Metformin: From Mechanisms of Action to Therapies. *Cell Metabolism* 20:1-14.

Fuente JA and Manzanaro S. (2003). Aldose reductase inhibitors from natural sources. *Natural Product Report* 20: 243–251.

Gaba M, Singh S and Gaba P. (2009). Dipeptidyl peptidase - 4 inhibitors: a new approach in diabetes treatment. *International Journal for Drug Development and Research* 1(1):146-156.

Gaoyan L, Xiaheng Z and Biao Y. (2014). Thioglycosides in carbohydrate research. *Carbohydrate Research* 403: 14-22.

Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM and Lawal A.(2010). Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry* 4(8): 142-151.

Hannah ACM and Krishnakumari S. (2005). Analysis of mineral elements, proximate and nutritive value in *Citrullus vulgaris* Schrad. (Watermelon) seed extracts. *The Pharma Innovation Journal* 4(8): 7-11.

Hannele YJ. (2004). Drug therapy thiazolidinediones. *New England Journal of Medicine* (351): 1106-1118.

Harish S, Krishna G and Baske PK. (2010). Plants used in the treatment of joint diseases (rheumatism, arthritis, gout and lumbago) in Mayurbhanj district of Odisha, India. *Report and Opinion* 2(9):24.

Hegde PS, Chandrakasan G and Chandra TS. (2002). Inhibition of collagen glycation and crosslinking in vitro by methanolic extracts of finger millet (*Eleusine coracana*) and kodo millet (*Paspalum scrobiculatum*). *Journal of Nutrition Biochemistry* 13:517-521.

Helio NM and Arthur GF. (2015). Plant alkaloids: Main features, toxicity, and mechanisms of action. *Plant Toxins* 1-15.

Hill BG, Dranka BP, Bailey SM, Lancaster JR and Darley VM. (2010). What part of no don't you understand? Some Answers to the Cardinal Questions in Nitric Oxide Biology. *Journal of Biological Chemistry* 285(26): 19699–19704.

Hollman PCH, Michel NCEB, Yvonne VG, Else EJC, Jeanne HMV and Martijn BK. (1999). The sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in man. *Free Radical Research* 31:569-573.

Idowu OA, Soniran OT, Ajana O and Aworinde DO. (2010). Ethnobotanical survey of antimalarial plants used in Ogun state, Southwest Nigeria. *African Journal of Pharmacy and Pharmacology* 4(2): 55-60.

International Diabetes Federation IDF. (2013). Diabetes Atlas, International Diabetes Federation, Brussels, Belgium 6th edition. Available at:www.idf.org/e-library/epidemiology-research/diabetes-atlas.html. Accessed 14th November 2018.

International Diabetes Federation IDF. (2015). Diabetes Atlas, International Diabetes Federation, Brussels, Belgium, 7th edition. Available at: www.diabetesatlas.org. Accessed 14th November 2018.

International Diabetes Federation IDF. (2014). Diabetes: Facts and Figures. 6th edition. Available at: www.idf.org/worlddiabetesday/toolkit/gp/factsfigures. Accessed 14th November 2018.

International Diabetes Federation IDF. (2017). Diabetes Atlas, 8th edition. Brussels, Belgium: Available at: www.diabetesatlas.org. Accessed 14th November 2018.

Islamiyat FB, Moruf OO, Sulaiman AO and Adeladun SA. (2016). A review of cyanogenic glycosides in edible plants, Toxicology Sonia Soloneski and Marcelo L. Larramendy, *IntechOpen.* Pp179-191.

Jain A, Soni M, Deb L, Rout S, Gupta V and Krishna K. (2008). Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Mormordica dioica*. Roxb leaves. 20(1):337-345.

Jasper EE, Yakubu MB and Baganjiya YP. (2017). Levels of essential and non-essential elements in commercially available moringa herbal teas sold in Nigeria. *Nigerian Journal of Chemical Research* 22(1): 1-8.

Jayaprasad B, Thamayandhi D and Sharavanan PS. (2012). Traditionally using antidiabetic medicinal plants in tamil nadu. *International Journal of Research in Pharmaceutical and Biosciences* 2(1): 1-8.

Joash BLT and Yau YL. (2014). Critical analysis of current methods for assessing the in vitro antioxidant and antibacterial activity of plant extracts. *Food Chemistry* 172:814-822.

Justin NK, Edmond S, Ally RM and Xin H. (2014). Plant Secondary Metabolites: Biosynthesis. *Journal of Pharmacy and Pharmacology* 2: 377-392.

Kabera JN, Semana E, Mussa AR and Xin H. (2014). Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *Journal of Pharmacy and Pharmacology* 2: 377-392.

Karan SK, Mondal A, Mishra SK, Pal D and Rout KK. (2013). Antidiabetic effect of Streblus asper in streptozotocin-induced diabetic rats. *Pharmaceutical biology* 51(3), pp.369-375.

Kalra S and Gupta Y. (2015). Recent advances in endocrinology. *Journal of the Pakistan Medical Association* 65(1):1-5.

Karamali K and Teunis R. (2001).Tannins: Classification and definition. *Natural Product Reports* 18: 641-649.

Keri RS, Srinivasa B, Ranjith KP and Geetha BR. (2014). Chromones as a privileged scaffold in drug discovery: A review. *European Journal of Medicinal Chemistry* 78: 340-374.

Kharb M, Jat RK and Gupta A. (2012). A review on medicinal plants used as a source of anticancer agents. *International Journal of Drug Research and Technology* 2(2): 177-183.

Kim DO, Jeong SW and Lee CY. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* 81(1): 321-326.

Konstantinos P, Nikolaos P, Maciej B, Dimitrios P and Michael E. (2016). Complications of Diabetes. *Journal of Diabetes Research*. 1-4.

Krishnaswami A, Ravi KS and John ML.(2010). Thiazolidinediones. *The Permanente Journal* 3(14): 64-72.

Larkins N and Wynn S. (2004). Pharmacognosy: phytomedicines and their mechanisms. *Veterinary Clinics Small Animals Practice* 34(1): 291–327.

Landgraf R. (2000). Meglitinide Analogues in the treatment of type 2 diabetes mellitus. *Drugs and Aging* 17(5): 411-425.

Latha MG, Srinivas P and Muralikrishna G. (2007). Purification and Characterization of Ferulic Acid Esterase from Malted Finger Millet (*Eleusine coracana*, Indaf-15). *Journal of Agricultural and food chemistry* 55: 9704-9712.

Letitia MM and Timothy J. (2002). Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the Indigenous Peoples of the North American boreal forest. *Journal of Ethnopharmacology* 82: 197 -205.

Liamis G, Tsimihodimos V and Elisaf M. (2015). Hyponatremia in diabetes mellitus: clues to diagnosis and treatment. *Journal of Diabetes and Metabolism* 6(6): 1-3.

Lian G, Zhang Xi and Biao Y. (2014). Thioglycosides in carbohydrate research. *Carbohydrate Research* 2(7): 1-10.

Lin Y and Sun Z. (2010). Current views on type 2 diabetes. *Journal of Endocrinology* .204. 1–11.

Lorenzati B, Chiara Z, Miglietta S, Lamberti F and Graziella B. (2010). Oral Hypoglycemic Drugs: Pathophysiological Basis of Their Mechanism of Action. *Pharmaceuticals* 3: 3005-3020.

Lorke D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology* 54: 275-87.

Maan H, Mohd AH and Inas MA. (2016). Superoxide Ion: Generation and chemical implications. *Chemical Reviews* 116: 3029-3085.

Madhavi DL, Deshpande SS and Salunkhe DK. (1996). Introduction. Food Antioxidants: Technological, toxicological, and health perspectives; Eds.; Marcel Dekker, Inc.: New York, NY. pp. 1-4.

Mahmoud AM. (2013). Hematological alterations in diabetic rats role of adipocytokines and effect of citrus flavonoids. *Experimental and Clinical Sciences Journal* 12:647-657.

Mahmoud RK, Azar B and Morteza R. (2013). Plants antioxidants: from laboratory to clinic. *Journal of Nephropathology* 2(2): 152-153.

Mahomoodally MF. (2013). Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence-based complementary and alternative medicine* 2: 4-15.

Mall TP and Tripathi SC. (2016). Millets-the nutrimental potent ethno-medicinal grasses: a review. *World Journal of Pharmacy Research* 5(2):495-520.

Mamta S, Jyoti S, Rajeev N, Dharmendra S and Abhishek G. (2013). Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry* 1(6).168-182.

Manisha WH, Richa R and Deepali J. (2017). Oxidative stress and antioxidants: an overview. *International Journal of Advanced Research and Review* 2(9):110-119.

Marcio C and Isabel CFR F. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology* 51:15-25.

Miriam MCK, Koning A, Gunter GCK, Peter N, Christopher LB, Andreas P, David AW, Jon MF, Alan AJ, Harry VG, Kenneth RO and Martin F. (2017). The reactive species interactome: evolutionary emergence, biological significance, and opportunities for redox metabolomics and personalized medicine. *Antioxidants and Redox Signaling* 10(27):684-712.

Maridass M and John DBA. (2008). Origins of Plant Derived Medicines. *Ethnobotanical Leaflets* 12: 373-387.

Martin CHG and Alan JS. (2012). The biology of reactive sulfur species (RSS). *Plant Physiology and Biochemistry* 59. 98-107.

Mathanghi SK and Sudha K. (2012). Functional and phytochemical properties of finger millet (*Eleusine coracana* L.) for health. *International Journal of Pharmaceutical Chemistry Biological Sciences* 2(4): 31-438.

Maxwell O, Omabe KN, Ezeani M and Nony MR. (2016). Incretin Mimetics and Type 2 Diabetes. *Prensa Medica Argentina* 1(102):1-5.

Martínez MC and Andriantsitohaina R.(2009). Reactive nitrogen species: molecular mechanisms and potential significance in health and disease. *Antioxidants and Redox Signaling* 11(3):669-702.

Maridass M and Britto AJD. (2008). Origins of Plant Derived Medicines. *Ethnobotanical Leaflets* (12): 373-387.

Mendoza RG, Prioletta A, Jiménez CLM and Aravind SFF. (2013). The role of nateglinide and repaglinide, derivatives of meglitinide, in the treatment of type 2 diabetes mellitus. *Archives of Medical Sciences*. 1-8.

Mkele G. (2013). Dipeptidyl peptidase-4 inhibitors: their role in the management of type 2 diabetes. *South African Pharmacy Journal* 8(2):18-20.

Mohammad TM, Majid AS, Mahmoud B and Mehrdad MS. (2016). Medicinal plants used for liver disorders based on the ethnobotanical documents of Iran: A Review. *International Journal of PharmTech Research* 9(5):407-415.

Mohammed S, Hamzat IT, Bashir MA, Saidu H, Yusuf H, Jibrin NM and Abdulrahman I. (2013). An overview of natural plant antioxidants: analysis and evaluation. *Advances in Biochemistry* 1(4): 64-72.

Moharram HA and Youssef MM. (2014). Methods for Determining the Antioxidant Activity: A Review. *Journal of Food Science and Technology* 11(1): 31-42.

Monier M and El-Ghani A. (2016). Traditional medicinal plants of Nigeria: an overview. *Agriculture and Biology Journal of North America* 7(5): 220-247.

Moradi MT, Samani AM, Bahmani M and Shahrani Mehrdad. (2016). Medicinal plants used for liver disorders based on the ethnobotanical documents of Iran: A review. *International Journal of Pharmaceutical Technology Research* 9(5):407-415.

Murray K. (1993). Normal function of the endocrine pancreas. The pancreas; Biology, Pathophysiology and Disease. Second edition. *Raven Press Limited New York*.751-758.

Murtaza N, Baboota RK, Jagtap S, Singh DP, Khare P, Sarma SM, Podili K, Alagesan S, Chandra TS, Bhutani KK, Boparai RK, Bishnoi M and Kondepudi KK. (2014).Finger millet bran supplementation alleviates obesity-induced oxidative stress, inflammation and gut microbial derangements in high-fat diet-fed mice. *British Journal of Nutrition*. 112(9):1447-1458.

Nasri H and Mahmoud RK. (2014). Metformin: Current knowledge. *Journal of Research in Medical Sciences* 19(7):658-664.

Nirmala M and Muralikrishn G. (2003). Three alpha –amylases from malted finger millet (Ragi *Eleusine coracana*, Indaf-15)-purification and partial characterization. *Phytochemistry* 62(1):21-30.

Nur AM, Nusrat JB and Rafiquzzaman M. (2012). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal* 21:143–152.

Obrenovich ME, Yi L, Parvathaneni K, Yendluri BB, Palacios HH, Leszek J and Aliev G. (2011). Antioxidants in health, disease and aging. *CNS and Neurological Disorders - Drug Targets* 10(2): 192-207.

Ogbera AO and Ekpebegh C. (2014). Diabetes mellitus in Nigeria: The past, present and future. *World Journal of Diabetes* 5(6): 905-911.

Olufemi AF and Samuel DJ. (2015). Diabetes Care in Nigeria. *Annals of Global Health* 6 (81): 821-829.

Olanipekun MK, Arowosegbe S, Kayode JO and Oluwole TR. (2016). Ethnobotanical survey of medicinal plants used in the treatment of women related diseases in Akoko region of Ondo-State, Nigeria. *Journal of Medicinal Plants Research* 10(20):270-277.

Oseghale IO, Imieje VO, Erharuyi O, Iheanacho C and Falodun A. (2017). A Review of the Phytochemistry and Pharmacology of *Eleusine coracana* Linn (Poaceae): (Poaceae):

A popular Nigerian edible grain. *Tropical Journal of Natural Product Research* 1(6):227-235.

Oyedemi SO, Adewusi EA, Aiyegoro OA and Akinpelu DA. (2011). Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic Wistar rats. *Asian Pacific Journal of Tropical Biomedicine* 1(5): 353-358.

Ozlem G and Giuseppe M. (2007). Saponins: Properties, Applications and Processing. *Critical Reviews in Food Science and Nutrition* 47(3): 231-258.

Palmer BF and Clegg DJ. (2015). Electrolyte and acid–base disturbances in patients with diabetes mellitus. *The New England Journal of Medicine* 373(6): 548-559.

Pavia DL, Lampman GM, Kriz GS and Vyvyan JR. (2001). Introduction to Spectroscopy: Infrared spectroscopy; Fourth edition. Brooks/cole. USA: pp 15-104

Pei-Chen W, Vin-Cent W, Cheng-Jui L, Chi-Feng P, Chih-Yang C, Tao-Min H, Che-Hsiung W, Likwang C, Chih-Jen W and the NRPB Kidney Consortium. (2017). Meglitinides increase the risk of hypoglycemia in diabetic patients with advanced chronic kidney disease: a nationwide, population-based study. *Oncotarget* 8(44): 78086-78095.

Pingle BR, Apte KG, Gupta M and Chakraborthy GS. (2011). Hepatoprotective activity of different extracts of grains of Eleusine coracana. *Pharmacologyonline* (2):279-286.

Poonia K, Chavan S and Daniel M. (2012).Fixed oil composition, polyphenols and Phospholipids of finger millet [*Eleusine coracana* (L.) Gaertn]. *Biological Forum- An International Journal* 4(1): 45-47.

Prashanth SMR, Shruthi RR and Muralikrishna G. (2014). Immunomodulatory activity of purified arabinoxylans from finger millet (*Eleusine coracana*, v. Indaf 15) bran. *Journal of Food Science and Technology* 52(9):6049-6054.

Rahimi R, Nikfar S, Larijani B and Abdollahi M. (2005). A review on the role of antioxidants in the management of diabetes and its complications. *Biomedicine and Pharmacotherapy* 59: 365–373.

Rajasekarana NS, Nithyac M, Rosec C and Chandraa TS.(2004). The effect of finger millet feeding on the early responses during the process of wound healing in diabetic rats. *Biochim Biophys Acta*.1689 (3):190-201.

Rajendiran D, Packirrisamy S and Gunasekaran. (2018). A review on role of antioxidants in diabetes. *Asian Journal of Pharmaceutical and Clinical Research* 11(2): 48-53.

Rajurkar NS and Hande SM. (2011). Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. *International Journal of Pharmaceutical Sciences* 73(2): 146-151.

Rastogi AK, Ramesh C, Arvind KS. (1997). Screening of natural products for hypolipidaemic and hypoglycaemic activities. International workshop on medicinal plants, their bioactivity, screening and evaluation. *Lucknow*.pp1-17.

Richard JW and Raskin P. (2011). Updated Review: Improved Glycemic Control with Repaglinide-Metformin in Fixed Combination for Patients with Type 2 Diabetes. *Clinical Medicine Insights Endocrinol Diabetes* (4): 29–37.

Rodolfo GM, Annamaria PLMAS and Franco F. (2012). The role of nateglinide and repaglinide, derivatives of meglitinide, in the treatment of type 2 diabetes mellitus.*Archives of Medicinal Sciences* 9(5): 936–943.

Ruby T and Rana CS. (2015). Plant secondary metabolites: a review. *International Journal of Engineering Research and General Science* 3(5):1-11.

Saikat S, Raja C, Sridhar C, Reddy YSR and Biplab D. (2010). Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. *International Journal of Pharmaceutical Sciences Review and Research*. 3(1): 91-100.

Salehi P, Asghari B, Esmaeili MA, Dehghan H and Ghaz I. (2013). α-Glucosidase and α-amylase inhibitory effect and antioxidant activity of ten plant extracts traditionally used in Iran for diabetes. *Journal of Medicinal Plants Research* 7(6): 257-266.

Sandhya B, Thomas S, Isabel W and Shenbagarathai R. 2006. Ethnomedicinal plants used by the Valaiyan community of Piranmalai hills (reserved forest), Tamilnadu, India. - a pilot study. *African Journal of Complementary and Alternative Medicines* (3): 101-114.

Sapna DD, Dhruv GD and Harmeet K. (2009). Saponins and their Biological Activities. *Pharma Times* 3(41): 13-16.

Sasidharan S, Chen Y, Saravanan D, Sundram KM and Yoga LL.(2011). Extraction, isolation and characterization of bioactive compounds from plant's extracts. *African Journal of Traditional Complementary and Alternative Medicine* 8(1):1-10.

Satish B N and Dilipkumar P. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *Royal Society of Chemistry* 5: 27986–28006.

Saxena M, Saxena J, Rajeev N, Singh D and Gupta A. (2013). Phytochemistry of Medicinal Plants. *Journal of Pharmacognosy and Phytochemistry Phytochemistry* 1 (6):168-182.

Saxena L, Iyer BK and Ananthanarayan L. (2010). Purification of bifunctional amylase/protease inhibitor from ragi (*Eleusine coracana*) by chromatogratphy and its use as an affinity ligand. *Journal of Chromatography* 879(19): 1549-1554.

Shahriar K and Robin JM. (2010). Monocyclic Phenolic Acids; Hydroxy- and Polyhydroxybenzoic Acids: Occurrence and Recent Bioactivity Studies. *Molecules* 15: 7985-8005.

Shobana S, Harsha MR, Platel K, Srinivasan K and Malleshi NG. (2010). Amelioration of hyperglycaemia and its associated complications by finger millet (*Eleusine coracana* L.) seed coat matter in streptozotocin-induced diabetic rats. *British Journal of Nutrition* 104:1787-1795.

Shukla A, Lalit A, Sharma V and Alam A. (2015). Screening of phytoconstituents and antimicrobial activity of hexane exract of pennisetum Glaucum (L.) R.BR. and *Eleusine coracana* (L.) Gaertn. *Applied Research Journal* 1(7):384-387.

Shukla K and Srivastava S. (2014). Evaluation of finger millet incorporated noodles for nutritive value and glycemic index. *Journal of Food Science and Technology* 51(3):527-534.

Singh H, Krishna G and Baske PK. (2010). Plants used in the treatment of joint diseases (rheumatism, arthritis, gout and lumbago) in Mayurbhanj district of Odisha, India. *Report and Opinion* 2(9):24.

Singh B and Sharma RA. (2015). Plant terpenes: defense responses, phylogenetic analysis,regulation and clinical applications. *Biotech* 3(5):129–151.

Singh N, Meenu G, Sekhar A, Abraham J. (2015). Evaluation of antimicrobial and anticancer properties of finger millet (*Eleusine coracana*) and pearl millet (Pennisetum glaucum) extracts. *The Pharma Innovative Journal* 3(11):82-86.

Soetan KO, Olaiya CO and Oyewole OE. (2010). The importance of mineral elements for humans, domestic animals and plants: A review. *African Journal of Food Science* 4(5):200-222.

Sofowora, A. (1982). Screening Plants for Bioactive Agents. In Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan: pp 128-161.

Sola D, Rossi L, Schianca GPC, Maffioli P, Bigliocca M, Mella R, Corliano F, Gian PF, Bartoli E and Derosa G. (2013). Sulfonylureas and their use in clinical practice. *Archives of Medical Science* 1(4): 840–848.

Soladoye MO, Chukwuma EC and Owa FP. 2012. An avalanche of plant species for the traditional cure of diabetes mellitus in South-Western Nigeria. *Journal of natural product and plant resource* 2(1): 60-72.

Sreeramalu D, Vijaya KRC and Raghunath M. (2009). Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India. *International Journal of Biochemistry and Biophysics* 46:112-115.

Sulaiman M, Hamzat IT, Bashir MA, Saidu H, Yusuf H, Jibrin NM, Abdulrahman I. (2013). An overview of natural plant antioxidants: analysis and evaluation. *Advances in Biochemistry* 1(4): 64-72.

Swati S, Nisheeth S, Madhusudan A, Megha J and Anish G. (2015). New Perspectives in Exploiting Secondary Metabolite Glycosides as Drug Leads. *Journal of Molecular Biomarkers & Diagnosis* 6(6): 1-4.

Taibur R, Ismail H, Islam MMT and Shekhar HU. (2012). Oxidative stress and human health. *Advances in Bioscience and Biotechnology* 3: 997-1019.

Thamizhmozhi M, Suraj B and Swarnalatha S. (2015). Evaluation of antiurolithiatic activity of whole plant of *Veronia cinerea* Linn. *International Journal of Experimental Pharmacology* 5(1):31-336.

Thenmozhi P, Prasanna SSG, Kannan M and Sathiyarajeswaran P. (2016). Overview on selected medicinal plants used in the management of diabetes mellitus. *World Journal of Pharmacy And Pharmaceutical Sciences* 5(10): 391-400.

Todt H and Fozzard HA. (1997). Principles of Medicinal Biology, Molecular and Cellular Pharmacology. *JAI Press Inc*. pp 501-518.

Trease EA and Evans WC. (2002). Pharmacognosy, 15th ed., Churchill Livingstone *Harcourt publishers Ltd*. London: pp. 204-393.

Tripathi BK and Srivastava AK. (2006). Diabetes mellitus: Complications and therapeutics. *International Medical Journal of Experimental and Clinical Research* 12(7): 130-147.

Tripathi V and Verma J. (2014). Different models used to induce diabetes: a comprehensive review. *International Journal of Pharmacy and Pharmaceutical Sciences* 6 (6): 29-32.

Tundis R, Loizzo MR and Menichini F. (2010). Natural Products as -Amylase and -Glucosidase Inhibitors and their Hypoglycaemic Potential in the Treatment of Diabetes: an update. *Mini-Reviews in Medicinal Chemistry* 10:315-331.

Vaidehi P and Rajesh P. (2016). The active constituents of herbs and their plant chemistry, extraction and identification methods. *Journal of Chemical and Pharmaceutical Research* 8(4):1423-1443.

Vetter J. (2000). Plant cyanogenic glycosides. *Toxicon* 38:11-36.

Villar AM, Heras B, Rodríguez B and Bosca L. (2003). Terpenoids: Sources, Structure Elucidation and Therapeutic Potential in Inflammation. *Current Topics in Medicinal Chemistry* 3: 53-67.

Vella A. (2012). Mechanism of action of DPP-4 inhibitors—new insights. *Journal of Clinical Endocrinology and Metabolism* 97(8): 2626–2628.

Visweswara RP, Madhavi K, Dhananjaya NM. and Gan SH. (2013). Rhinacanthus nasutus improves the levels of liver carbohydrate, protein, glycogen, and liver markers in streptozotocin-induced diabetic rats. *Evidence-Based Complementary and Alternative Medicine* 1-7.

Wada O. (2004). What are trace elements? Their deficiency and excess states. *Japanese Medical Association Journal* 47(8): 351–358.

Wink M. (2015). Modes of action of herbal medicines and plant secondary metabolites. *Medicines* 2: 251-286.

World Health Organization, 2013. In: World Health Organization (6th Ed.), WHO Traditional Medicine Strategy 2014–2023. WHO Press, Geneva, Switzerland 1-78.

World Health Organization (WHO). 1976. Afro Tech. Rep. Series. Brazaville, 3-4.

World Health Organization. (1994). Management of diabetes mellitus standards of care and clinical practice guidelines. Diabetes prevention and control 1-35.

World Health Organization. (2002). W.H.O Traditional medicine strategy 2002-2005.W.H.O Publication 1-6.

Winterbourn CC. (2013). The Biological Chemistry of Hydrogen Peroxide. *Methods in enzymology* 528: 3-25.

Yaro AH, Aliyu M, Borodo SB and Balarabe NA. (2018). Antihyperglycaemic and antihyperlipidaemic activities of ethanol leaf extract of *Eleusine coracana* (Linn.) Gaertn. in alloxan-induced hyperglycaemic rats. *Journal of Applied Pharmaceutical Science* 8(7): 28-32.

Yi L and Zhongjie S.(2010). Current views on type 2 diabetes. *Journal of Endocrinology* 204: 1–11.

Young EE, Okafor CN, Okwara CC. (2016). Diabetes mellitus, associated co-morbidities and complications - *A review. Journal of Medicine and Medical Sciences* 7(3): 47-55.

APPENDIX 1

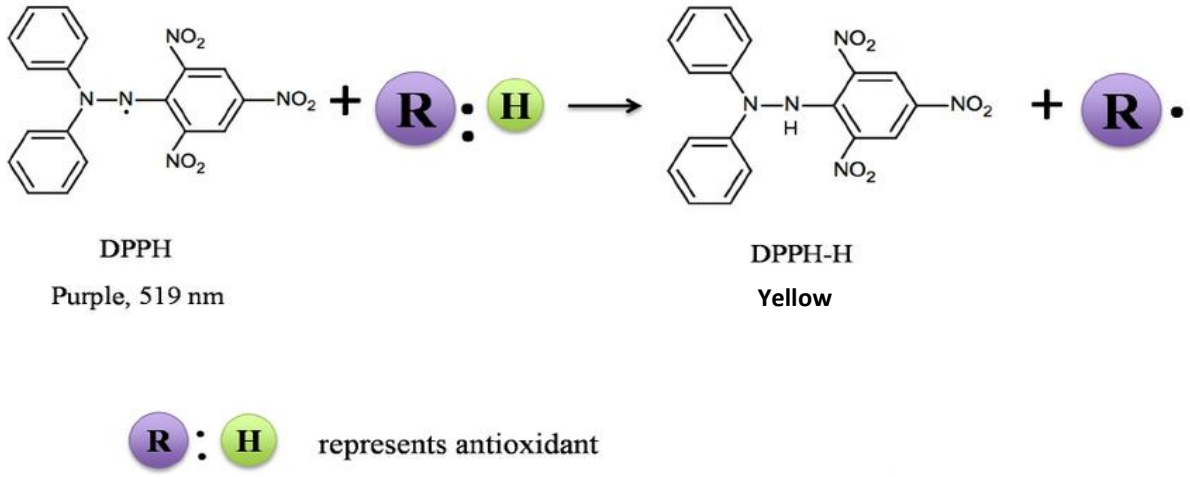


Figure 1: 1, 1 diphenyl, 2, picrylhydrazyl (DPPH) free radical scavenging activity of antioxidants.

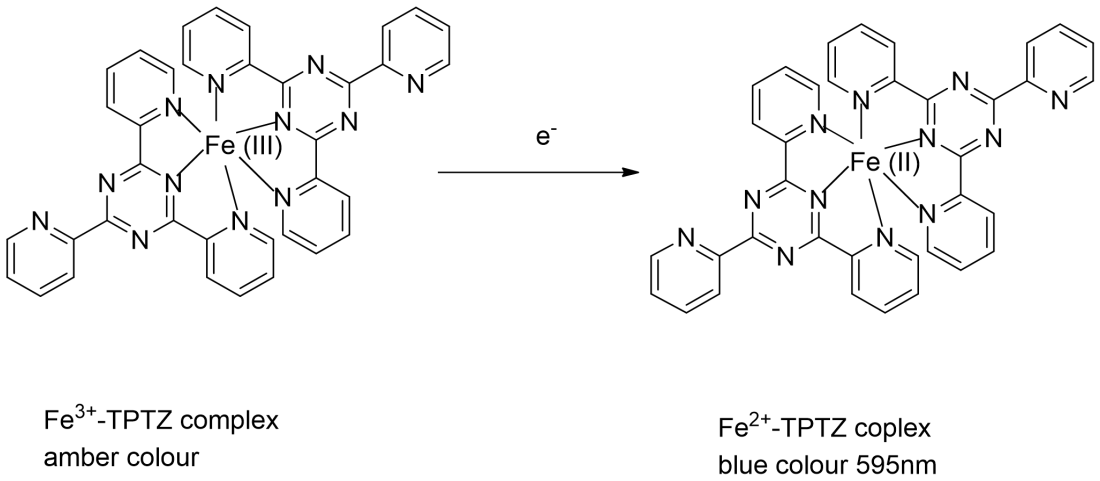


Figure 2: Reaction between ferric ion in tripyridyltriazine complex and antioxidant

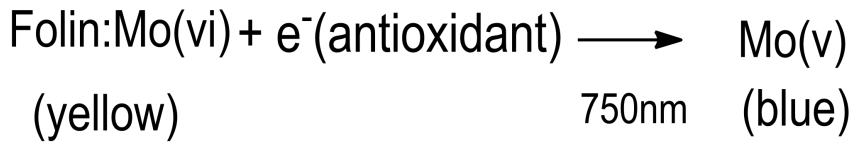


Figure 3: Reaction between antioxidants and Folin Ciocalteu reagent

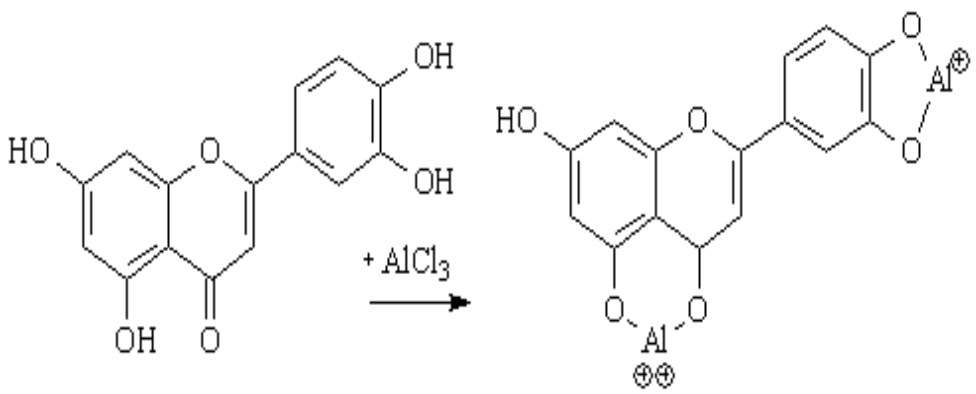


Figure 4: Reaction of flavonoid with aluminium chloride forming a colour complex

APPENDIX 2

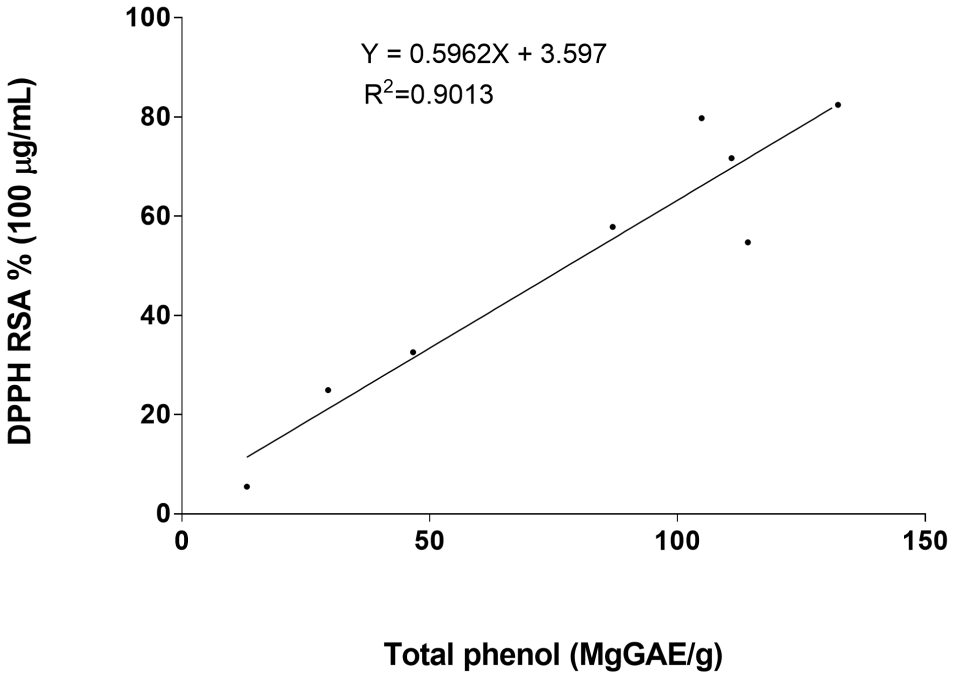


Figure 5: Correlation plot between total phenol content and percentage DPPH free radical scavenging activity of *Eleusine coracana* seed.

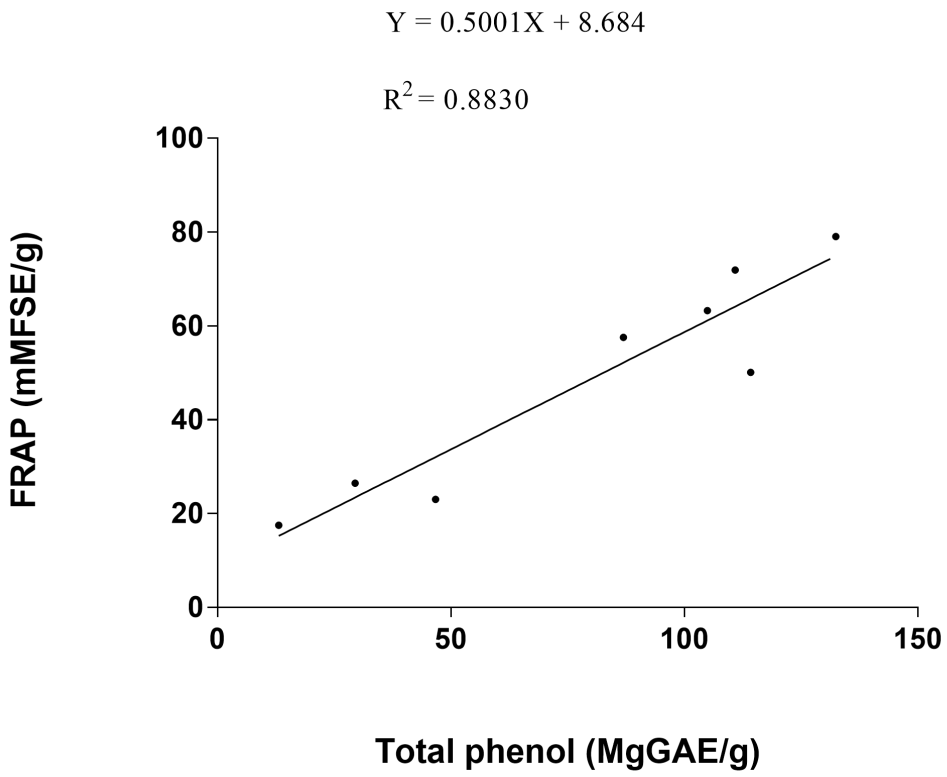


Figure 6: Correlation plot between total phenol content and ferric reducing antioxidant power of *Eleusine coracana* seed.

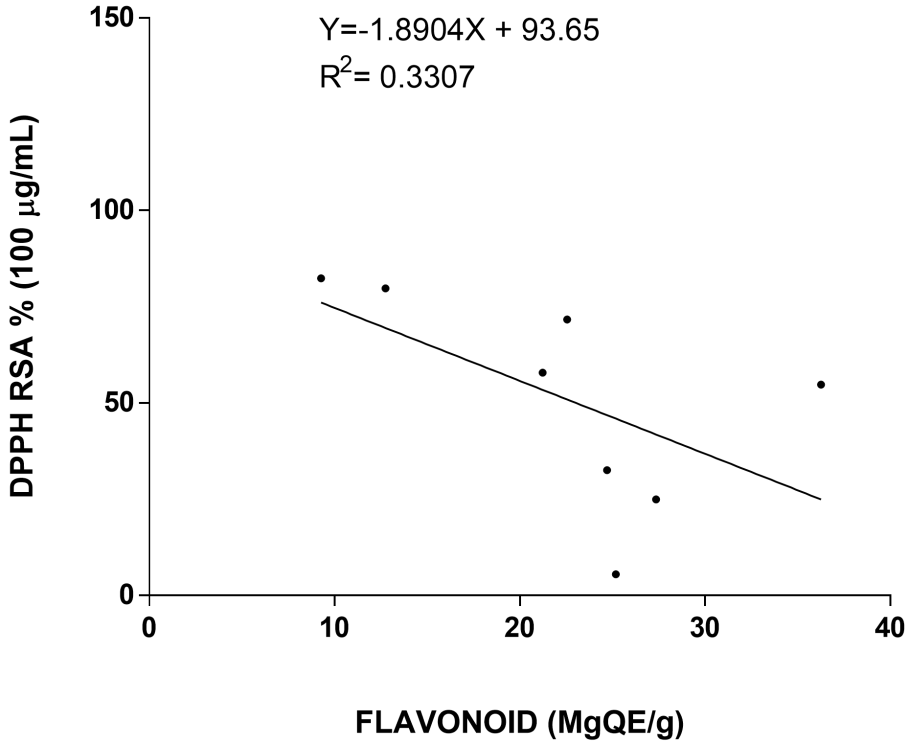


Figure 7: Correlation plot between flavonoid content and percentage DPPH free radical scavenging activity of *Eleusine coracana* seed.

APPENDIX 3

Table 1**:** Elemental constituents of *Eleusine coracana* seed

Metals	**Concentration1 (PPM)**	**Concentration2 (PPM)**	**Concentration 3 (PPM)**
Sodium (Na)	13.86	11.24	9.88
Potassium (K)	19.30	20.50	19.35
Calcium (Ca)	380.00	350.00	356.00
Magnesium (Mg)	1.06	1.05	1.06
Zinc (Zn)	0.34	0.37	0.33
Cadbium (Cd)	0.00	0.00	0.00
Lead (Pb)	0.01	0.01	0.00
Iron (Fe)	2.68	2.82	3.01
Copper (Cu)	0.20	0.16	0.20
Nickel (Ni)	0.00	0.00	0.00
Chromium (Cr)	1.32	1.09	1.29

APPENDIX 4

Table 2: Acute toxicity profile of *Eleusine coracana* seeds

Group	Weight (g)	Volume (mL)	Strength (mg/kg)	24 hr mortality	14 days mortality
I	16.6 16.0 15.0	0.35 0.33 0.31	10	0/3	0/3
II	22.3 20.0 17.8	0.47 0.42 0.37	100	0/3	0/3
III	30.0 26.6 19.1	0.63 0.54 0.40	1000	0/3	0/3
IV	18	0.38	1600	0/1	0/1
V	16	0.33	2900	0/1	0/1
VI	32	0.67	5000	0/1	0/1
VII (Control)	16	0.5	nil	0/1	0/1