CHEMICAL CONSTITUENTS AND MEDICINAL APPLICATIONS OF SELECTED PLANT SEEDS AS POTENTIAL THERAPY FOR DIABETES MELLITUS

BY

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ABSTRACT

The use of synthetic drugs for management of diseases such as diabetes is associated with medical challenges such astoxicity, cost and availability. Previous studies have reported the use of stems, leaves and barks of plants as recipes for managing diabetes. Due to underutilisation of selected plant seeds in the recipes for the management of diabetes mellitus, there is need for further investigations. Therefore, the aim of this study was to evaluate the chemical constituents and medicinal applications of some locally sourced plant seeds.

Seeds of *Erythrococc aanomala* (EA)*, Cyperus esculentus* (CE), *Butyrospermum paradoxum* (BP)*, Blighia sapida* (BS), *Crotonpenduliflous* (CP)*, Parkia biglobosa* (PB), *Momordic acharantia* (MC), *Monodora myristica* (MM), *Monodora tenuifolia* (MT) and *Picralima nitida* (PN) were purposively collected and identified at the Herbarium Unit of Botany Department, University of Ibadan. The seeds were cleaned, air-dried, pulverised, digested and analysed for constituents elements by atomic absorption spectrophotometry. Extraction was carried out with methanol and concentrated on rotary evaporator. Qualitative and quantitative phytochemical and antioxidant constituents were determined following standard procedures.Fatty acid and bioactive contents of the extracts were analysed by Gas chromatography flame-ionization detector and Gas chromatography/Mass spectrophotometer, respectively. *In vitro* anti-diabetic assay was conducted on all the extracts using Bio-Tek ELIZA reader and Omega Flurostar ®. The most potent extracts were further subjected to *in vivo* test in wistar rats with alloxan-induced type 2 diabetes. Data was analysed using ANOVA at $\alpha_{0.05}$.

The concentrations in mg/kg of the predominant elements in the seeds of the plants were Mg, 99.13±0.17 (EA); Ca, 108.35±9.27 (BP); Fe, 83.47±6.80 (CE); K, 99.18±1.20 (CP); Ca, 86.79±1.97 (MT); K, 87.95±1.40 (BS); K, 52.94±1.47 (MM); Ca, 86.79±1.97 (MT); K, 98.98±1.05 (PN) and K, 83.99±0.06 (PB). Flavonoids, saponins, terpenoids, phenol, carbohydrates and sterols were detected in all the extracts. The phenols content ranged from 48.27±1.95 to 146±61 mgGAE/g, while flavonoids was 6.29±0.26 to 29.54±0.40 mgQE/g. PN displayed highest antioxidant activity against DPPH and FRAP with IC_{50} (µg/mL) of 12.20±0.75 and 1.04±0.03, respectively. Non essential fatty acids such as palmitic (1.16% to 13.46%), palmitoleic (0.27% to 6.26%), stearic (0.57% to 11.34%), arachidic (0.27% to11.06%), and oleic (2.50% to 30.70%) were confirmed in all the plant seeds. Similarly, essential fatty acids were linoleic (2.18% to 42.72%) and linolenic (0.38% to 6.40%). Octadec-9-enoic acid (54.80%, BP); 9, 12-Octadecadienoic acid methyl ester (53.68%, CE); Hexadecanoic acid methyl ester (7.82%, CP); Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl (17.99%, EA); Octadecanoic acid methyl ester (22.68%, MC); α-Cubebene (4.93%, MT); 8, 11-Octadecadienoic methyl ester (28.41%, BS); Octadecanoic acid methyl ester (34.86%,MM); Octadec-9-enoic acid (65.14%,PB) and Oleyl alcohol (23.96%,PN) were identified. *In vitro* screening revealed that all the extracts have antidiabetic activities, while *in vivo* test showed that BS, MM, PB and PN significantly lowered the fasting blood glucose.

The seeds of *Blighia sapida*, *Monodora myristica*, *Parkia biglobosa* and *Picralima nitida* have antioxidant, essential elements, fatty acids and phytochemicals which might be useful for the development of appropriate drugs for diabetes management.

Keywords: Antidiabetic activity, Phytochemical screening, Essential fatty acid, Antioxidant activity
Word count: 488

CERTIFICATION

I certify that this thesis was undertaken by **RAJI, AKEEM ADEWALE** under my supervision in the Department of Chemistry, Faculty of Science, University of Ibadan, Ibadan, Nigeria.

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DEDICATION

This thesis is dedicated to the Almighty God, the Creator of the heaven and the earth and to my entire family.

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CHAPTER ONE

INTRODUCTION

The large dependence of both human and animals on plants have made plants a very essential and indispensable commodity. Plants have acted as a source of food, shelter and clothing to both human and animals and these advantages have been exploited since olden days. Many plants and their part have been employed as an adjuvant in food diets for the treatment of several diseases even without having the proper knowledge of their composition and functions. This practice is due to the scarcity, cost and side effects of many drugs that are synthesised (Balaraman *et al.,* 2010). The medicinal value of plants cannot be over-looked especially before the invention of modern medicines. Though many drugs were designed and developed for the treatment of ailments but the safety and 100% efficiency of most of these drugs are yet to be established. Medicinal plants or foods are widely used even when their active ingredients or the bioactive compounds are yet to be identified because they are readily available, cheap, effective, and considered safe (Dewanjee *et al.,* 2009).

World Health Organization (WHO) has recommended the evaluation of traditional plants being used for the cure of several diseases and they have been considered effective and found to be the best for oral therapy (Shokeen *et al.,* 2008). The search for plant bioactive compounds for the treatment of diseases has gained considerable attentions in the world (Okwu, 2001). Herbal preparations form the basis of many therapeutic drugs and are the first materials being employed for the treatment of aliment for many of the world's population, because of their availability (Okpara *et al.,* 2007). Herbal medicinal products are assuming greater roles globally in the face of global challenges of drug toxicity, resistance, adverse effects and increase in the costs of synthetic drugs (Mbi and Bilikha, 1998). In Nigeria, thousands of plant species have been reported to have medicinal properties and are being exploited for the treatment of several diseases (Iweala and Oludare, 2011). Plants are highly nutritional due to the presence of fats and oil, protein content, carbohydrates, minerals, vitamins and water

which are essential for the growth and development in man and animals. Plants of medicinal values are essential source of bioactive compounds with potential therapeutic effects (Kuhn and Winston, 2000). Presently, plants and their parts play major roles in diabetes management, most especially in Africa where 80% of the people have limited access to synthetic antidiabetic drugs (Acharya and Shrivastava, 2008).

Furthermore, plants seeds are good sources of edible oils and fat diet because of their nutritive and calorific values (Akubugwo *et al.,* 2008). They have also been used as part of food component since antiquity (Parry *et al.,* 2005). The seeds and seed oils can also be used for domestic purposes and medicine. Seeds are essential part of diet, and their required daily intake can prevent diseases; their consumption have been linked to low occurrence of cancer mortality rate (Willet, 1994), and heart diseases. There are numerous literature data on the mineral composition; proximate composition and other analysis on some seeds that have been reported by Ajayi and Aghanu (2011); Ajayi *et al.* (2012); Ajayi and Raji (2013) and Ajayi (2014) but information on the therapeutic uses of plant seeds are lacking. Presently, 90% of plant seeds are being discarded which could be used as raw materials for pharmaceutical industries for the production of drugs and other products. Economic viability of majority of the seeds could be achieved by the effective utilization of the plant seeds. And to achieve this, more information on the use of seeds and their chemical composition is essential and thus, necessitating the investigation of some selected seeds for their antidiabetic activity.

Diabetes Mellitus (DM) is known to be a metabolic disorder characterised by hyperglycemia caused by deficiency in insulin, it causes protein and lipid alteration in the body and in carbohydrate metabolism (Patel *et al.,* 2012). It is a well-recognized disease in developed countries with high prevalence. It is divided into two types: a disorder based on genetics and a disorder related to the dietary (Grover *et al.,* 2002). Among the reported cases of diabetes, type 2 diabetes was reported to be more common (Balaraman *et al.,* 2010). In most of the developed countries, diabetic patients are above sixty years of age, unlike developing countries where it affects people in their prime. A considerable progress has been achieved in diabetes management and its mechanisms understanding. However, diabetes and its related complications continue to be on the increase (Tiwari and Madhusudana, 2006). Diabetes effects have been found to be mediated via oxidative stress with rise in reactive oxygen species

production and impaired antioxidant defence systems that are cause by glucose oxidation, lipid peroxidation, antioxidant enzymes alteration and metabolism of many digestive enzyme (Dewanjee *et al.,* 2009). The scarcity, cost, side effects and unwanted materials of synthetic antidiabetic agents for diabetes treatment have made medicinal plants/foods widely prescribed for diabetes patients (Dewanjee *et al.,* 2009).

1.1 Research Justification

Globally, diabetes mellitus prevalence has been on the increase and most especially in adults who form a global public health burden. Diabetes management without significant side effect is a problem to medical community. Presently, no satisfactory potent therapy is available for diabetes mellitus cure. Efforts have also been on the increased towards developing and refining treatments as well as addressing the underlying causes of the disease. Though, insulin and different synthetic drugs have been used for the management of diabetes, it has numerous side effects like brain atrophy, insulin resistance, fatty liver and anorexia nervosa after chronic treatment. Regardless of the progress recorded so far in the management of diabetes using synthetic drugs, traditional medicine for the treatment is still in use throughout the world. Large number of the countries in the world has a documented history of plants that are used for the treatment of diabetes; these can be attractive and be an alternative to expensive pharmaceutical medicines. New therapies are also required due to inability of current drugs to control all of the pathological areas of diabetes, and plant-based medicines are required as a result of the poor availability and the high cost of the drugs for the people in local areas, particularly developing countries.

However, scientific investigation of medicinal plants for diabetes treatment may serve as leads for novel drugs development. Accordingly, there is an increasing interest in identifying new phytochemicals with proven pharmacological effects on diabetes from plants. In Nigeria, the selected plants are readily available, not expensive and several parts (stems, leaves, roots and barks) have been used for years back for the treatments of diabetes and related diseases but their seeds are underutilised. In this work, plant seeds of *Butyrospermum paradox*, *Blighia sapida*, *Cyperus esculentus*, *Erythrococca anomala*, *Croton penduliflorous, Momordica charantia, Monodora myristica*, *Monodora tenuifolia, Parkia biglobosa,* and *Picralima nitida* were selected with the aim of providing scientific information regarding their chemical composition, *in vitro*

inhibition against some enzymes linked to diabetes and its related diseases and *in vivo* antihyperglycemic effect.

1.2 Aim and objectives

The general aim of this study was to determine the chemical constituents and medicinal application of ten selected plant seeds.

The specific objectives for this study are categorised into three viz:

1. Chemical composition of the seeds determination:
   - Screening of the phytochemical constituents of the seeds
   - Estimation of metal content of the seeds
   - Establishment of the antimicrobial activities of the selected seeds against some pathogenic bacteria and fungi
   - Determination of the seed oil/extracts fatty acid components

2. Evaluation of antioxidant activity of the selected seeds which involved
   - 2,2-diphényl-1-picrylhydrazyl (DPPH) radical scavenging activity of the seeds
   - Ferric reducing power (FRAP) of the seed samples
   - The seeds total phenols and flavonoids contents

3. Antidiabetic property and cytotoxic effect of the selected seeds:
   - Identification of new inhibitors of α-glucosidase, β-glucosidase, aldose reductase, aldehyde reductase, antiglycation and pancreatic lipase porcine enzymes
   - Evaluation of inflammatory (15-LOX) and cytotoxicity effect of the seeds
   - Oral glucose and sucrose tolerance test
   - Investigation of antihyperglyceamic effect of the most active seeds enzymatically in type 2 diabetes to be modeled in mice or rat

CHAPTER TWO

LITERATURE REVIEW

2.1 *Butyrospermum paradoxum* (Sapotaceae)

Butyrospermum paradoxum (Shea butter) is a deciduous plant of *Sapotaceae* family. The plant is a native to the Central Africa and West-African savannas. *B. paradoxum* tree bark is rough and corky (deeply cracked) (Ajayi *et al*., 2015). The plant has milky latex in the branches and stems. The fruits are greenish yellow and are of great economic values. The tree is majorly available in the North, East and Western parts of Nigeria (Adgidzi, 1999). The physical and mechanical properties of the plant are being affected by the moisture content (Sitkei, 1986). Moisture content also has high effects on the plant storability, handling and processing of any of the biomaterials. The main source of shea butter is the kernel that is extracted during several hours of processing, to make a kilogram of the butter. The shape of the fruits is like large plums and skin are very smooth and has an oval shape.

The shea butter from the shea nuts contains high amount of minerals and vitamins that are required daily to improve people's well-being. It can also be used to improve the digestibility of some main dishes, texture and tastes. Shea butter can also serve as source of income for livehood. It can also be processed into cosmetics, chocolates, soap and can also be applied for various pharmaceutical uses. It can be used for the production of wax, for dressing of hair and candle production. It can be used to produce margarines, and also as component of animal feeds. The bark is used in children to cure skin diseases, scratches and cuts. It also has anti-inflammatory property and it also has the ability to lower cholesterol in human. The ointment has also been reported to have anti-inflammatory activity (Akihisa *et al*., 2010).

2.2 *Blighia sapida* (Sapindaceae)

B. sapida belongs to Sapindaceae family and its origin could be traced to Western Tropical Africa. The plant was brought to Jamaica in 18th century and spread to America tropical areas, but largely grown in Jamaica more than any other country. In Nigeria, *Sapida* is known as Ackee and commonly called Isin (Yoruba), Okpu (Igbo) and Gwanja Kusa (Hausa). The tree is evergreen and it is about 33 to 40 ft (10-12 m) tall and the branches are densely spread. Ackee (*B. sapida*) is also believed to be an underutilized spread throughout tropical and subtropical areas of Africa (Assi, 1988). The height of the tree can be up to 10 m or more with distinctive heavy dark green foliage and bright red fruits (Keay *et al.,* 1989). *B. sapida* is used for the treatment of several classes of disease in developing countries. In traditional system of medicine, the pulp from the bark can be used to treat pains in Ivory Coast. The leafy and pulp from the bark can be applied on the eyes for the treatment of conjunctivitis. The roots from the plant are used in combination with *Xylopia aethiopica* to remove pregnancy. The seeds aqueous extract has been used to chase parasites in Brazil. The leaves and bark stomachic properties have been reported by Morton (1987).

The plant extract was reported by Antwi *et al*. 2009 to have anti-diarrheal activity. The bark can also be employed for the cure of skin diseases. The fruits are rich in saponins, with haemolytic property. The dried husks ashes and the seeds can be processed into soap. The combination of *B. sapida* stem with *Allium capa L.* and *Capsicum frutescens* L, (Solanaceae) can be made into powder to treat gonorrhea. The fruits are good repellant of insects (Khan and Gumbs, 2003). The shape of the fruit is capsule-like (Olorode, 1984) with up to three fleshy valves which split open when it ripe; the seeds are elongated black. The seeds from the fruits are not edible, only flesh that is edible. The aril from the fruit can be eaten raw or after frying in oil. Beside the food usage of the plants, there are other additional benefits of the three economically. The aril can be processed into lather in water, which can be used for coloring fabrics locally. The fragrant from the flower can be used as cosmetic and the light hardwood commonly used as timber. Oil from the seeds is being used to making soap and husks are employed in making soap because of their high content of potash (Olapade, 1997).

2.3 *Cyperus esculentus* (Cyperaceae)

C. esculentus is a well known yellow nut sedge crop that grown in temperate region and tropical areas around the globe; is widely grown in United State of America (Moore *et al.,* 1998), Italy and Spain as the temperate zone. It is found in abundance in the tropic area of India and West Africa. It is a plant growing to length of 90 cm, it is a perennial or annual plant and the stems are always growing from the tube (Coskuner *et al*., 2002). The production of the plant is by seeds, tubers and the rhizomes. The shapes of the stems are triangle with slender leaves of 3-10 mm wide. The colour varies from straw to gold-brown colour and it produces up to 2420 seeds per plant. The foliage of the plants is very tough and fibrous and always mistaken for a grass. The diameter of the tubers is between 0.3-1.9 cm with various colours between yellow, black and brown (Oderinde and Tairu, 1988). *C. esculentus* can produce between several hundred to multiple fold of thousand of tubers during a particular season. During cool weather the roots, rhizomes foliage and basal bulbs die but the tubers survive when the soil temperature is above 6 ℃ and survive up to several years later.

C. esculentus is popular nut with different applications. It can be used to replace almonds in confectionery. The oil from nuts is known as chufa oil with suitable application in cooking and industries for the manufacture of hair and body creams. The powder from the dried nuts is incorporated into the foods. The presence of high amount of soluble glucose, oleic acid and energy content in the nuts has made the plants to be very important in health sector. They are also rich in nutritional elements required for body growth (Belewu and Belewu, 2007). The sugar free milk from the nuts has been reported to be good for diabetic patient. The presence of high amount of arginine in the nuts is good for the production of insulin. High content of oleic acid in the nuts have also been reported to have good effect on cholesterol thus preventing heart attack and risk of cancer reduction. The milk from the nut has been used in China as liver tonic heart stimulant and cure for several diseases. The black species of *C. esculentus* is also good for the treatment of breast lumps and cancer (Adejuyitan et *al*., 2009). The tubers have good antioxidant activity as a result due to significant amount of glycosides and flavonoids (Chukwuma *et al*., 2010). The tuber of the nuts also has ability to protect or fight against respiratory infections.

2.4 *Croton penduliflorous* (Euphorbiaceae)

C. penduliflorous is a shrub-like tree that originated from China and other Asia countries it belongs to Euphorbiaceae family. The seeds are used for medicinal purposes and can be harvested during autumn; the seeds are sun dried and make into powder and roasted (Azuzu, 1989). *C. penduliflorous* comprises of different 1200 species and come out during the warmer regions of the world. Up to 65 species can be found in continental Africa and nothing less than 125 in Madagascar. One of the species can be found in East of Sierra Leone to Nigeria, Central Africa Republic and Gabon (Keay, 1958). The seeds are of economic important and are being sold locally in Nigeria and the herbal laxative can also be produced from the seeds (Ashafa, 2012).

C. penduliflorous is a monoecious, medium sized tree with the height up to 25 m and with glabrous branches. The shape of the leaves are alternate, stipules tiny, simple, it also has petiole which is about 2-5.5 cm long, blade elliptical to ovate- elliptical, glabrous; 6-14 cm x 3.5-8 cm, base rounded to cuneate, with 2 glands on 2-3 mm long stalk, glabrous to sparsely short, apex shortly and abruptly acuminate, margins toothed, and hairy (Keay, 1958). The flowers are inflorescence with few female flowers and elongated terminal raceme 28-42 cm long, pendulous, with numerous flowers male intersperse. The unisexual flowers are 5-merous, regular, white, male flowers with ovate sepals, the length is about 1.5 mm it is hairy, the petals obviate is 2 mm while the upper margin is hairy, woolly with 12 free stamen; female flowers and oblong sepals, 2 mm long, petals are not present, ovary superior, rounded, densely hairy 3 celled, styles 3, apex 2. The plant is common in lowland forest, mostly in rocky or dry areas (Keay, 1958).

In Ghana and Cote diovie the leaf infusion of *C. penduliflorous* is used to treat menstrual disorders and fever. The plant seed extract can be used orally for the cure of uterine tumours, stomach and can also be used for abortion (Odesanmi *et al*., 2006). The combination of the seeds and cassava are taken as a purgative agent (Asuzu *et al*., 1989). Rafts are made from the wooden yellow part of the plant. The oil crystal from the seeds has significant protection against gastric and duodenal ulcer when orally administered at a moderate dose in rats (Asuzu and Chineme, 2006). The oil crystals also reduced sleeping time in mice when induced with pentobarbitone and also the analgesic action intensity of the opioids has prolongment action (Asuzu *et al*., 1989).

2.5 *Erythrococca anomala* (Euphorbiaceae)

E. anomala belongs to the family of Euphorbiaceae which is made up of 50 species of Africa origin and four out of the species are peculiar to Southern Africa. It can also be found in Cameroon (Burkill, 1985) and Gabon. *E. anomala* is commonly called wild pepper which is about 3.0 m in height (Burkill, 1985). The bark could be brown and flaky, and brown. The flowers from the plant are minute, unisexual; most of the flowers are whitish to pale yellow in colour and calyx 4-lobed corolla absent. The flowers which are males have 9-15 stamens while the female types of the flowers contain superior ovary in 3 lobed. *E. anomala* fruit is 2-3 lobed capsules and are red when they are fully matured. The seeds are globular shaped, pitied, and rapped by a thin orange of red aril. The plant is one of the fast growing heliophile flowers during the end and beginning of both rainy and dry seasons. *E. anomala* is widely distributed and not in danger of any genetic erosion.

The plant has analgesic and antiseptic properties. In Ivory Coast, its leaf sap is instilled into the nose and eyes or ears for sinusitis, colds, ophthalmias and inflammations of the outer ear (Burkill, 1985). The pulp of the fruit is rubbed topically for the treatment of localized pain; a decoction of leafy twigs is used to cleanse sores, ulcers, craw-craw, and yaws ulcers. Leaves powdered with maleguetta pepper furnish a snuff for chronic headaches. The Igbo of Nigeria use the bark for arthritic and rheumatism conditions (Adjanohoun and Ake-Assi, 1979). Leaf sap is dripped into the eyes for eye troubles in Sierra Leone. The Akye of Ivory Coast reduce the leaves to a powder with those of *Cephaelis penduncularis* (*Rubiaceae*) and some kaolin and rub the mixture with a little water onto the body of infants suffering from malaria and meningitis (Adjanohoun and Ake-Assi, 1979).

A decoction of the plant may also be used on feverish children, as a purgative agent. In Guinea, the plant has a high reputation and is used for the treatment of tapeworms. An extract from the leaf can be employed to treat toothache. The pulp from the fruit and the root barks are used as tonic when the body is weak. Twigs and leaves are reported to contain 0.1% total alkaloids. Over 1% concentration of the alkaloid recorded for the roots and up to 1% in the bark and a very strong presence has been found in the seeds of the Nigerian sample. In the Danane area of Ivory Coast the plant has the magical power to protect the followers of the snake from snake bite.

2.6 *Momordica charantia* (Cucurbitaceae)

M. charantia L. (Bitter gourd) has been known for long time for its food and medicinal purposes (Chakravarty, 1990). It is an important plant with both medicinal and economical values. It is from a family of Cucurbitaceae and popularly referred to as bitter melon or bitter gourd in English. It derived its Latin name from *Momordica* known as "to bite" from the look of the leaves. The leaves have jagged edges which appear as if they have been bitten; China and India is the main centre of domestication (Yang and Walters, 1992). *M. charantia* is called different names because it grows well in tropical areas like tropical Africa, America, India, Malaysia, China, Middle East, (Sultana and Bari, 2003) and Thailand. The plant is of two varieties which have been mostly cultivated in India. One of the varieties *is* charantia that has large fruits with fusiform shape; the second type is *muricata* that can be identified by small, round fruit (Chakravarty, 1990).

The plant adapts to different climate although it perform better in hot areas. The whole plant is bitter and the mode of propagation is by seeds, bitter gourd produces flowers within 30 days of planting, and produce mature fruits about 20 days after that. The fruits that are not mature are consumed as vegetables and known to be good source of phosphorus and iron, vitamin A and C (Paul *et al.*, 2009). *M. charantia* is highly medicinal and different medicinal properties of the tree have been reported. It has been reported by Kubola and Siriamornpun (2008) that the fruit of the plant has antioxidant property. Anti-inflammatory and immune modulatory was reported by Manabe *et al*. (2003). It also has the following properties; hypoglyceamic, hypolipidiamic activity (fruit) and anticancer antileshmania and antiallergic. The melon aids digestion like most bitter foods. In 2006, Kirtikar and Basu reported the usefulness of the fruits for the treatment of leprosy, jaundice and pile. The seeds and fruits also have anti-HIV, antimicrobial and antidiabetic properly. *M. chrantia* vegetable can also be used to cure diseases of liver and skin ailments (Chakravarty, 1990).

2.7 *Monodora myristica* (Annonaceae)

M. myristica (Annonaceae) is a plant that grows in most of the African countries like Nigeria. The tree is popularly known as Ariwo in the Southern part of Nigeria (Fournier *et al.,* 1999). It is highly nutritional and is used as a seasonal agent because of its flavour. The kernel is a spicing agent (Ekeanyawu *et al.,* 2010). The aromatic nature of the seeds has made it a stimulant being added into snuff (Uwakwe and Nwaoguikpe, 2008). The bark can be employed for the treatment of ailment such as stomach-aches, haemorrhoids, febrile pains and eye diseases and can also be employed as an antisickling agent (Weiss, 2002). The seeds are also used as condiment for headache and hypertension treatment in Central Africa (Koudou *et al.,* 2007).

M. Myristica has a lot of nutritional values as a result of its seasoning agent due to the aromatic flavuor. The seeds kernels are known as a popular condiment used in Africa as spicing agent. The seeds are used as stimulating agent in snuff (Ekeanyawu *et al*., 2010). The seed powder can also be used as condiments in food with a flavour similar to that of nutmeg. The Central African Republican is also using the seeds as condiment for the treatment of hypertension and headache. The bark can also cure stomach aches, eye diseases, febrile pains and haemorrhoids treatment. *M. myristica* has antisickling effect demonstrated by Weiss in 2002. The kernel can be made into soup by acting as a stimulant to relieve constipation, passive uterine and haemorrhage control in women after child birth. Many studies have reported the economic important of every part of *M. myristica* which include carpentry, housing fittings, joinery and the seeds can be made into necklaces (Ngueface *et al*., 2004).

2.8 *Monodora tenuifolia* (Annonaceae)

M. tenuifolia (Annonaceae) is a tree with different names in Nigeria which includes African nutmeg in English, Igbo; Ehuru ofia, Yoruba; Abo-lakoshin. The tree which is about 17.0 m tall can be found in the evergreen and fringing forest, and in secondary and regenerating thickets, occurring from Guinea to West Cameroons and Fernando Po and from Cameroun and Gabon to Zaire. The seed has many important medicinal values (Ezenwali *et al,* 2010). The toxicity of flavonoids of the plant was examined on albino rats (Ekeanyanwu, 2013). The seed oil fatty acid composition was also analysed and its suitability as edible oil was determined by feeding the oil to albino rats (Ajayi *et al.,* 2012). It was discovered from the experiment that seed oil is safe because there

was no report of anaemic from the result of heamatological analysis of the blood sample conducted or mortality during the study.

M. tenuifolia is well known in West Africa where it being used for both medicinal and ornamental plant (Burkill, 1985). It can be used to treat diseases such as toothache, dysentery, dermatitis, headache and vermifuge (Adeoye *et al.,* 1986). The seeds can be used as parts of herbal ingredient due to its aromatic nature. The seeds can also be used as a spice in food and as flavouring agent. The roasted powdery seeds can be rubbed on the skin for the treatments of skin infections. The oil from the plant parts have been reported to have antimicrobial activity (Njoku *et al.,* 2005).

2.9 *Parkia biglobosa* (Leguminosae)

P. biglobosa is a legume plant that belongs Leguminosae family and sub family of Mimosoidaea. It is well known in Nigeria as African locust beans in English, dawadawa in Hausa and Igba/Iyere in Yoruba. The trees can be seen in the savannah area of Nigeria but can be found in populations of two or more (Hopkins, 1983). It is readily available and widely distributed within savanna belt of Nigeria, and is the most common species of the parklands agro-Forestry system (Scande and Clethero, 2007). It is a deciduous tree with the height ranging from 7 to 20 m but can reach 30 m under exceptional conditions (Hopkins *et al.,* 1984). It is grown in Western India including some tropical regions in America (Hopkins, 1983). The seeds contain 30 % of protein as well as vitamins and minerals while the pulp contains 60 % sugar when ripe (Sacande and Clethero, 2007). During the dry season, the striking red spherical inflorescences are always appearing and often used as games by children (Burkill, 1995). The powder inside the seed pods are sweet and can be made into drinks and eaten without preparation.

It has high medicinal and antimicrobial importance (El Mahmood and Ameh, 2007). The black liquid used for sealing floors can be made from the pods. Dawadawa a black, strong smelling, tasty food that is high in protein can be produced from the seeds when fermented (Steinkraus, 1996). In Nigeria, the locust bean is processed into consumable delicious food locally and is a part of traditional dishes in different parts of the country. The leaves, fruits, nuts and oils obtained from the plants are foods for livestock, human and wildlife (Latif *et al.,* 2002). *P. biglobosa* roots, barks, leaves, stems, flowers, fruits and seeds are used for the treatment of different ailments such as

diarrhea, ulcers, pneumonia, burns, coughs, jaundice etc (Sacande and Clethero, 2007). The *P. biglobos*a trees have been used for ecological purposes like nutrients recycling from deep soils. It can also provide shades for farmers (Campbell-Platt, 1980). The trees have economical uses, which include production of pestles, mortars, bows, seats and hoe handles. The fruit can form part of ingredient for production of different stews, sausages and soups for cereals consumption made into cakes and also for the production of some indigenous drinks

2.10 *Picralima nitida* (Apocynaceae)

P. nitida is well known as genus of Picralima which is related to *Pleiocarpa* and *Hunteria.* The tree is well known as Picralima, Akuamma or Pile plant which belongs to family of apocynaceae. It has distribution in deciduous forest of countries in West-Central Africa (NNMDA, 2008). *P. nitida* is an understory plant which is about 4-35 m tall with diameter of the trunk close to 5-60 and cylindrical. The flowers are white and the wood is pale yellow. The leaves are long, oblong and broad with lateral nerves close to pairs of 14 to 24 (Burkill, 1985). The tree applications are very varied in all the countries within West Africa folk medicine. All the plants parts are being used for the treatment of diseases like hypertension, malaria, jaundice and intestinal disorders (Burkill, 1985). The extracts from the plant and parts have been reported to have some important pharmacological activities which have led to the increase in the ethno-medicinal use of the plant. Bioactive compound like Indole alkaloids have been isolated from the plant with analgesic activity (Ezeamuzie *et al.,* 1994).

P. nitida is a plant of high medicinal values with various uses; the parts have been employed as herbal extract for the treatment of ailments in human. The seeds are commonly used in Nigeria, Cote d'Ivoire and Ghana as aphrodisiac for malaria, chest, pains and pneumonia treatment (Kouitcheu *et al*., 2008). The seeds are applied for the treatment of diseases externally in Gabon, while the decoction from the seeds can be applied to treat enema in Ghana. The fruit is used in most of the West Africa countries for gastrointestinal disorder and dysmenorrheal treatment. In Ghana, the combination of the fruit shell with palm wine is used as fever remedy. The leaves and leaf sap can be used for the treatment of otitis in the ears. The bark has laxatives; anthelmintic and purgative properties while the decoction from the bark has jaundice property (Burkill, 1985). The root has cure for fevers, gastrointestinal disorder and pneumonia.

2.11 Phytochemicals

There are different definitions for phytochemicals. Phytochemicals are bioactive chemical components of plants which are non-nutrient in nature. They are medically active and protect plants from various infections either from microorganism or pests (Doughari *et al*., 2009). They can be defined as compounds derived from plants, hypothesized to be protecting plants and animal against diseases (Arts and Hollman, 2005). Hasler and Blumberg (1999) defined phytochemicals as biologically active, chemical compounds that occur naturally with the main sources in plants of high health benefits. They are available in all the plant parts such as seeds, barks, fruits leaves, roots, grains and rhizomes. The phytochemicals include alkaloids, cardiac glycosides, flavonoids, saponins, terpenoids, tannins, phenol and carbohydrate etc.

Phytochemicals are secondary metabolites of various physiological and biological activities. The common properties of these phytochemicals are antioxidant, enzymes detoxification, prevention of infections by preventing growth of microbes, immune system stimulation, and anticancer. Furthermore, phytochemical analysis of medicinal plants has revealed enormous compounds in plants being used traditionally for medical purposes. Most of these chemical constituents are strong bioactive chemicals in plant parts which could serve as starting materials for the synthesis of drugs with high values medicines (Sofowora, 1993). The level of phytochemicals in plant species depends on some factors such as varieties, conditions required for the plants to grow, processing conditions and cooking method. Presently over 4,000 bioactive chemical compounds have been isolated and characterised from plants with different chemicals and physical properties (Meagher and Thomson, 1999). Extensive studies have been carried out to establish plants efficacy and their mechanism of actions. Some studies have revealed that phytochemicals have the ability to reduce fasting blood glucose in diabetes and coronary heart disease and others like anticancer, enzymes inhibition and radical scavenging (Meagher and Thomson, 1999). The medicinal value of plants to the health of communities and individuals is based on the bioactive chemical substances that exert a specific physiological action in the body system.

Phyotochemical are products produced along metabolic process in plants and are known as metabolites (alkaloids, Cardiac glucosides, flavonoids, saponins, terpenoids, tannins, phenol and carbohydrate). The most crucial ones among them are the bioactive components like phenolic compounds, flavonoids, tannins and alkaloids (Edoga *et al.,* 2005). Motivation of people towards herbs is increasing due to their concern about the side effects of drugs and the ones prepared from synthetic materials. Prior to the plant extracts pharmacological evaluation, phytochemical screening is essential and the first stage in understanding the medicinal plants active principles.

2.11.1 Phenols

Phenols are the largest class of phytochemicals which are abundant in plant. They are very large and complex component of plants (Harborne, 1980). Polyphenols are compounds of the plant secondary metabolism that can accumulate in certain plant organs such as leaves, fruits, roots and stems. As a large group of bioactive chemicals, they have diverse biological functions. Because they are essential to plant life, they can provide defense against microbiological attacks and make food unpalatable to predators and other herbivores (Vogt, 2010). The consumption of fruits and vegetables, rich in phenolic compounds, has been associated to healthy diets and to the prevention of several chronic diseases because of the antioxidant properties of these compounds, which act as reducing or metal chelating agents, hydrogen donors and singlet oxygen quenchers (Tsao and Yang, 2003). Moreover, phenolic compounds can prevent disease through mechanisms that differ from antioxidant function, such as cellular signaling, gene expression, and modulation of enzymatic activity (Milenkovic *et al*., 2011).

They are compounds with different hydroxyl groups (-OH) that are directly bonded to aromatic hydrocarbon. Phenols are grouped into three and the most important ones are the dietary phenolic compounds. Phenols show differ properties which are highly beneficial to humans and their antioxidant activities are very essential in determining their protecting nature against free radical-mediated disease processes (Milner, 1994)
. This group of phytochemical is mostly studied. Some are in form of hydroxybenzoic and hydroxycinnamic acids. They are classified as polymers of high molecular weight which could be hydrolysable or condensed class of tannins.

2.11.2 Flavonoids

Flavonoids are ubiquitous class of polyphenolic compounds. Over 4,000 types of flavonoids are found in fruits, beverages, tea, vegetables and fruits that have been fully recognized (Robard, 1997; Cheynier, 2005). Since ancient time, flavonoids have been playing a crucial role for the treatment of different diseases and also antioxidant agent up till present time. They are found in plant parts normally eaten by animals and different classes of the compounds have been reported. Aglycones are the flavonoids normally found in small amount without sugar in plants. The flavones and the flavonols are known to be six-membered ring condensed with the benzene ring. The benzenoid substituent positions always classify the flavonoids. Majority of them occur naturally in association with conjugated sugar (Pretorius, 2003).

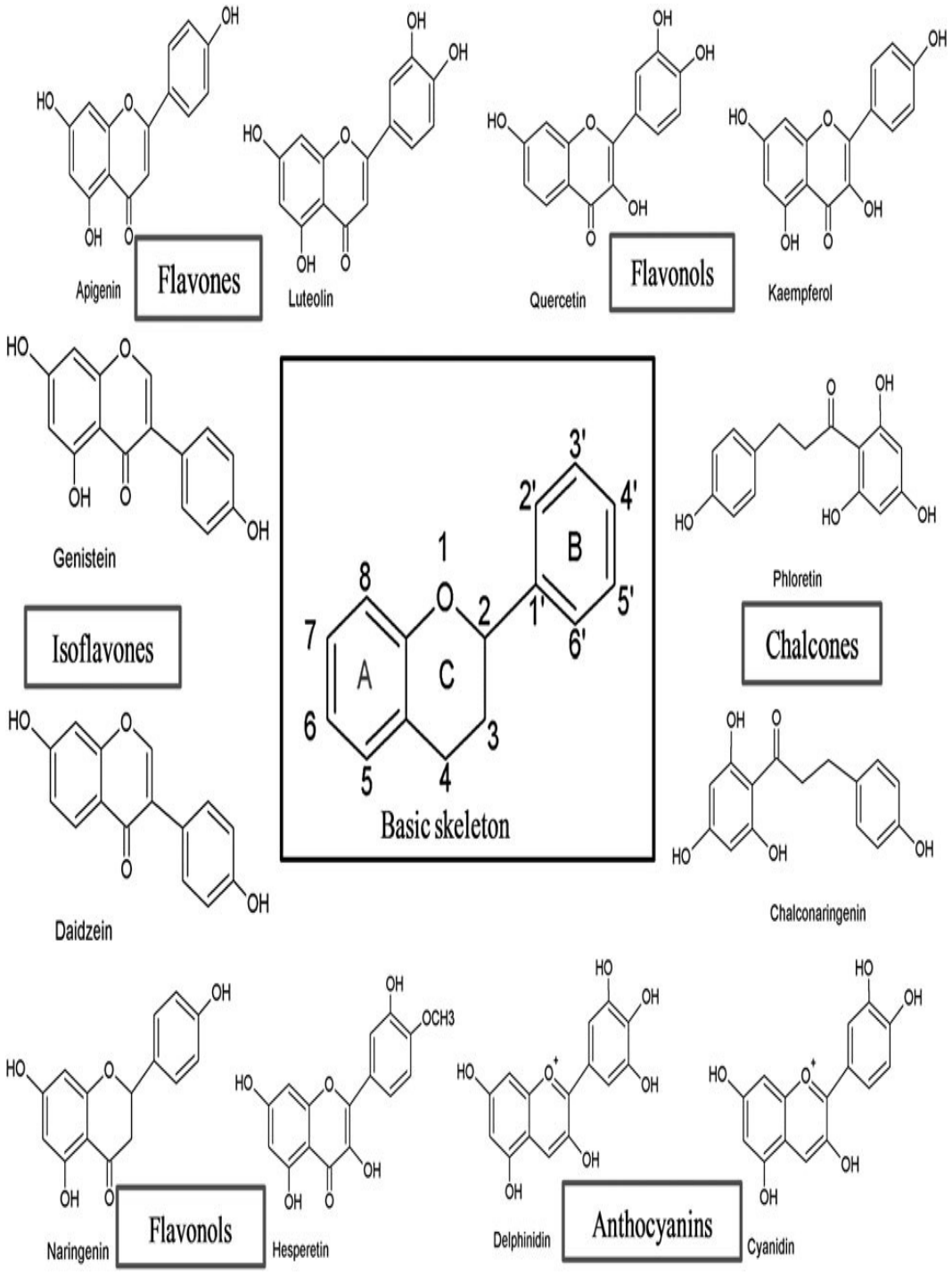


Figure: 2.1. Basic skeleton structure of flavonoids and their classes ((Panche *et al.*, 2016)

2.11.3 Antioxidant role of phytochemicals

Antioxidants are chemical substances that shield cells away from destructive effects of radicals like super oxide, peroxynite, hydroxyl radicals, singlet oxygen and peroxyl radical known as reactive oxygen species (ROS) (Mattson and Cheng, 2006). Antioxidants from the natural origins are good in maintenance of body health system, prevention and generation of chronic diseases e.g. atherosclerosis, cardiac and cerebral ischema and diabetes. Antioxidants act by scavenging radicals out of the body cells by creating very stable compounds. These are chemicals in metastable condition, which could trap electrons from any molecule within their vicinity. If the radicals are left not scavenged in time, they may cause damage to some molecules like proteins, lipid, DNA, and membranes protein during diabetic conditions.

Diabetes and some other diseases have been linked to the presence of the ROS in the body. Insufficient production of antioxidant within the body system to prevent oxidative stress could be compensated for by the use of natural antioxidants from plants and some other essential plant natural products. Plants have different types of natural products that have high antioxidant activity which can easily get rid of reactive oxygen species within the body system (Cai and Sun, 2003). Vitamin C and some bioactive compounds (flavonoids, phenols) from leafy vegetables and citrus fruits have the potential to scavenge free radicals. Plants with high amount of phenols, ascorbic acids and flavonoids are potential antioxidant and are required to reduce occurrence of different diseases (Anderson and Teuber, 2001). Antioxidants are always introduced into food to avoid initiation of long chain radical reaction of oxidation. As a result of safety issues of conventional drugs, food industries are now making use of plant natural antioxidants for the replacement of synthetic compounds. In addition, the consumers demand for natural antioxidants from plants are now on the increase, which have given more opportunity to explore natural antioxidants from plant materials.

2.11.4 Phytochemicals mechanism of action

The modes of action of plants phytochemical constituents have been reported to be in different forms. Phytochemical constituents in plants have some mechanisms of actions with strong potential to inhibit growth of microorganisms, interference in process of metabolism, and modulation of gene expression and transduction of pathways signal. Phytochemicals can be used as agents for chemo prevention to

inhibit, retard or reverse tumorigenesis. The phytochemicals which are chemo-preventives have been used for the treatment of cancer (Sarkar and Li, 2006). Extracts from plants and other essential oils can show different action modes against different bacterial strains either by interfering with cell lipid layers with the increased cellular permeability or cellular constituents' loss, destruction of enzymes involved cellular energy production and structural components synthesis and genetic material activation (Kotzekidou *et al.,* 2008). There are different mode of actions proposed through which the plant phytochemicals can regulate the level of blood glucose. The action exerted by a particular plant can correspond to the plant potential or the principle of the active ingredients in it, to reduce the blood glucose level either by the interference with one or more of the processes related to glucose homeostasis. The suggested mode of actions reported for most of the plants with antidiabetic properties are very similar to the ones suggested for conventional antidiabetic agents (Bnouham *et al.,* 2006). The suggested modes of actions include:

i) Insulin synthesis stimulation from beta-cells of the pancreas
ii) Regeneration of pancreatic beta cells
iii) Insulin sensitivity improvement
iv) Plants ability to act as insulin (Mimicking insulin)
v) Enzymes activity alteration
vi) Retardation of carbohydrates absorption from the gut

2.11.5 Phytochemicals safety concerns

Plants are power house of natural products that could be employed in treating various diseases that are free of possible adverse effects caused by conventional drugs (Fennell *et al.,* 2004). WHO reported that 70% of the world population still relies on the use of herbal products as a result of its acceptability culturally, compatibility, and minimal side effects. The over dependent on conventional medicines with high level of unwanted materials which is the cause why the side effects is higher has been the cause of re-introduction of natural medicines for safer ailments treatment. The challenges of geographical locations of the plant growth and different vernacular names have made WHO to publish the standards for herbal products so as to ensure their safety (WHO, 1999). Medicinal plants have been used for food and medicinal purposes with the information in circulation from different set of people to another (Adedapo *et al.,*

2005). This is based on the information that herbs are relatively safe (WHO, 1999). Although, plants that have medicinal values have been reported to be safe to use, but the combination with conventional drugs should be carried out with care. The use of herbal medicines and synthetic drugs should not be encouraged or it should be with care (Liu *et al.,* 2009). The combination of the two may cause either pharmacokinetic or pharmacodynamic interactions with the adverse herbal-drug interactions. Simultaneous use of conventional drugs with some herbal medicines may cause reduction in blood plasma concentration and possibly resulting in suboptimal therapeutic amounts, or accumulation of toxic materials in the blood. Trace amount of phytochemicals in fruits and vegetables have the potential to boost the immune system and protect tissue cells against risk of cancer.

2.12 Fatty acid

Fats and lipids are component of foods that performs crucial roles in man, they have high health benefit. Lipids work by reducing the risk of diseases and also enhance promotion of good health (Roberfroid, 2000). The conjugated linoleic acid, lipids like Omega-3, phytosterol, triglycerides and omega-6 are commonly recognized lipids known as functional lipids, because they have several health effects to human beings; they are used in treatment of diseases such as blood pressure, obesity, diabetes and in management of depression (Moreau, 2011). More so, the available scientific reports have disclosed encouraging improvements and beneficial health effects of the use of functional lipids as dietary supplement in patients and healthy people. Free fatty acids (FFAs) which functions as source of energy in form of nutrients and signaling molecules in many cellular process have also been linked to secretion of insulin that involve plasma glucose elevation response and various modifying factors in insulin secretion (Henquin, 2000). Recently, several reports propose that polyunsaturated fatty acids and their metabolites could also play a highly significant role in management of diabetic retinopathy (DR), known as an acute complication of long standing diabetes mellitus. In addition, the metabolism of fatty acids like α-linolenic acid and linolenic acids and their derivate metabolites could be, incorporated into the phospholipids part cell membrane in reacting to numerous stimuli like growth factors, free radicals and cytokines. Arachidonic acid forms starting material to pro-inflammatory prostaglandins. Linoleic acid (LA) and arachidic acid (AA) have also been reported to prevent damage of retinal vascular endothelial by high glucose-induction (Shen *et al.*,

2012). High oleic acid in plant or vegetable oils has been reported to be good and it works by reversing the negative effects of inflammatory in non insulin dependent diabetes mellitus and obesity (Vassiliou *et al.,* 2009).

Oleic acid also has the tendency to lower blood pressure (Teres *et al.,* 2008) and low density lipoprotein cholesterol level (O'Byrne *et al.,* 1997), and high ratio of oleic to linoleic acids increases the seed shelf life of the seeds. Fats and lipids are part of food component and offer many beneficial effects to human health during development of any ailments such as obesity, diabetes, blood pressure and some others. They are also regarded as subset of known functional foods and have been reported to have benefits in physiologically reduction of chronic disease risk beyond basic nutritional functions (Moreau, 2011). More so, the available scientific reports have disclosed encouraging improvements and beneficial health effects of the use of functional lipids in dietary supplement in diabetic patients and healthy people. Free fatty acids (FFAs) which functions as source of energy inform of nutrients and signaling molecules in many cellular process have also been linked to insulin secretion from β cells of pancreas in response to plasma glucose elevation and various modifying factors in insulin secretion (Henquin, 2000). Though, FFAs are regarded to promote insulin in an acute phase with little understanding of the mechanisms (Dobbins *et al.,* 1998).

2.13 Mineral elements

These are elements that available in minute amount in the tissues cells but are essential for life process (Guidotti *et al.,* 2010). A large number of these elements are needed in high amount by the body and are known as macro elements (Simsek *et al.*, 2007) with the minimum daily intake of 100 mg/kg per each element such as sodium, potassium and magnesium. On the contrary, mineral elements with daily intake lower than that of macro element are known as micro-elements. Generally, mineral elements are necessary for different physiological functions in the body. Metabolic functions of micro mineral elements in the body strictly based on their required quantity in the tissue cells; their imbalance can affects pancreas beta cells and eventually diabetes development (Chen *et al.,* 2009). Mineral element also has profound effect on the production and generation of radicals (ROS) during diabetes. Most of the metals are readily available in soluble forms as salt that regulate biofluids composition and their normal metabolic function depends on their normal range in the tissue cells (Guidoti *et al.,* 2010). The deficiencies of some of the elements have been reported to have some

effects during diabetic condition, e.g. Cr deficiency can lead to blood glucose elevation in the tissues. Toxic metals reaction with different tissue proteins can modify their functions and kinetics (Flora, 2009). The presence of some toxic metals in the body tissue may induce toxicity that can cause derangement of mechanism of antioxidant and high reactive oxygen species (ROS) generation (Guidoti *et al.,* 2010).

2.14 Antimicrobial activity

Any active substances that kill or inhibit microbes' growth like bacterial, fungal and viruses are known as antimicrobial. It kills microbes (microbial) or prevents the growth of microbes (microbistatic). They play significant roles in infectious disease treatment and as one of the most essential weapons in combating infections from bacterial and fungal. They have served enormous benefits to the health related quality of well-beings of human ever since their production. However, for the past decades, the health benefits of readily available antibiotics has become a threat due to their resistant or ineffectiveness against some illness and their toxic nature to the host cells; and intestinal and gut microbes could create a hostile environment by making the antidiabetic drugs resistant or inefficient. It is eminent to investigate newer antimicrobial activity of the antidiabetic plants for their better efficiency. In addition, the roles of derived drugs from natural sources are significant in human diseases treatment. In most of the developing countries, one of their primary healthcare systems is traditional medicine (Houghton, 1995).

Plants natural products may serve as better source of new antimicrobial agents with new action (Runyoro *et al.,* 2006). The plant extracts effects on bacterial and fungal have been studies by a numerous researchers in the world (Reddy, 2001). WHO proposed that 800% of the population in the worlds depends on the plant extracts or their active compounds. Plants are source of bioactive molecules which are diverse in range, well known as rich based of different classes of medicines. For decades, plants have been serving as valuable natural products source for taking care of human health and control of health problems. Despite the availability of conventional drugs, there is still a continual search for new antimicrobial agents with low side effects; hence, plants are the only hope of antimicrobial agents' source. Antibiotics are one of the most essential weapons in combating infections from bacterial and fungal.

They have served enormous benefits to the health related quality of well-beings of human ever since their production. However, for the past decades, the health benefits of readily available antibiotics has become a threat due to their resistant or ineffectiveness against some illness and their toxic nature to the host cells. During diabetic, the roles of drugs or plants with significant antimicrobial is an added advantage to prevent the intestinal and gut microbes to develop hostile environment for the antidiabetic agents, thereby making it resistance to the specific action in diabetic patients. Higher plants natural products may serve as a new antimicrobial agent's source. The plant extracts effects on bacteria and fungi have been studies by a numerous researchers in the world (Reddy, 2001). World health organization (WHO) estimates that about 800% of the populations in the world rely on the plant extracts or their active compounds.

2.15 Uses of plants and plant products

2.15.1 Food additives

Vegetable oils can serve as food additives or preservatives that support processing and production and maintain the properties of food (Foulk *et al*., 2002). Their functions in food industry include; the improvement of the nutritional value of food, e.g. vitamins, minerals, and other nutrients, maintenance of palatability and wholesomeness of food, e.g. preservatives, antioxidants and sequestrates, provision of leavening or control of acidity/alkalinity, e.g. leavening agents, acidifiers, alkaline, buffers, and acid regulators and also and enhancement of colour and flavor (Sharma, 2015**)**. It can also serve as a preservative agent. The oil from vegetable can also be used as antimicrobial or antioxidant agent. The oils as an antimicrobial always prevent the microorganisms' growth like moulds, yeasts, and bacterial. If the vegetable oils are used as antioxidant, it prevents rancidity of foods, browning and the development of black spots (Bennion, 1995).

2.15.2 Cosmetic

Application of oil from vegetable in cosmetic industries and other related products have different benefits such as a pleasant favour, ensuring microorganisms' protection and in some cases; it improves cosmetic products properties and preservation (Manou *et al*., 1998). Scalp problems like dandruff and seborrheic dermatitis caused by *Malasseizia furfur* could be cured using tea tree oil due to the presence of terpinen-4-ol

which has been proved effective. However, application of vegetable oils as antimicrobial agents in cosmetic preparations is often discouraged because of their milder action compared to classical preservatives (Kabara, 1984), their properties which might add to the final product and a potential loss of antimicrobial action due to their volatility and lipophilicity.

2.15.3 Health care application

Plants and their possible products have been employed for years in healing, altering of moods, and enhancement of consciousness (Wagner *et al*., 1998). It affects the emotional, mental, spiritual, and physical state of human being. When they are diffused, it can get to the brain through olfactory system. Impulses are transported simultaneously to the limbic system and olfactory sensory centre at the brain base where they are being passed between pineal gland and pituitary when absorbed, and where body's natural healing system will be stimulated. Data have revealed compounds that are aromatic in nature can impart effects that are very strong to the brain, especially on the limbic system and hypothalamus. Some vegetable oils can increase oxygenation dramatically and their activity in the brain can cause increased negative ions and ozone (Scarpeci *et al.,* 2008), thereby prevent growth of bacterial and can alter nontoxic chemicals by interfering with their structure at molecular level.

2.16 Diabetes mellitus

Diabetes Mellitus (DM) is a metabolic disorder which occurs by the failure of beta cells of the pancreas to produce enough insulin or when the insulin produce by the pancreas cannot be effectively utilized by the body. Insulin is defines as an hormone produced by the pancreas that enhances the body cells to make use of glucose in the blood for energy. Insulin production failure or in proper action or both can increase the blood glucose levels in the body (IDF, 2011). The main manifestation of diabetes clinically is hyperglycemia. However, deficiency of insulin and/or resistance of insulin have also been linked to protein and lipid metabolism abnormalities, disturbance of electrolytes and minerals in the body system. Diabetes is one of the oldest known diseases. Some of the common symptoms associated with diabetes are excessive urination, excessive appetite and thirst (Cooke and Plotnick, 2008). These signs are as a result of high amount of glucose and failure of cells to make use of glucose as energy source.

It is one of the top five diseases that are highly significant in developed countries and mankind, the third killer after cardiovascular and cancer diseases due to its high prevalence, mortality and morbidity (Li *et al.,* 2004). It is now recognized that developing countries is presently facing the greatest burden of diabetes. The prevalence of diabetes is expected to be on increase within Asia and Africa, where more people will likely be affected by 2030. This is due to ageing population structure and the global increase in obesity as well as sedentary lifestyle (Vats *et al.,* 2002). Evidence from epidemiology study suggests that if preventive measures are not properly implemented, the prevalence will continue to be on increase.

Diabetes can be classified into three types Viz; type 1 diabetes, type 2 diabetes and gestational diabetes, and depends on the manifested clinical disorder. Type 1 is known as insulin-dependent (DM) that can occur due to beta cells of the pancreas destruction immunologically thereby causing insulin deficiency. It is also known as juvenile onset diabetes because it occurs in adolescents and young adults (Anderson *et al.,* 2006). Type 2 (DM) develops gradually and is characterized by the in ability of the body to make use of the available insulin or inability of pancreas to produce enough insulin by that can be utilised by the tissue cells (Ahmed, 2006). Type 2 diabetes has been on the increase rapidly and is becoming a global problem. This is most common in adults and the elderly; and is the most prevalence type of diabetes accounting for 90-95 % of all diabetic cases. Gestational (DM) is referred to as glucose intolerance which occur during pregnancy as a result of in effective ultilisation of carbohydrates and disappears after delivery. Resistance of insulin is one of the physiological changes that can take place during pregnancy.

2.16.1 Management of diabetes mellitus

Diabetes treatment is predominantly directed at microvascular and macrovascular levels with the use of synthetic drugs (Roman and Harris 1997). Presently, six types of oral drugs available for the treatment of diabetes are: sulfonylureas, biguanides, thiazolidenediones, meglitinides and dipeptidyl peptidase IV inhibitors, α-glucosidase inhibitors (Nathan, 2007). They are glucose lowering drugs which exert their effects through a variety of mechanisms. Presently, DM can now be managed by design of inhibitors or drugs that could inhibit or slow down some enzymes that are peculiar to some physiological reaction in the body.

2.16.2 Enzymes role in diabetes management

Enzymes are natural proteins functioning as catalysts that speed up chemical reactions by lowering the activation energy (Neet, 1998). They are also been referred to as biological catalysts that accelerate chemical reactions without equilibrium alteration. They are secreted to part take in digestion processes (Peter, 2015). They are available in the stomach, small intestine and could also be secreted by the pancreas to aid digestion of carbohydrates lipids, proteins and several minerals. Enzymes literally chop down large compounds such as starch into smaller pieces (maltose and dextrin) and eventually into component building blocks or units (glucose). These simple compounds are then absorbed to be used by the animal for maintenance and production purposes. Recently, enzymes inhibition is of the strategies that can be employed to design and develop drugs for some ailments like diabetes (Kim *et al.,* 2008) e.g. acarbose against diabetes ((Wehmeier and Piepersberg, 2005) and clavulanic acid which is used as inhibitors of reverse transcriptase. Thus, the evaluation of the selected seeds as natural inhibitors of α-glucosidase, β-glucosidase aldose reductase (ALR1), aldehyde reductase (ALR2) which has been linked to onset of diabetes and diabetes complications (Chatzopoulou *et al.,* 2011). Glucosidase enzyme is a class of enzymes that involves in the hydrolysis of the α-1, 4- and α-1, 6-glucosidic bonds of glycogen, yielding glucose. This type of enzyme is involved in breaking down of carbohydrate into glucose and plays a crucial role in diabetes development and management. They are of two types viz: α and β-glucosidase enzymes**.**

Aldo keto reductase is another type of enzyme that is associated with the development and control of diabetes complication due to inability to control sugar level (Jez *et al*., 1997). Pathogenic mechanism leading to complications of diabetes has been explained by a series of hypotheses and the most prominent theory proposes that under hyperglycemia condition, a significant amount of glucose enters polyol pathway in which aldose reductase is the key rate-limiting enzyme (El-Kabbani *et al*. 2005). They are also involves in reduction of aldehydes and ketones to alcohols of primary or secondary functional which is involved in phase (1) of xenobiotic bearing carbonyl groups and endogenous compounds metabolisms (Carper *et al*., 1989). Most of these reactions are often catalyzed by aldo-keto reductases. Aldo-keto reductases (AKRs) are present in almost phylla; they are soluble monomeric proteins (34-37KDa) and depend on NADPH for their oxidoreductases reactions (El-Kabbani *et al*. 1998). Aldo-keto

superfamily is further divided into 14 different types of sub-families, which starts from AKR1 and ends at AKR14 which are further sub divided into sub-families depending upon the type of catalytic reaction involved. They involve in catalysis of sugar (Bohren *et al.,* 1992), lipid aldehyde, Keto Steroids, Keto-prostaglandins and carcinogenic chemicals like derived nicotine nitrosamines.

Lipases are class of enzymes that are responsible for catalysis of triacylglycerols (TAGs) hydrolysis by releasing diacylglycerols and monoacylglycerols, with fatty acids as co-products (Beisson *et al.,* 2000). Modern biotechnology has designed numerous lipases classes with their applications in pharmaceuticals, foods and detergents while 15-Lipoxygenase enzyme also known as 15-LOX, an enzyme that catalyzing polyunsaturated fatty acids oxygenation, to produce carbon-cantered free radicals and some peroxyl radicals which are potential linked to atherosclerosis and diabetic complication (Radmark and Samuelsson, 2009). In addition, there is also a reaction which is non-enzymatic that is also peculiar to the to diabetes management known as Advanced glycation endproducts (AGEs) formation**.** This is a process whereby glucose reacts with protein amino groups to form Schiff bases. Formation of AGEs in the tissue can cause tissue damage during diabetic condition therefore, it management is essential to be able to control diabetes effectively. AGEs accumulation at various sites of diabetic patient like heart and blood vessels has been implicated in several pathologies which are associated to diabetes and ageing (Aldini *et al.,* 2013**)**. However, formation of AGEs occurs during ageing process, and its formation being accelerated under hyperglycemic condition. Glycation changes conformation of protein and stability and also induces protein aggregation and cross linking (Kumar *et al.,* 2004).

Though, the design of drugs with anti-AGEs activity is a challenge due to complexity of the reactions involved in AGEs formation. A hydrazine compound which is nucleophilic in nature (aminoguanidine) has showed a promising result for AGEs formation inhibition (Thornalley, 2003), it has entered three clinical trials. However, it was later stopped due to numerous safety concerns. Other agents like pyridoxamine (Voziyan and Hudson, 2005), carnosine, taurine and phenyl thiazolium bromide (Kim and Spiegel, 2013) have been experimented both *in vitro* and *in vivo* with a promising results but only pyridoxamine is now on clinical trial.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection and preparation

The seeds selection were based on the hypoglyceamic activity of some parts (leaves, flowers, and roots) used as additives in foods and herbal remedies for diabetes and some other related diseases to diabetes cure. *Erythrococca anomala*, *Picralima nitida*, *Monodora myristica*, *Momordica chrantia* and *Croton penduliflorous* seeds used for the study were purposively purchased in July, 2013 from a local market known as Bode in Ibadan metropolis, Oyo State. Seeds of *Parkia biglobosa, Butyrospermum paradox*, *Blighia sapida,* and *Cyperus esculentus* were collected from Saki West Local Government of Oyo State in January, 2014 while *Monodora tenuifolia* seeds were collected from the Botanical Garden, University of Ibadan, Oyo State. All the seeds were identified in Botany Department Herbarium Unit, University of Ibadan. The seeds were properly cleaned to remove sandy materials and later dried at room temperature, weighed and then grounded to coarse powder using a commercial grinder. The pulverized seeds samples were weighed and then stored at 25 ^{o}C for extraction.

3.2 Sample documentation

. The (ten) 10 selected plant seeds were documented as shown on Table 3.1.

Table 3.1: Seeds' name and code

No.	Plant name	Family	English name	Local name	Code
1	*Butyrospermum paradoxum*	Sapotaceae	*Shea butter*	Ese	BP
2	*Blighia sapida*	Sapindaceae	Ackee	Isin, Okpu, Gwanja kausa	BS
3	*Cyperus esculentus*	Cyperaceae	Earth almond		CE
4	*Croton penduliflorous*	Euphorbiaceae	Croton, Turks cap	Aworoso	CP
5	*Erythrococca anomala*	Euphorbiaceae	Cocca	Iyere-igbo	EA
6	*Momordica chrantia*	Cucurbitaceae	African cucumber, Bitter gourd, Balsam pear	Ejinwere, alo-ose, kakayi	MC
7	*Monodora myristica*	Annonaceae	Calabash nutmeg	Ariwo, Ehuru	MM
8	*Monodora tenuifolia*	Annonaceae		Ehina-wo, sinin	MT
9	*Parkia biglobosa*	Leguminosae	African locust beans	Igba/Iyere (Yoruba), Nere (Bambara	PB
10	*Picralima nitida*	Apocynaceae	Picralima	Erin, eso abere, Osu, igwe	PN

Butyrospermum paradoxum

Blighia sapida

Cyperus esculentus

Croton penduliflorous

Erythrococca anomala

Momordica chrantia

Monodora tenuifolia

Monodora myristica

Parkia biglobosa

Picralima nitida

Plate 3.1: Photograph of the seed samples sourced from Bode market in Ibadan and Saki west LGA of Oyo State

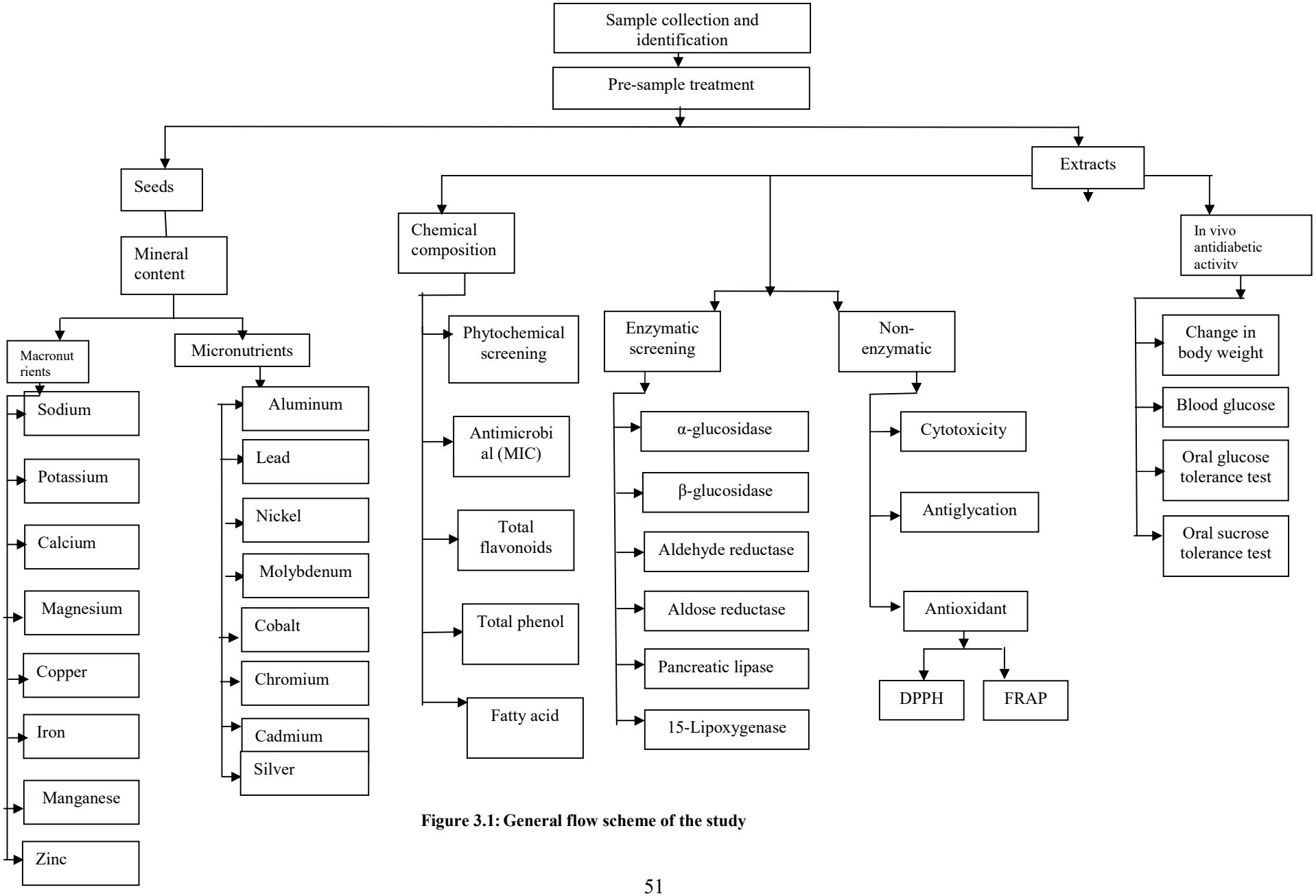


Figure 3.1: General flow scheme of the study

3.3 Equipment

The equipment used during the undertaken of this study are: rotary evaporator, centrifuge, GCMS, GCFID, ELIZA reader, Omega flurostar, UV-spectrophotometer, CO_2 incubator, 96 well plate, Permeable membrane, gluconometer, weighing balance and gluconometer strips.

3.4 Chemicals

The reagents and chemicals employed for this study were of grade required for standard analysis. All the solvents: hexane, chloroform, methanol, DMSO, 2, 2-diphényl-1-picrylhydrazyl (DPPH), potassium ferricyanide [$K_3Fe(CN)_6$], ferric chloride ($FeCl_3$), trichloroacetic acid (TCA), linoleic acid, hydrochloric acid (HCl), bovine serum album (BSA), gallic acid, quercetin, and Sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany), while enzymes and substrates: α-glucosidase (*Saccharomyces cerevisiae*), β-Glucosidase (Almonds), 15-lipoxygenase (15-LOX) isolated from soybean, *P*-nitrophenyl-α-D-glucopyranose, acarbose, *P*-nitrophenyl-β-D-glucopyranoside (pNPG), NADPH and pancreatic lipase porcine obtained were from Sigma-Aldrich (St. Louis, USA).

3.5 Extraction

The pulverised seeds of each selected plant were used for the extraction. This was done by packing 500 g ground seeds samples into an aspirator bottles and soaked with 2.0 L methanol for seven days with continous stirring. On the day seven, the extract was separated by filteration using vacuum pump connected with Buchner funnel under a reduce pressure. Rotary evaporator was used to concentrate the filtrate at 40 ^{o}C and 626 mmHg (pressure) quantity obtained was measured and refrigerated at -4 ^{o}C (Ajayi *et al*., 2015)

3.6 Phytochemical screening

The seed extracts were screened phytochemically to determine the presence of chemical constituents like flavonoids, tannins, saponins, alkaloids, reducing sugar and glycoside which could be responsible for their biological and physiological properties following the method previously used by Ugochukwu *et al.* (2013).

3.7 Macronutrient and micronutrient elements content of the seeds

This was achieved by digesting the seed powders obtained from the selected plants seeds differently in a digestion bottle of 100.0 mL according to Khemnani *et al.* (2012) described method. 10.0 mL conc. HNO_3 was introduced into the tubes to be used for the digestion with 1.0 g of the plant seeds kept for 24 h and later heated for additional 4 h at 50 ^oC. Each solution with the mixture of concentrated acids; HCl and HNO_3 in a ratio of 1:5 was boiled for 4 h to ensure dissolution of all the organic content that might be in the mixture. The mixture was separated by filtering into 25.0 mL standard flask after been cooled. The sample final volume was made up to the standard flask mark with deionized water. Atomic Absorption Spectrophotometer (AAS), Perkin Elmer (USA) with WINLAB 32 reading software was used after calibration with different concentrations of the standards of the metals to be analysed.

3.8 Antimicrobial activity

3.8.1 List of microorganisms used

The clinical isolates of bacterial flora such as *Staphylococcus aureus, Pseudomonas aerugonisa, Escherichia coli, Klebsiellae pneumonia, Salmonella typhi Bacillus subtilis and Bacillus subtilis were* the tested bacterial.and fungal such as *Aspergillus niger, Candida albicans*, *Rhizopus stolonifer* and *Penicillum notatum* were used in this study. All the organisms were supplied by the Department of Pharmaceutical Microbiology, University of Ibadan. The pure bacterial strains were cultured over night at 37 ^oC, the bacterial were cultured in a nutrient agar while the fungal were prepared over night at 28 ^oC using potato dextrose agar.

3.8.2 Antimicrobial activity assay

The seed extracts antimicrobial activity on the selected microorganisms was determined based on the guidelines for the clinical laboratory standards. Bacterial was cultured in Mueller Hinton (MH) broth over night at 37 ^oC and Potato Dextrose broth (PDB) was used to culture fungal for 72 h at 28 ^oC. The visible growth of the organisms cultured over night was tested on agar plates with various concentrations of the seed extracts (0-200 mg/mL) and standard antibiotics using disk diffusion prescribed procedures (Ajayi *et al.,* 2015). Antibacterial and antifungal activity of seed extracts was determined using the disc diffusion method of clinical laboratory standards. Muller Hinton agar plates for bacterial and Potato Dextrose broth (PDB) for

fungal were seeded with $2.0x10^8$ CFU/mL (0.5 McFarland's standard) of each of the test organism and sterile filter paper disks (6 mm diameter) containing 6.25-200 mg/disk of the extracts was placed on the agar surface and plates incubated at 37 °C for 18-24 h. The diameter of the zone of inhibition was measured to the nearest a millimeter using a transparent ruler. This was taken as an index of the degree of sensitivity of the test organisms to the extracts. All the seed extracts were reconstituted in DMSO, The positive control used was Gentamycin and Tioconazole for both bacterial and fungal respectively while extraction solvent was used as the negative control.

3.8.3 Minimum inhibitory concentration (MIC) of the seed extracts

Minimum inhibitory concentration for both bacterial and fungal selected for antimicrobial activity was carried out according to Ajayi *et al.* (2015). The most sensitive concentration of each seed extract was used for MIC determination using dilution method. Cells from each of the pure isolates were harvested from 18 h nutrient broth culture and washed in phosphate buffer buffer (0.05M, p^H 7.0) and resuspended in normal saline with turbidity adjusted to 0.5 McFarland's turbidity level (about $2.0x10^8$ CFU/mL. The broth dilution technique was utilized and a serial dilution to working concentrations between 0.3125 mg/mL and 10.00 mg/mL were made from the stock concentrations of the seed extracts used for antimicrobial activity determination in nutrient agar and later inoculated with 1.0 mL suspension of the test organisms. After 48 h at 28 °C for fungal and 18 h of incubation at 37 °C for bacterial and then, plates were observed for turbidity.

3.9 Determination of fatty acid composition of the seed extracts using GCFID

The seed extracts fatty acid content was determined by the conversion of seed extracts into fatty acid methyl esters (FAMEs). FAMEs of the seed extracts were made by the addition of *n*-hexane (1 mL) to 40.0 mg of the seed extracts followed by the addition of 200.0 µL sodium methoxide (2.0 M). The mixture was incubated in a water bath at 50 ^oC for 2 min followed by the addition of 200 µL of 2.0 M HCl solution (Fatnassi *et al*., 2009). The reaction mixture top layer was obtained and used for the determination of the fatty acid composition. The top layer from the reaction mixture (1.0 µL) was put into gas chromatograph (HP7820A, Agilent) equipped with flame ionization detector (FID) and HPINNOWAX 15m x 0.25 mm x 0.20 µm (HP) at the gradient temperature of 120 ^oC.

3.10 Determination of the seed extracts chemical composition using GCMS

The seed extracts chemical constituents were determined by Agilent 6890N (California, USA) gas network chromatographic system coupled with author injector 7683B and inert mass selective detector with a HP-5 MS capillary of a stationary phase of 5 % phenyl polysiloxane. The length of column was 30.0 m, i.d. 0.25 mm and thickness film of 0.25 µm. 1 µL from the prepared solutions of each of the seed extracts were injected into GCMS with split mode ratio of 1:20 at a temperature of 275 °C. Carrier gas was helium and used at a constant flow rate of 5 µL/min. Program temperature was at 50 °C initially and held for 1 min and then increased at the rate of 50 °C/5 mins up to 280 °C and kept constant for 5 min. Ionization energy of electron ionization mode was 70 eV with mass scanned range of 40–500 m/z. The identification of the mass spectra was done by comparing the peaks with those of the NIST mass spectra library (Muhanned *et al.,* 2015)

3.11 Evaluation of the seed extracts antioxidant activity

3.11.1 DPPH antioxidant assay

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging by the extracts was determined according to Ilyas *et al.* (2014) method, using Omega flurostar for the measurement of the absorbance. From the seed extract solutions, 10.0 µL each in triplicate was put into a 96 well plate; this was then followed by the addition of 90.0 µL of 330 mM DPPH methanolic solution. The reaction medium was incubated for 30 mins at a temperature of 37 ^{o}C and absorbance was taken at 517 nm with Omega fluoro star. Methanol and vitamin C were used as both negative and positive control for the assay. Inhibition percentage was calculated according to the formula below:

Inhibition (%) = [Absorbance $_{control}$ − Absorbance $_{test}$/ Absorbance $_{control}$] × 10

A serial dilution was further made for the seed extracts with percentage antioxidant activity above 50 % while the GraphPad Prism software (version 5.0) was used to determine the IC_{50} values.

3.11.2 Ferrous reducing power activity of the seed extracts

Ferrous reducing power activity of the extract was performed based on the procedure described by Singhal *et al.* (2011). 25.0 µL each of the seed extracts was mixed with 25.0 µL of phosphate buffer (pH 7.2) in a 96 well plate and 50.0 µL of 1.0 % $K_3(Fe(CN)_6$ solution was added latter. The mixture was incubated for 10 mins at 50 ^oC followed by the addition of 25.0 µL of 10.0 % TCA (% w/v) solution and 100 µL distilled water was added into it after this absorbance was taken. Finally, 25.0 µL of 0.2 % $FeCl_3$ solution freshly prepared was added into the resulting solution and the increased in absorbance was taken at wavelength of 700 nm on a UV spectrophotometer. Quercetin was used as the positive control and ability of the extract to reduce ferrous was determined by using the formula below:

Inhibition (%) = [Absorbance $_{control}$ − Absorbance $_{test}$/ Absorbance $_{control}$] × 100

Extracts with the inhibition percentage above 50 % were further dilluted to seven different concentrations for the determination of IC_{50} using GraphPad Prism software version 5.

3.12 Estimation of total phenols contents in the seed extracts

3.12.1 Preparation of gallic acid calibration curve for total phenols content

The total phenols content of the seed extracts was determined by using Folin-Ciocalteu method previously used by Hossain and Al-Saeedi (2015) with slight modification. The calibration curve for the measurement was made from the combination of 200 µL each of the six different concentrations of gallic acid in six separate test tubes, followed by the addition of 1.5 mL of 10.0 % solution of Folin-Ciocalteu reagent and then kept in a dark cupboard at room temperature for 5 mins. After then, 1.5 mL of 6 % Na_2CO_3 solution was added to each of the test tube and mixture was further kept in a dark incubator at a temperature of 40 ^oC for 2 h. The absorbance was determined with UV-visible spectrophotometer at a wavelength of 760 nm. Uwin software was used to plot the graph of the absorbance against the respective concentrations of gallic acid.

3.12.2 Seed extracts total phenols content determination

The seed extracts total phenols were determined from the prepared calibration curve (Fig. 3.1). This was carried out by weighing 1.0 mg each of the extracts into 2.5 mL of methanol, and then mixed thoroughly to obtained homogenous solution. 200.0 µL each from the seed extract solutions was introduced in triplicate into three different test

tubes and 1.5 mL of 10.0 % Folin-Ciocalteu reagent added, the mixture in the dark for 5 min. After then, 1.5 mL of 6 % Na_2CO_3 solution was added to each of the test tube and incubated at 40 ℃ for 2 h. Mixture absorbance in each test tube was determined under the calibration curve prepared at wavelength of 760 nm. The total phenols content was determined as (mg GAE/g dry sample).

3.13 Estimation of total flavonoids contents in the seed extracts

3.13.1 Preparation of quercetin calibration curve

The calibration curve was prepared from the combination of 500.0 µL of six different concentrations of the standard quercetin solutions in six separate test tubes, followed by the addition of 100.0 µL of 10.0 % aluminum chloride, 100 µL of 1.0 M potassium acetate; 2.8 mL of distilled water was then added to each of the test tube and mixed thoroughly. The solutions were kept in a dark place for 30 min at room temperature and absorbance was taken at 415 nm using UV-visible spectrometer curve. Measurement and the plot of the absorbance against quercetin concentration were done with uwin software installed in the PC couple to the spectrophotometer (Hossain and Al-Saeedi, 2015).

3.13.2 Seed extracts total flavonoids content determination

The total flavonoids content of the seed extracts were estimated by Hossain and Al-Saeedi (2015) method. The extract solutions and the blank were mixed with methanol (1.5 mL) in test tubes in triplicate, then 100.0 µL of 10.0 % aluminum chloride, 100 µL of 1.0 M potassium acetate and 2.8 mL of distilled water were added to each of the test tubes and mixed properly. The solutions were kept at 0 ^{o}C in a dark place for 30 mins and absorbance was taken at 415 nm by using UV-visible spectrometer couple with computer installed with uwin software for taking the absorbance and plotting of calibration curve while the blank solution was used for correcting the absorbance followed by each samples and the total flavonoids content was measured as mg QE/g dry sample.

3.14 Determination of antiglycation activity of the seed extracts

3.14.1 Antiglycation assay

The entire seed extracts antiglycation assay was carried out by the method previously used by Matsuura *et al.* (2002). Mixture in eppendorf tube containing 500 µL of albumin was incubated with 400.0 µL of glucose in the presence of 100 µL of all test

extracts for a week at the temperature of 37 ^{o}C. After a week, the samples were taken out and cooled at 0 ^{o}C and then terminated by the introduction of 10 µL of 100 % TCA (Trichloroacetic acid). The mixture was centrifuged at 10000 rpm at temperature of 4 ^{o}C for 10 mins and unbounded glucose in the supernatant, inhibitor and substances that could interfere with the reaction were removed after centrifugation. Pellets obtained at the bottom of the tubes were re-dissolved in 500 µL PBS (pH 7.4). Spectrofluorometer was used for the comparison of fluorescence intensity at 370 nm excitations and emission at 440 nm and standard inhibitor was rutin (Atta-ur-Rahman *et al.,* 2007). Inhibition was determined using the calculation below

% inhibition = 100-(OD (sample) /OD (blank) x 100

3.15 Enzymatic study

3.15.1 Extraction and purification of aldehyde reductase (ALR1) from bovine Kidney

Freshly slaughtered bovine kidneys were removed and cortex area of the kidney was dissected carefully and 3 volume of the sodium phosphate buffer pH of 7.2 with 10.0 mM containing sucrose 0.25 M, β-mercaptoethanol at 2.5 mM (homogenisation buffer) and ETDA dipotassium salt 2.0 mM were used for the tissues homogenisation. The homogenate was further centrifuged at 12000 rpm for 30 mins at 4 °C. The precipitate was discarded as it contains remaining insoluble lipids. As to obtain required ALR1, 40 % saturation with ammonium sulphate was subjected to the supernatant and then centrifuge again at 12000 rpm for 30 min at 4 °C. Supernatant was again subjected to 50 % saturation with ammonium sulphate after removal of the precipitate followed by centrifugate again at 12000 rpm and at temperature of 4 °C for 30 min. At the last step of centrifugation, 75 % saturation is obtained with the powdered ammonium sulphate with centrifugation at 12000 rpm at 4 °C for 30 mins resulting in ALR1 precipitation. The supernatant obtained was discarded and the pellet was dissolved in the buffer containing sodium phosphate 10 mM at pH 7.2 with β-mercaptoethanol at 2.5 mM and ETDA dipotassium salt 2.0 mM. Final product obtained from the last step was dialysed in the dialysis membrane over night. The dialysis membrane mixture which contains the extracted ALR1 enzyme was aliquoted and all were stored at -80 °C for further analysis (Silvio *et al.,* 2015).

3.15.2 Isolation and purification of aldose reductase (ALR2) enzyme from bovine lenses

Isolation of ALR2 enzyme was done based on the previous method of Silvio *et al.* (2015) with little adjustment. ALR2 enzyme was isolated from the bovine lenses immediately after being slaughtered and frozen until needed. The lenses were homogenized in 3 volumes of homogenisation buffer for 20 mins. Homogenate obtained was later centrifuged at 10000 rpm for 15 mins at 4 ºC to separate the unwanted materials. Precipitated material was discarded as it contained lipids. Supernatant layer was separated and ammonium sulphate salt was added to make the saturation up to 30 %. It was centrifuged at 10,000 rpm for 15 mins; and again precipitate was discarded. A pure ALR2 enzyme was precipitated by the addition of powdered ammonium sulfate to 80 % saturation. Supernatant was discarded after centrifugation and isolated enzyme was precipitated in 50 mM NaCl and dialyzed in 4.0 L of 50 mM NaCl over night. The volume of the suspension was recorded and the sample was dialyzed overnight against 50 mM NaCI (double replacement of dialysis solution). After dialysis, obtained enzyme volume was recorded and then stored in the eppendorf tubes in deep freezer at -80 ºC (Silvio *et al.,* 2015).

3.15.3 Protein determination in crude enzymes of ALR1 and ALR2

Protein quantification in the crude extract of ALR1 and ALR2 enzymes was performed using biuret test according to the method described by Gerardo *et al.* (2011) assay, spectrophotometrically. The method was reported as accurate and efficient for determination of protein content in solution or compounds having two or more peptide bonds due to formation of purple complex with biuret reagent.

3.15.3.1 Preparation of biuret reagent and calibration curve

To prepare biuret reagent, 1.51 g sodium potassium tartarate ($NaKC_4H_4O_6$) and 0.375 g of cupric sulphate ($CuSO_4.5H_2O$) were dissolved in 100.0 mL of distilled water. 100 mL of 7.5 % NaOH (CO_2-free, boiled water) with constant stirring was then added to the above mixture. The resulting solution was transferred into a 250 mL flask made up to the mark with distilled water and vortex. The solution was transferred into an amber bottle and stored at -5 ^oC and carefully observed for the formation of precipitation. The calibration curve was made by the subjection of the protein standard solutions made to biuret test and the bovine solutions of 1.0 mL of each concentration was added to 4.5

mL of biuret reagent. The resultant solution was vortex. A light blue colouration solutions were formed. 1.0 mL of distilled water was added to 4.5 mL of biuret reagent in a test tube and used as control to zero the absorbance. The same processes were repeated for the quantification of protein in both ALR1 and ALR2 enzymes (Silvio *et al.,* 2015).

3.15.4 Characterization and inhibition of ALR1 and ALR2 enzymes

The amount of the crude enzyme to be used in the assay mixture was determined by carrying out the eznymes optimisation experimentally. In this, different volumes of the crude enzymes ranging from 30 μL to 100 μL were used. Absorbance at all different volumes of crude extract of the enzymes used in the assay was then taken and the difference of absorbance before and after the addition of cofactor (NADPH) was determined. The volume of the enzymes used in the assay mixture was determined from the change in the absorbance (Silvio *et al.,* 2015).

3.15.4.1 Optimization of substrate (D, L-Glyceraldehdye)

D, L-Glyceraldehdye optimization was performed at different volumes, ranging from 10 µL to 90 µL against enzymes extracted and after 5 mins, the absorbance was noted. The reaction mixture contained 20 µL of 100 mM phosphate buffer, 70 µL of the dialyzed enzymes with different substrate concentration. The mixture was incubated for 5 mins at 37 °C and for the initiation of the enzymatic reaction 50 µL of 0.1 mM NADPH was added as the cofactor and absorbance was taken at 340 nm. Incubated again at 37 °C for 10 mins and absorbance was again meaured at the repective UV range in ELIZA plate reader. Values for K_m and V_{max} for the enzyme was graphpad prism software (version 5.0) (Silvio *et al.,* 2015).

3.15.4.2 Optimization of cofactor (NADPH)

The cofactor used in the aldehyde reductase assay was NADPH, different concentration of NADPH was used ranging from 10 µL to 70 µL having molarity of 0.1 mM per well. Phosphate buffer of 20 µL of 100 mM along with 70 µL of the dialyzed enzyme was used with different volumes of NADPH and change in absorbance was determined after every 5 mins (Silvio *et al.,* 2015).

3.16 Enzyme inhibition assays

3.16.1 ALR1 enzyme inhibition assay

The inhibitory activity of all the seed extracts against ALR1 was performed using spectrophotometry which determines the decrease in NADPH absorption that follow NADPH oxidation by the enzymes catalysis (Silvio *et al.,* 2015); which determines NADPH consumption at 340 nm spectrophotometrically. The reaction mixture was made up of 20.0 µL of the inhibitor, 0.5 mM NADPH, 100.0 M sodium phosphate buffer (pH 6.2), 10.0 mM of sodium D-glucoronate and enzyme to make a total volume of 200 µL. Substrate was added to the reaction mixture to initiate the reaction after the incubation at temperature of 37 ^{o}C for 10 mins. An absorbance change was determined on a microplate reader at a wavelength of 340 nm after incubation for 5 mins at 37 ^{o}C. Inhibition percentage was determined from the equation below;

$$\text{Inhibition (\%)} = 100 - (OD_{testwell}/OD_{control}) \times 100$$

Inhibitor dose-response curves at 50 % were obtained and IC_{50} was accurately measured using graphpad prism.

3.16.2 ALR2 enzyme inhibition assay

The seed extracts ALR2 activity was conducted according to Silvio *et al.* (2015) method. The reaction consists of 40 µL of 10 mM DL-glyceraldehyde and the rest of the reaction reagents were introduced according to the procedure used for ALR1 assay. In this regards, positive control was 20 µL of quercetin (1 mM). The reaction was done in triplicate. The reaction absorbance was obtained by using ELIZA reader and the result was determined using PRISM 5.0 to calculate the IC_{50} values of the tested seed extracts with percentage inhibition above 50 %. Inhibition percentage was determined using the equation below:

$$\text{Inhibition (\%)} = 100 - (OD_{testwell}/OD_{control}) \times 100$$

Dose-response curves of the seed extracts as the potential inhibitors of the enzyme (≥50 %) were obtained and IC_{50} values were determined with GraphPad prism (Version 5.0 software Inc., San Diego, California, USA).

3.16.3 α-Glucosidase inhibition assay

Assay method of α-glucosidase activity by Amin *et al.* (2016) was followed. The activity was performed with the pre-incubation 10 µL of enzyme with the seed extracts (10 µL) in 70 µL of buffer at temperature of 37 ^{o}C for 5 mins. After the first step, 10 µL of *p*-NPG (10 mM) was introduced into each of the well in a 96 well plate and further put into the incubator at 37 ^{o}C for additional 30 mins. Negative control was methanol and acarbose was the positive control. The seed extracts activity against α-glucosidase enzyme was determined by measuring amount of *p*-nitrophenol that was released at a wavelength of 405 nm using ELIZA microplate reader. Activity of the seed extract against the enzyme was determined with the following equation:

% inhibition = 100-(OD (sample) /OD (blank) x 100.

Dose-response curves of the seed extracts with percentage inhibition (≥ 50 %) against α-glucosidase were obtained and values of IC_{50} was determined by Graphpad prism.

3.16.4 β-Glucosidase enzyme inhibition assay

β-glucosidase activity of the seed extracts was done according to the previous method of Pérez *et al.* (2008) with slight modifications. The assay was performed by the introduction of 10 µL of the inhibitor (extract) to 70 µL of buffer and 10 µL of enzyme (2.0 U/mL) already in the buffer followed by pre incubation for 5 min at 37 °C. After the incubation, 10 µL of the substrate was added to the reaction mixture for the initiation of the assay, further incubated for 30 mins at temperature of 37 °C. 10 µL of acarbose and distilled water was employed as positive and negative control respectively. The activity of seed extracts against β-glucosidase enzyme was done by measuring *p*-nitrophenol that was released at a wavelength of 405 nm. The percentage inhibition was calculated using the following equation:

Inhibition (%) = 100-(OD (sample) /OD (blank) x 100.

IC_{50} values of the seed extracts with the percentage inhibition above 50 % were calculated.

3.16.5 Porcine pancreatic lipase assay

The inhibitory ability of the seed extracts against pancreatic porcine lipase was carried out according to the modified method of Kim *et al.* (2007). The enzyme assay was done in a 96 well plate containing 20.0 μL each of the seed extract, positive (orlistat) and negative controls, 164.0 μL of assay buffer and 6.0 μL of the porcine lipase enzyme mixture, incubated at 37 ^{o}C for 15 mins. After the incubation, 10.0 μL of 10.0 mM *p*-NPB (*p*-nitrophenylbutyrate) substrate was introduced and further incubated for additional 15 mins. The seed extracts inhibitory activities were evaluated by taking measurement of amount of the *p*-NPB to *p*-nitrophenol liberated at a wavelength of 405 nm using BIO-TEK ELISA reader.

$$\text{Inhibition (\%)} = 100\text{-(OD (sample) /OD (blank) x 100.}$$

The equation above was used to determine the percentage inhibition and IC_{50} was determined by Graphpad prism.

3.16.6 15-Lipoxygenase inhibition assay

Lipoxygenase (15-LOX) activity of the extracts was carried out according to the described method of Aamer *et al.* (2016) with slight modifications. The total assay volume in a 96 well plate was 200.0 µL containing 145.0 µL of 100.0 mM KH_2PO_4 buffer with pH of 7.6, 20.0 µL of the seed extracts and 10.0 µL of the 42.5 units of 15-LOX enzyme per well. The mixture was pre-incubated for 10 mins at a temperature of 25 ^{o}C. Addition of 25.0 µL of linoleic (substrate) to the solution was followed for the initiation of final reaction. The mixture was further incubated under the same condition for an additional 10 mins and final absorbance was measured; the positive control used was quercetin. The percentage inhibition of the extracts and control was reported as a mean of independent experiments in triplicate, determined with the formula below:

$$\text{Inhibition (\%)} = 100 - (OD_{testwell}/OD_{control}) \times 100$$

The equation above was used to determine the percentage inhibition and IC_{50} was determined by Graphpad prism.

3.17 Cytotoxicity evaluation of the seed extracts

3.17.1 Cell lines and cell culture

HeLa cell lines were used and prepared in a medium consisting RPMI-1640 supplemented with penicillin (100 UmL−1), streptomycin (100 µgmL−1) and L-glutamine (2.0 mM), followed by 10.0 % FBS and kept in the incubator supplied with 5 % CO_2 at 37 ^oC for two days. The cells were cultivated in 96 well plates to conduct cytotoxicity assays after the confluence of the adherent cell lines (Skehan *et al.,*1990).

3.17.2`Cytotoxicity assay

The ability of each seed extract to inhibit growth of HeLa cancer cell lines was determined by sulforhodamine B (SRB) assay using a cytotoxicity method (colorimetric) which measures the quantity of cellular protein of the cells as described by Skehan *et al.* (1990). HeLa cells were seeded at a density of $5x10^3$ cells/well in a 200 µL of growth medium in a humidified atmosphere of 95 % air and 5 % CO_2 and temperature of 37 °C for 24 h in a 96 well plate; adopted concentrations of all the seed extracts were prepared in DMSO and added to the culture medium (RPMI-1640 buffered with 2.2 g/L $NaHCO_3$ and supplemented with 5 % FBS), penicillin (100 U/mL) and streptomycin (100 µg/mL) and inoculated into wells of the 96 well containing the cells. +3It was then further incubated for 48 h at a 37 °C and humidified CO_2 incubator. In the same manner, the blank and positive control wells of culture medium and vincristine (VCN) were also prepared. After the completion of incubation time, the cells were fixed with TCA solution of 50 % of ice cold (50 µL) for an hour at 4 ^oC. PBS was used to wash the plate for 5 times, air dried, stained with sulforhodamine B dye of 0.4 % (w/v) made in 1 % acetic acid solution and then left to stand for 30 mins at room temperature.

The wells of the plate were further rinsed with acetic acid solution of 1 % w/v to cater for unbound dye and left to dry up. The bounded dye was solubilised with 10 mM of Tris base solution and later added to the wells, after which it was left for 10 mins at room temperature. Elx800 Biotek Microplate Reader was used for the measurement of the absorbance at a wavelength of 490 nm by subtracting the background absorbance measured at 630 nm from it. Antiproliferative activity of the extract was calculated by comparing the absorbance of wells containing the extract with those of the control

wells of the negative and positive control wells of culture medium and vincristine (VCN).

$$\% \text{ inhibition} = 100-(\text{OD (sample)} /\text{OD (blank)} \times 100$$

3.18 Animal study

3.18.1 Oral glucose and sucrose tolerance test using albino rats

Oral glucose and sucrose tolerance test were performed on BS, PB and PN seed extracts using a set of 40 wistar rats with the average weight of 100-200 g housed in clean polypropylene cages and acclimatized for seven days. The rats were fasted overnight for 12 h with free access to water and divided into eight groups (n=5). The seed extracts were suspended in saline water and administered orally at doses of:

Group I-VI: 500 mg/kg/b.wt-1000 mg/kg/b.wt of BS, PB and PN
Group VII: Glibenclamide at a dose of 10 mg/kg
Group VIII: Vehicle at a dose of 10 mL/kg)
20 min after the drugs and extracts administration, the rats were loaded with glucose at a dose of 4.0 g/kg/b.wt and 2.0 g/kg/b.wt for sucrose. The tails of the rats were snipped for blood glucose estimation at 0 min before administration of the extracts/drugs and glucose/sucrose loading and at 30, 60, and 120 min after sucrose and glucose loading (Ye *et al.,* 2002).

3.18.2 *In vivo* antidiabetic activity on mice

Ninety eight (98) mice of average weight 30-45 g supplied by COMSAT, Institute of Information Technology Abbottabad, Pakistan were housed in clean polypropylene cages in different groups with each group containing 7 mice. The animals were kept at a temperature of 32 ^{o}C in a daily and light cycle and acclimatized for seven days before the induction of diabetes. All the animals were fed with pelletized diet and water *ad-libitum* under strict hygienic conditions. All procedures were performed in accordance with the University of Ibadan Ethics Committee (UI/EC) guidelines with the protocol approval number (14/0059/UIECRA). Diabetes was induced in the mice that were fasted for 12 h by using alloxan monohydrate in salt water that was injected (i.p.) at a single dose of 150 mg/kg/b.wt after taking their fasting blood glucose (Nagappa *et al.,* 2003). All the injected animals were given 20 % of glucose solution to prevent drug-induced hypoglycemic mortality. Three days after the induction of

alloxan, the mice were screened and diabetes was confirmed in animals with blood glucose level of 200 mg/dL and above; measured with the gluconometer (ACCu-Chek active, Roche, Diagnostics, USA).

In vivo antidiabetic activity was tested on BS, PB, MM and PN seed extracts based on their *in vitro* enzymatic activity. The study was carried out in batches; the first was carried out with the combination of PB and PN while BS and MM was the second batch conducted together under the same condition. The seed extracts and reference drugs were prepared in saline and orally administered to diabetic mice as shown below:

Group I-II: Diabetic mice treated with PB at doses of 100 and 200 mg/kg/b.wt
Group III-IV: Diabetic mice treated with PN at doses of 100 and 200 mg/kg/b.wt
Groups V: Non diabetic mice treated with vehicle solution.
Groups VI: Diabetic mice treated with vehicle solution.
Group VII: Diabetic mice treated drugs at 10 mg/kg/b.wt. The same procedure was repeated for another set of mice using BS and MM seed extracts.

3.19 Statistical analysis

All the results were analysed and expressed as mean ± standard deviation (SD) of n=3 for chemical analysis on the seed powders and extracts while it is n=7 for animal experiment on the seed extracts. The significant differences between the means of the results were determined using one-way analysis of variance (ANOVA) test with the accepted significant level ($P \leq 0.05$).

CHAPTER FOUR

RESULTS AND DISCUSSSION

4.1 Result of phytochemical screening of the seed extracts

The phytochemical composition of the tested seed extracts are presented on Table 4.1. The results showed the presence of different bioactive constituents in the seed plants and it could be observed that alkaloids, flavonoids and phenols were present in all the seed extracts. Phenolic compounds are among the plant secondary metabolites with different biological activities; anti-inflammation, cardiovascular protection, ant-aging, antiapoptosis, antiatherosclerosis and endothelia improvement; also antioxidant activity of the several phenolic compounds was also reported. The common plant antioxidants are always the phenol compounds like flavonoids, tocopherol and phenolic acids. Availability of phenol in the selected seed samples suggests the ability of most of the seeds to act as an antioxidant agents.

Flavonoids are phenolic hydroxylated compounds known which can be produced in plants to prevent infection of microorganisms and over 4000 have been reported so far Flavonoids have different biological activity such as anti-inflammatory, antiviral, antibacterial and anti-allergic activity. They also have known to have capacity to neutralize free radicals that can contribute to heart disease, aging and cancer. The presence of alkaloids in the plant seeds could have be the reason for their high potency against bacterial and fungal tested and some of its properties such as analgesic and antispasmodic have also been reported by Edeoga and Enata, (2001). In addition, alkaloids are known to be effective detoxifying and antihypertensive agents. The seed extracts also contain saponin known to possess inhibitory activity against inflammation. It has also been confirmed that saponins also have immense significant as hypotensive, cardiac depressant and antihypercholesterol properties and it also has the ability to precipitate and coagulate red blood cell. Saponins are cardiotonic in nature and have been reported to possess antidiabetic property (Kamel, 1991). Tannins, cardiac glycosides and carbohydrate were detected in all the seed extracts except BS seed extract but reducing sugar and sterols were not detected in seed extract of only CP. (Tannins do bind to proline protein by interfering with synthesis of protein and

also; fungal from growing and could be useful in the treatment of wounds, bruise, sprains and bleeding arrest (Akinpelu and Onokoya, 2006)). Availability of tannins in all the seed extracts also suggests their ability to play a significant role as antidiarrhoea and antihaemorrhagic agents.

Table 4.1: Phytochemical constituents of the seed extracts

	Seed extracts									
Phytochemical	**BP**	**BS**	**CE**	**CP**	**EA**	**MC**	**MM**	**MT**	**PB**	**PN**
Alkaloids	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	-	+	+	+	-	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Saponins	+	-	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+
Tannins	+	-	+	+	+	-	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	-	+	+	+	+
Reducing sugar	+	+	+	-	+	+	+	+	+	+
Sterols	+	+	+	+	+	+	+	+	+	+

+: indicates the presence of phytochemical constituent and - : indicates absence of tested constituent. BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida*

4.2 Macronutrients elements content of the seeds

The nutritional elements in the seeds of the selected plants were determined because of their significant physiological roles they play in the body system during metabolism processes and in the treatment of diseases. Elements analysed are: calcium, magnesium, potassium, copper, sodium, zinc, manganese and iron and amount of each element present in the seeds were presented on Table 4.2. In the pulverized seeds of PN, the most abundant element was potassium (98.98±0.05 mg/kg) followed by calcium (55.89±2.21 mg/kg), iron (53.62±4.64 mg/kg), magnesium (19.23±1.53 mg/kg), zinc (5.72±0.18 mg/kg) and manganese (0.45±0.06 mg/kg) being the least abundant element in the seeds of PN. Like the seeds of PN, potassium was also the most predominant element in the seeds of PB, and BS with concentrations of 83.99±0.06 mg/kg and 87.95±1.40 mg/kg respectively. In PB seeds, the next most abundant after potassium was iron (71.12±3.96 mg/kg) followed by calcium (63.88±0.61 mg/kg) and Magnesium (63.88±5.76 mg/kg) and lastly manganese (2.43±0.03 mg/kg) while the second most predominant mineral elements in BS seeds was iron (73.15±7.22 mg/kg) followed by calcium (63.82±0.61 mg/kg). Concentration range of 0.38±0.01 mg/kg was obtained for manganese as the least abundant mineral elements in BS seeds (Table 4.2).

The variation of the concentrations of the nutritional elements observed in most of these plant seeds could be due to some factors like; the soil type and the element nature. Calcium was found in highest concentration in PB seeds (108.35±9.27 mg/kg). The next most abundant mineral element in PB seeds is potassium with the value of 99.07±0.06 mg/kg followed by iron (29.34±3.04 mg/kg) and magnesium (21.88±3.03 mg/kg). Manganese and copper were found in low concentration in PB seeds with the obtained values of 0.21±0.01 mg/kg and 0.20±0.01 mg/kg respectively. From Table 4.2, it can be seen that EA have highest concentration of magnesium (99.13±0.17 mg/kg), the next most abundant in the seeds of EA was potassium (88.17±2.4 mg/kg), calcium (86.38±10.72 mg/kg) followed by iron (49.69±5.18 mg/kg). Manganese was the least abundant element in the seeds of EA with the concentration of 0.30±0.01 mg/kg. In the same way like PN and PB seeds, the most abundant element in CP seeds was potassium (99.18±0.00 mg/kg) followed by calcium (92.76±4.4 mg/kg), Magnesium (28.64±0.84 mg/kg) and lastly iron (27.14±3.31 mg/kg). Manganese was the least abundant element in the seeds of CP 0.3±0.02 mg/kg.

The seeds of MC and MT have calcium (67.20±4.11 mg/kg and 86.79 ± 1.97 mg/kg) as the most abundant element. Unlike other seeds, iron was the next most abundant element in the seeds of MC with concentration of 48.35±5.44 mg/kg while potassium (33.19±0.25 mg/kg) was the next most abundant in MT seeds. The lowest manganese concentrations were found in MC (0.15±0.01 mg/kg) and MT (0.22±0.01 mg/kg) respectively. The highest iron concentration was found in CE (83.47±6.08 mg/kg) followed by calcium (60.37±2.94 mg/kg) magnesium (5.54±0.53 mg/kg) and lastly potassium (5.06±0.77 mg/kg). Manganese (0.27±0.04P mg/kg) was the least abundant element in CE. The seeds of MM were rich in calcium (45.96±6.17 mg/kg), potassium (52.94±0.04 mg/kg) and iron (42.41±4.33 mg/kg). MM was low in copper (0.79±0.08 mg/kg) and Manganese (0.22±0.04 mg/kg). The comparison of the seeds mineral elements composition revealed that PN, CP, MM, PB, BS and CA seeds have potassium as the most prominent element. The seeds also have high concentrations of calcium, magnesium, iron and sodium.

Magnesium is the most abundant nutritional element that is needed in the body system for the optimal performance of about 300 enzymes to play their physiological roles (Swaminathan, 2003). Many of the activity are performed by the enzymes containing Mg (Swaminathan, 2003), therefore its deficiency may contribute to decrease in insulin mediated glucose uptake. Magnesium also prevents insulin resistance and risk of developing diabetes. Mn acts as a cofactor for different enzymes that part take in bone marrow production, protein, fats and carbohydrate metabolism (Orbea *et al.,* 2002). Mn is also a cofactor for pyruvate carboxylase that is involved in non-carbohydrate compounds conversion to glucose through gluconeogenesis for their use. The insulin synthesis secretion and alteration in insulin metabolism also required Mn and its deficiency have been implicated in the development of diabetes (Kazi *et al.,* 2008). Another essential mineral element is Cu that is required for different functions that is peculiar to biological activity. The catalytic activity of superoxide dismutase (SOD) also required Cu; for cells protection from superoxide radicals. Cu deficiency can also influence the development of cardiovascular diseases (Klevay, 2000).

Among the mineral element required in trace amount for essential cell processing activity is Zn. It is involved in apoptosis and cell division in the body. It also responsible for different biochemical pathways (Karamouzi *et al.,* 2002); and also play significant roles in insulin secretion (Wijesekara *et al.,* 2010). A number of these essential mineral elements levels regulation are required for normal body functions, for example iron is an essential element necessary for the synthesis of two essential functional proteins (heamoglobin and myoglobin), required for the transportation of molecular oxygen during respiration. In the blood stream, serum iron is being transported by a glycoprotein (known as transferring) in to the cells (Wish, 2006). And elevation of iron level oxidizes bionucles like nucleic acids, lipids and proteins which may contribute to diabetes development by reducing insulin secretion from beta-cells of the pancreas with increase in insulin resistant.

Table 4.2: Macronutrients elements composition of the seeds (mg/kg)

	Seeds									
Parameters	**BP**	**BS**	**CE**	**CP**	**EA**	**MC**	**MM**	**MT**	**PB**	**PN**
Sodium	2.61±0.62^{de}	2.74±0.11^{de}	3.74±0.12^{cd}	3.352±0.32^{cde}	3.95±0.93^{bc}	5.45±0.51^{a}	4.04±0.26^{bc}	3.209±0.47^{cde}	2.38±0.25^{e}	5.00±1.2^{ab}
Potassium	99.07±0.06^{a}	87.95±1.40^{b}	5.06±0.77^{e}	99.180±0.00^{a}	88.17±2.43^{b}	27.78±0.48^{d}	52.94±0.47^{c}	33.19±0.25^{d}	83.99±0.06^{b}	98.98±0.05^{a}
Magnesium	21.88±3.03^{e}	35.29±0.17^{c}	5.54±0.53^{f}	28.64±0.48^{d}	99.13±0.17^{a}	5.164±0.72^{fg}	29.23±0.08^{d}	2.71±0.07^{g}	39.58±1.39^{b}	19.23±1.53^{e}
Calcium	108.35±9.27^{a}	63.82±0.61^{c}	60.37±2.94^{c}	92.76±4.14^{b}	86.38±10.72^{b}	67.20±4.11^{c}	45.96±6.17^{d}	86.79±1.97^{b}	63.88±5.76^{c}	55.89±2.21^{cd}
Copper	0.21±0.01^{e}	0.78±0.08^{ed}	0.58±0.00^{d}	0.62±0.37^{d}	0.73±0.04^{cde}	0.57±0.09^{d}	0.79±0.08^{b}	0.63±0.11^{cd}	0.30±0.07^{e}	1.35±0.28^{a}
Iron	29±3.04^{d}	73.15±7.22^{ab}	83.47±6.80^{a}	27.14±3.31^{d}	49.69±5.18^{c}	48.35±5.44^{c}	42.41±4.33^{c}	26.45±1.77^{d}	71.12±3.96^{b}	53.62±4.64^{c}
Manganese	0.20±0.01^{gh}	0.38±0.01^{bc}	0.27±0.01^{ef}	0.3±0.02^{cd}	0.30±0.01^{de}	0.15±0.01^{h}	0.22±0.04^{fg}	0.22±0.01^{fg}	0.43±0.03^{ab}	0.45±0.06^{a}
Zinc	2.42±0.13^{d}	3.07±0.19^{c}	1.61±0.06^{e}	7.57±0.36^{a}	1.66±0.15^{e}	2.66±0.30^{cd}	2.29±0.37^{d}	1.80±0.11^{e}	2.50±0.06^{d}	5.72±0.18^{b}

Values are expressed as mean±SD (n=3); values of common superscript within the same row are not significantly different at $p<0.5$. BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida

4.3 Micronutrients elements content of the seeds

The level of the micronutrients metals in the seeds were analysed and the result showed that micronutrients metals concentration in all the selected seed plants were within the range reported by WHO and FAO for medicinal plants and food material products. In the plant seed samples, aluminum, molybdenum, and chromium were the most abundant with concentrations range of 11.12±0.24-4.94±1.17 mg/kg, 4.29±0.01-1.26±0.25 mg/kg and 14.01±6.00-2.03±0.01 mg/kg respectively (Table 4.3). The obtained value for lead, cobalt and silver in this study for all the seed samples were low and within the range of 0.41±0.24 -0.15±0.01 mg/kg for lead, 0.80±0.00-0.6±0.01 mg/kg for cobalt and 0.22±0.25-0.02±0.00 mg/kg for silver. Accumulation of some heavy metals such as Pb, Nickel, Cd and As in the body system have been linked to glucose disruption and alteration in molecular mechanism in glucose regulation in diabetic patients.

Many of the elements are in soluble states for the regulation of biofluids composition, metabolic function of these elements are based on their range in different body tissues. As a result of their diversification characteristics and functions, some of them are considered to be important for human health, and their imbalance has negative effect on pancreatic islet beta cells and diabetes formation (Chen, 2009). The production of reactive oxygen species (ROS) during diabetes; has also been linked to their imbalance (Chen, 2009). The season of sample collection of the plants, the age, atmosphere, pollution and condition of soils have significant effects on the level of the elements in the plants.

Deposition of some toxic metals (Pb, Ni, Cd and As) in the tissues are non-degradable. Thus, when the metals spend too much time in the tissues and it's very difficult to be removed because tissues have minimum amount of metals to be retained, and beyond the threshold limits tissues get destroyed as a result of the metal toxicity. Among the toxic micronutrients metal are As, Pb, Ni, Cd and As (Chiu *et al.,* 2004). The reactions of toxic metal with various body proteins may modify their function and kinetics. Also, when toxic metals are in abundance, they compete with some essential metals required for enzyme activity and numerous physiological functions in the body. The presence of some toxic metals in the body tissue may induce toxicity that can cause derangement of mechanism of antioxidant and high ROS generation. The biological activity of Cr is solely relies on its oxidation state. When it in trivalent form, Cr has strong biological

activity required for optimal glucose uptake by cell (Tudan *et al.,* 2011). Cr roles in regulation of insulin and blood glucose cannot also be ruled out. Antioxidant imbalance may lead to proteins nucleic acids and lipid peroxidation degradation while the ROS oxidative attack of components of cells is a risk to pathogenesis of large number of diseases including diabetes. High levels of Cd reduce calcium absorption and results into bone and kidney loosed known as Itai-Itai disease. It is important to note that desirable benefit for human health depends on obtaining the correct amount of supplement in the right form and at the right time.

Table 4.3: Micronutrients elements composition of the seeds (mg/kg)

	Seeds									
Parameters	**BP**	**BS**	**CE**	**CP**	**EA**	**MC**	**MM**	**MT**	**PB**	**PN**
Lead	0.38±0.01^{a}	0.18±0.01^{cd}	0.25±0.02^{bc}	0.21±0.04^{b}	0.41±0.24^{a}	0.39±0.03^{a}	0.20±0.00^{bcd}	0.15±0.01^{d}	0.27±0.01^{b}	0.25±11^{bc}
Cobalt	0.06±0.01^{g}	0.14±0.02^{de}	0.07±0.00^{fg}	0.80±0.00^{a}	0.64±0.02^{b}	0.15±0.05^{de}	0.12±0.01^{ef}	0.18±0.01^{bc}	0.60±0.01^{g}	0.21±0.00^{a}
Chromium	3.40±0.14^{e}	25.97±0.66^{a}	7.42±0.31^{d}	14.07±0.00^{b}	3.01±0.26^{ef}	2.03±0.01^{f}	8.91±1.09^{c}	3.04±0.01^{ef}	3.08±0.11^{e}	2.51±1.39^{ef}
Cadmium	0.17±0.01^{bc}	0.16±0.00^{bc}	0.17±0.01^{bc}	0.92±0.01^{b}	0.56±0.05^{bb}	0.14±0.01^{bc}	0.08±0.01^{d}	0.50±0.13b^{cd}	0.17±0.02^{bc}	3.29±0.02^{a}
Silver	0.02±0.02^{c}	0.05±0.01^{c}	0.11±0.00^{c}	0.37±0.00^{ab}	0.50±0.01^{a}	0.01±0.00^{c}	0.06±0.01^{abc}	0.22±0.25^{bc}	0.02±0.00^{b}	0.14±0.01^{c}
Nickel	0.17±0.01^{g}	3.17±0.27^{b}	2.07±0.09^{cd}	1.85±0.04^{d}	0.04±0.00^{fg}	1.42±0.04^{e}	2.33±0.04^{c}	2.00±0.01^{d}	0.51±0.08^{f}	4.70±0.21^{a}
Aluminum	11.12±0.24^{abc}	8.38±1.22^{bcd}	10.14±0.24^{bcd}	16.06±0.27^{a}	16.35±0.30^{a}	9.14±0.55^{bcd}	7.45±0.60^{cd}	5.16±0.47^{cd}	13.99±1.27^{ab}	4.94±1.17^{d}
Molybdenum	4.40±0.13^{a}	3.47±0.18^{b}	4.29±0.01^{a}	2.99±0.14^{d}	3.04±0.17^{d}	2.37±0.04^{e}	2.55±0.01^{de}	1.26±0.25^{f}	3.72±0.07^{b}	2.72±0.10^{cd}

Values are expressed as mean±SD (n=3); values in the same column with common super script are not significantly different at *p<0.05.* BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida

4.4 Antimicrobial activity of the seed extracts

In this present era, drug resistance human pathogenic organisms emergency are on increase, thus demanded an urgent need for effective management of infectious diseases. In this regards, one of the important approach is to search for new effective, less toxic and inexpensive drugs from plants, for all the possible antimicrobial properties. In this study, the methanolic extracts of the plants seeds were tested against six clinical isolated drugs-resistant Gram negative and positive bacterial and four fungal. The bacterial; *P. aeruginosa*, *E. coli* and *S. aureus*, *K. pneumonia*, *S. typhi*, and *B. subtilis* and the fungal; *P. notatum*, *R. stolonifer*, *A. niger* and *C. albicans* showed varying degrees of sensitivity to studied seed extracts. The seed extracts antimicrobial activities were very encouraging as all the tested extracts were found to be highly potent against the tested organisms, though the seed extracts inhibitory activity were dose dependent and strain specific. The seed extract of PN was highly active against *S. aureus* and *E. coli* with maximum inhibition zones of 28.50±0.71 mm and 28.00±0.00 mm respectively. *B. subtilis* and *K. pneumonia* were the second most susceptible pathogens with the same inhibition zone of 17.50±0.71 mm. Among the class of the bacterial, *S. typhi* was the least sensitive to PN seed extract having 16.50±0.71 mm as the zone of inhibition. PN seed extracts activity against fungal was found to be lower than the values recorded for all the bacterial. *P. aeruginosa* had maximum inhibition zone of 19.50±0.71 mm while minimum inhibition zone of 14.50±0.71 mm was observed for PN seed extracts against *P. notatum* at concentration of 200 mg/mL (Table 4.4). *A. niger* and *R. stolonifer* sensitivity was found to be moderate with 16.50±0.91 mm and 15.00±1.4 mm zones of inhibition respectively.

The seed extract of CP showed highest inhibition zone against *E. coli* (28.00±0.00 mm); followed by *S. aureus* (26.00±0.00 mm). The inhibition zones of the CP extract against *B. subtilis* and *K. pneumonia* were 17.50±0.71 mm and *P. aeruginosa* and *S. typhi* were 17.00±1.41 mm and 14.00±0.00 mm respectively. The sensitivity of CP seed extract against fungal was in the order of *C. albicans* (18.00±0.00 mm) followed by *A. niger* (16.50±0.71 mm), *R. Stolonifer* (15.00±1.41 mm) and lastly *P. notatum* (14.50±0.71 mm) at 200 mg/mL concentration of the seed extract.

Table 4.4: Antimicrobial activity of PN and CP seed extracts against the indicated microorganisms

Sample	conc. (mg/mL)	Diameter of zones of inhibition (mm)									
		Gram +Ve			Gram -Ve					Fungal	
		SA	BS[b]	KB	EC	ST	PA	CA	AN	RS	PN[f]
PN	200	28.50 ± 0.71[b]	17.50 ± 0.71[b]	17.50±0.71	28.00±0.00[b]	16.50±0.71	19.50±0.71[b]	18.00±0.00	16.50±0.71	15.00±1.41	14.50±0.71
	100	25.00 ± 1.41[e]	16.00 ± 0.00[c]	15.50±0.71	24.50±0.71[e]	14.00±0.00	17.00±1.41[c]	14.50±0.71	13.00±1.41	12.50±0.71	12.00±0.00
	50	19.50 ± 0.71[d]	14.50 ± 0.71[d]	13.00±1.41	20.00±0.00[d]	12.50±0.71	13.00±1.41[d]	12.50±0.71	11.00±1.41	10.50±0.71	9.50±0.71
	25	16.50 ± 0.71[e]	12.50 ± 0.71[e]	12.00±0.00	17.50±0.71[e]	10.50±0.71	12.00±0.00[d]	10.50±0.71	9.00±1.41	-	-
	12.5	12.50 ± 0.71[f]	10.00 ±0.00[f]	10.00±0.00	12.00±0.00[f]	-	11.00±0.00[d]	-	-	-	-
CP	200	26.00 ± 0.00[b]	17.50 ±0.71[b]	17.50±0.71	28.00±0.00[b]	16.50±0.71	19.50±0.71[b]	18.00±0.00	16.50±0.71	15.00±1.41	14.50±0.71
	100	22.50 ± 0.71[c]	16.00±0.00[c]	15.50±0.71	24.50 ± 0.71[e]	14.00±0.00	17.00±1.41[c]	14.50±0.71	13.00±1.41	12.50±0.71	12.00±0.00
	50	17.50 ± 0.71[d]	14.50±0.71[d]	13.00±1.41	20.00±0.00[d]	12.50±0.71	13.00±1.41[d]	12.50±0.71	11.00±1.41	10.50±0.71	9.50±0.71
	25	13.50 ± 0.71[e]	12.50±0.71[e]	12.00±0.00	17.50±0.71[e]	10.50±0.71	12.00±0.00[d]	10.50±0.71	9.00±1.41	-	-
	12.5	12.50 ± 0.71[e]	10.00±0.00[f]	10.00±0.00	12.00±0.00[f]	-	11.00±0.00[d]	-	-	-	-
	+Ve	39.50 ± 0.71[a]	37.50±0.71[a]	37.50±0.71	40.00±0.00[a]	40.50 ±0.71	38.00±0.00[a]	28.00±0.00	27.50±0.71	25.50±0.71	27.00±1.41
	-Ve	-	-	-	-	-	-	-	-	-	-

SA = *Staphylococcus aureus;* BS[b] = *Bacillus subtilis*; KB = *Klebsiellae pneumonia;* EC= *Escherichia coli*; ST= *Salmonella typhi*; PA = *Pseudomonas aeruginosa;* CA = *Candida albicans*; AN = *Aspergillus niger*; RS = *Rhizopus stolonifer*; PN[f]= *Penicillum notatum*; +Ve = Gentamicin (5 g/mL) (bacterial) and tioconazole (70 %) (Fungal); -Ve = Dimethylsulfoxide (DMSO); (-) = No activity; PN = *Picralima nitida;* CP = *Croton penduliflorous*

Table 4.4 showed the sensitivity of the tested pathogens against seed extract of MM, the maximum inhibition zone of 21.00±1.41 mm was recorded for *S. aureus* at maximum concentration of the seed extract, followed by *P. aeruginosa* (19.00±1.41 mm), *E. coli* (19.50±0.71 mm), and *R. stolonifer* (17.00±1.41 mm). *A. niger's* sensitivity to MM seed extract was 14.00±0.00 mm inhibition zone. The seed extract of CE, on the other hand was found to be very active in preventing the growth rate of *S. aureus*, *E. coli* and *C. albicans* with inhibition zones of 25.00±1.41 mm, 21.50±0.71 mm, and 18.00±0.00 mm respectively. In addition, CE also inhibited *B. subtilis* growth with 18.00±0.00 mm zone of inhibition. The inhibition zone recorded for *A. niger, R. stolonifer* and *P. notatum* was 14.00±0.00 mm zone of inhibition zone each.

PB seed extract was also found to be highly active against *S. aureus* (27.00±1.41 mm); *E coli* (24.50±0.71 mm) and *B. subtilis* (20.00±0.00 mm). The growth of *S. typhi* and *P. aeruginosa* was inhibited with 17.00±1.41 mm as the minimum inhibition zones against all the pathogenic bacterial. On the contrary to the rest of seed extracts. PB inhibited the growth of *C. albicans* with the zone of 19.00±1.41 mm as the only sensitive fungal to the seed extract at 200 mg/mL concentrated solution (Table 4.5). Among all the tested pathogens against BS seed extract, *K. pneumonia* (+ve gram bacterial) and *S. typhi* (–ve gram bacterial) were found to be resistant to the seed extract of BS. The maximum zone of inhibition was recorded against *S. aureus* (24.50±0.71 mm) as the most susceptible pathogen, followed by *E. coli* and *C. albicans* (20.00±0.00 mm) and lastly *B. subtilis* (19.00±1.41 mm). *A. niger*, R. *stolonifer and P. notatum* showed inhibition zone of 16.50±0.71 mm, 15.50±0.71 mm and 14.50±0.71 mm respectively.

Table 4.5: Antimicrobial activity of MM and CE seed extracts against the indicated microorganisms

Sample	conc. (mg/mL)	Diameter of zones of inhibition (mm)									
		Gram +Ve			Gram -Ve			Fungal			
		SA	BS[b]	KB	EC	ST	PA	CA	AN	RS	PN[f]
MM	200	17.50±0.71	18.00±0.00	16.00±0.71[b]	19.50±0.71[b]	21.00±1.41[b]	19.00±1.41[b]	15.00±1.41	14.00±0.00	17.00±1.41	16.00±0.00
	100	16.00±0.00	16.00±0.00	14.50±0.71[c]	18.00±0.00[c]	18.00±0.00[c]	17.00±1.41[b]	11.50±0.71	13.50±0.71	13.50±0.71	13.50±0.71
	50	14.00±0.00	13.50±0.71	12.00±1.41[d]	14.50±0.71[d]	12.00±0.00[e]	13.00±0.00[c]	10.00±0.00	11.00±1.41	12.00±0.00	11.00±1.41
	25	10.00±0.00	10.00±0.00	11.00±0.71[de]	12.00±0.00[e]	10.00±0.70[f]	11.00±0.00[cd]	-	9.00±1.41	10.00±0.00	-
	12.5	-	-	10.00±0.00[e]	10.00±0.71[f]	-	9.00±1.41[e]	-	-	-	-
	+Ve	39.00±1.41	38.00±0.71	40.00±00[a]	40.00±0.00[a]	39.00±0.00[a]	38.50±0.71[a]	29.00±0.00	28.00±0.00	29.00±0.00	28.00±0.00
CE	200	25.00±1.41[b]	18.00±0.00	14.00±0.00	21.50±0.71[b]	14.00±0.00	15.50±0.71	18.00±0.00	14.00±0.00	14.00±0.00	14.50±0.71
	100	21.00±1.41[c]	15.00±1.41	12.00±0.00	19.00±1.41[c]	12.00±0.00	14.00±0.00	16.00±0.00	11.50±0.71	12.00±0.00	12.50±0.71
	50	17.50±0.71[d]	11.50±0.71	10.00±0.99	16.50±0.71[d]	10.00±0.00	12.50±0.71	13.50±0.71	10.00±0.00	9.50±0.71	10.00±0.00
	25	15.00±1.41[e]	9.50±0.71	-	14.50±0.71[d]	-	10.00±0.00	11.00±1.41	-	-	-
	12.5	12.50±0.71[f]	-	-	12.00±0.00[e]	-	-	9.00±1.41	-	-	-
	+Ve	40.00±0.00[a]	39.00±1.41	40.00±0.00	39.00±1.41[a]	37.08±1.41	25.50 ± 0.71	28.00±0.00	27.00±1.41	28.00±0.00	40.00±0.00
	-Ve	-	-	-	-	-	-	-	-	-	-

SA = *Staphylococcus aureus;* BS[b] = *Bacillus subtilis*; KB = *Klebsiellae pneumonia;* EC= *Escherichia coli*; ST= *Salmonella typhi*; PA = *Pseudomonas aeruginosa;* CA = *Candida albicans*; AN = *Aspergillus niger*; RS = *Rhizopus stolonifer*; PN[f]= *Penicillum notatum*; +Ve = Gentamicin (5 g/mL) (bacterial) and tioconazole (70 %) (Fungal); -Ve = Dimethylsulfoxide (DMSO); (-) = No activity; MM = *Monodora myristica*; CE = *Cyperus esculentus*

Table 4.6: Antimicrobial activity of PB and BS seed extracts against the indicated microorganisms

Sample	conc. (mg/mL)	Diameter of zones of inhibition (mm)									
		Gram +Ve			Gram -Ve				Fungal		
		SA	BS[b]	KB	EC	ST	PA	CA	AN	RS	PN[f]
PB	200	27.00±1.41[b]	20.00±0.00	16.00±0.00	24.50±0.71[b]	17.00±1.41	17.50±0.71	19.00±1.41	-	-	-
	100	21.00±1.41[c]	18.50±0.71	13.50±0.71	21.00±1.41[c]	14.00±0.00	15.00±1.41	16.50±0.71	-	-	-
	50	19.00±1.41[c]	13.50±0.71	12.00±0.00	16.50±0.71[d]	12.50±0.71	13.50±0.71	13.00±1.41	-	-	-
	25	14.50±0.71[d]	12.00±0.00	10.50±0.71	14.50±0.71[d]	9.00±1.41	12.00±0.00	11.50±0.71	-	-	-
	12.5	12.50±0.71[d]	10.00±0.00	-	12.00±0.00[e]		10.00±0.00	10.00±0.00	-	-	-
	+Ve	39.50±0.71[a]	40.50±0.71	40.00±0.00	38.00±0.00[a]	39.00±1.41	37.00±1.41	27.00±1.41	26.00±0.00	26.00±0.00	26.00±0.00
BS	200	24.50±0.71[b]	19.00±1.41[b]	-	20.00±0.00[b]	-	16.00±0.00	20.00±0.00[b]	16.50±0.71	15.50±0.71	14.50±0.71
	100	20.00±0.00[c]	15.00±1.41[c]	-	17.50±0.71[c]	-	14.50±0.71	17.50±0.71[c]	14.00±0.00	14.00±0.00	12.50±0.71
	50	17.00±1.41[d]	13.50±0.71[cd]	-	14.00±0.00[d]	-	12.00±0.00	13.50±0.71[d]	13.00±1.41	12.50±0.71	9.50±0.71
	25	13.50±0.71[c]	11.50±0.71[de]	-	11.50±0.71[e]	-	9.00±1.41	12.00±0.00[bd]	10.50±0.71	10.00±0.00	-
	12.5	12.00±0.00e	10.00±0.00[e]	-	10.00±0.00[f]	-	-	10.50±0.71[d]	10.00±0.71[d]	-	-
	+Ve	39.50±0.71[a]	40.50±0.71	38.00±00	38.00±0.00	-	37.00±1.41	27.00±1.41a	26.00±0.00	26.00±0.00	26.00±0.00
	-Ve	-	-	-	-	-	-	-	-	-	-

SA = *Staphylococcus aureus;* BS[b] = *Bacillus subtilis*; KB = *Klebsiellae pneumonia;* EC= *Escherichia coli*; ST= *Salmonella typhi*; PA = *Pseudomonas aeruginosa;* CA = *Candida albicans*; AN = *Aspergillus niger*; RS = *Rhizopus stolonifer*; PN[f] = *Penicillum notatum*; +Ve = Gentamicin (5 g/mL) (bacterial) and tioconazole (70 %) (Fungal); -Ve = Dimethylsulfoxide (DMSO); (-) = No activity; PB: *Parkia biglobosa*; BS: *Blighia sapida*

The seed extracts of EA and BP inhibitory activity against the pathogenic microorganisms is shown in Table 4.6. Maximum zones of inhibition for EA seed extract were 27.50±0.71 mm against *S. aureus;* followed by *E. coli* (26.50±0.71 mm), *B. subtilis* (25.50±1.41 mm) and *S. typhi* (20.50±1.4 mm) and then *P. aeruginosa* (21.00 ± 0.71 mm). Minimum zone of inhibition was recorded against all the tested fungal, *C. albicans*, *A. niger* and *P. notatum* at inhibition zone of 15.50±0.71 mm while *R. stolonifer* zone of inhibition was 14.00±0.00 mm. The seed extract of BP showed maximum zone of inhibition against *S. aureus* and *E. coli* at zone diameters of 26.50±0.71 mm and 23.50±0.71 mm respectively. The second most inhibited pathogens were *B. subtilis* and *C. albicans* with 21.00±0.4 mm inhibition zone. Among the tested bacterial *S. typhi*, *P. aeruginosa* and *K. pneumonia* were found to be least susceptible with inhibition zone in the range of 16.00±0.00-18.00±0.00 mm. The second most inhibited fungal were *P. notatum* (19.00±14.0 mm) followed by *R. stolonifer* (17.70±1.41 mm) and lastly *A. niger* (15.50±0.71 mm).

The seed extracts of MC was found to have high potency against *S. aureus* (23.50±0.71 mm), *E. coli* (22.00±0.28 mm) and *B. subtilis* (21.00± 1.41 mm). MC seed extract also inhibited the growth of *S. typhi* with 17.00±1.41 mm zone of inhibition. 14.00±0.00 mm zone of inhibition was also recorded for MC seed extract against *K. pneumonia*. *P. aeruginosa* was found to be inhibited by MC seed extract with 18.00±0.00 mm zone of inhibition. MC seed extract showed no activity against the following fungal: *R. stolonifer* and *P. notatum* and the zones of inhibition recorded against *C. albicans* and *A. niger* were 16.50±0.71 mm and 14.00±0.00 mm respectively (Table 4.7).

Like seed extracts of PN, CP, and BP; MT seed extract was found to be highly active against all the tested bacterial with the moderate activity against fungal. Maximum zone of inhibition (21.00±0.01 mm) was recorded against *E. coli* followed by *S. aureus* (20.00±0.00 mm) and (20.00±0.00 mm) respectively. The least susceptible among the bacterial were *K. pneumonia* and *S. typhi* at inhibition zone of 18.00±0.00 mm. *P. notatum* was the most sensitive fungal, with inhibition zone of 19.00±1.41 mm followed by *R. stolonifer* (16.00±0.00 mm), *C .albicans* (15.00±1.41 mm) and lastly *A. niger* (14.00±0.00 mm).

Table 4.7: Antimicrobial activity of EA and BP seed extracts against indicated microorganisms

Sample	conc. (mg/mL)	Diameter of zones of inhibition (mm)									
		Gram +Ve				Gram -Ve				Fungal	
		SA	BS[b]	KB	EC	ST	PA	CA	AN	RS	PN[f]
EA	200	27.50±0.71[b]	25.50±1.41[b]	16.50±0.71	26.50±0.71[b]	20.50-±0.71	21.00±1.41	15.50±0.71	15.00±1.41	14.00±0.00	15.00±1.41
	100	25.00±1.41[c]	21.00±1.41[b]	15.00±1.41	23.50±0.71[c]	17.50±4.95	17.00±1.41	14.00±0.00	14.00±0.00	12.00±0.00	12.50±0.71
	50	19.50±0.71[d]	16.00±0.00[c]	12.00±0.00	21.00±1.41[d]	12.50±0.71	14.00±0.00	12.00±0.00	12.00±0.00	10.50±0.71	9.50±0.71
	25	18.00±0.00[d]	14.00±0.00[d]	10.00±0.00	19.00±1.41[d]	9.00±1.41	12.00±0.00	10.00±0.00	10.00±0.00	-	-
	12.5	14.00±0.00e	12.00±0.00[e]	-	16.50±0.71[e]	-	11.00±1.41	-	-	-	-
	+Ve	40.00±0.00[a]	37.00±1.41[a]	27.50±0.71	40.00±0.00[a]	40.00±0.00	37.50±0.71	27.00±1.41	28.00±0.00	28.0-0±0.00	27.00±1.41
BP	200	26.50±0.71[b]	21.00±1.41	16.00±0.00	23.50±0.71[b]	18.00±0.00	18.00±0.00	21.00±1.41[b]	15.50±0.71	17.70±1.41	19.00±1.41
	100	23.00±1.41[c]	18.00±0.00	13.50±0.71	20.00±0.00[c]	16.50±0.71	14.00±0.00	18.00±0.00[c]	14.00±0.00	13.00±1.41	15.00±1.41
	50	19.00±1.41[d]	15.00±1.41	11.50±0.71	17.00±1.41[d]	15.00±1.41	12.00±0.00	15.00±1,41[d]	11.00±1.41	11.50±0.71	12.00±0.00
	25	18.00±0.00[d]	12.00±0.00	10.50±0.71	14.00±0.00[c]	12.00±0.00	10.00±0.00	13.50 ±0.71[e]	9.00 ± 1.41	9.50±0.71	9.50±0.71
	12.5	14.00±0.00[e]	10.00±0.00	-	11.00±1.41[f]	11.00±0.00	-	12.00±0.00[e]	-	-	-
	+Ve	40.00±0.00[a]	39.00±1.41	40.00 ±0.00	39.00±1.41[a]	37.00±1.41	25.50±0.71	28.00±0.00[a]	28.00±0.00	28.00 ± 0.00	40.00±0.00
	-Ve	-	-	-	-	-	-	-	-	-	-

SA = *Staphylococcus aureus;* BS[b] = *Bacillus subtilis*; KB = *Klebsiellae pneumonia;* EC = *Escherichia coli*; ST = *Salmonella typhi*; PA = *Pseudomonas aeruginosa;* CA = *Candida albicans*; AN = *Aspergillus niger*; RS = *Rhizopus stolonifer*; PN[f]= *Penicillum notatum*; +Ve = Gentamicin (5 g/mL) (bacterial) and tioconazole (70%) (Fungal); -Ve = Dimethylsulfoxide (DMSO); (-) = No activity; EA: *Erythrococca anomalo;* BP: *Butyrospermum paradoxum*

Table 4.8: Antimicrobial activity of MC and MT seed extracts against the indicated microorganisms

Sample	conc. (mg/mL)	Diameter of zones of inhibition (mm)									
		Gram positive			Gram negative					Fungal	
		SA	BS[b]	KB	EC	ST	PA	CA	AN	RS	PN[f]
MC	200	23.50±0.71[b]	21.00±1.41[b]	14.00±0.00	22.00±2.82[b]	17.00±1.41	18.00±0.00	16.50±0.71	14.00±0.00	-	-
	100	21.00±1.41[c]	16.00±0.00[c]	12.00±0.00	17.50±0.71[bc]	14.00±0.00	14.00±0.00	14.50±0.71	12.50±0.71	-	-
	50	19.50±0.71[c]	14.00±0.00[d]	10.00±0.00	15.00±1.41[bc]	12.00±0.00	12.00±0.00	12.00±0.00	10.50±0.71	-	-
	25	14.00±0.00d	12.00±0.00[e]	-	14.60±0.00[bc]	10.00±0.00	10.00±0.00	10.00±0.00	-	-	-
	12.5	12.00±0.00[e]	10.00±0.00[f]	-	10.00±6.36[c]	-	-	-	-	-	-
	+Ve	40.50±0.71[a]	37.00±1.41[a]	27.50±0.71	40.00±0.00[a]	40.00±0.00	37.50±0.71	27.00±1.41	27.50±0.71	-	-
MT	200	20.00±0.00[b]	20.00±0.00[b]	18.00±0.00	21.00±0.1[b]	18.00±0.00	19.00±1.41[b]	15.00±1.41	14.00±0.00	16.00±0.00	19.00±1.41
	100	17.00±1.41[c]	18.50±0.71[bc]	14.00±0.00	17.00±1.41[c]	15.00±1.41	16.00±0.00[c]	13.00±0.00	12.00±0.00	14.00±1.41	14.00±0.00
	50	14.00±0.00[d]	17.00±1.41[c]	12.50±0.71	13.00±1.41[dc]	12.00±0.00	14.00±0.00[d]	12.00±0.00	9.00±0.00	12.00±0.00	12.00±0.71
	25	12.00±0.00[d]	14.00±0.00[d]	9.00±1.41	12.00±0.00[de]	10.00±0.00	12.00±0.00[e]	9.00±0.00	-	10.00±0.00	10.00±0.00
	12.5	9.00±1.41[e]	11.00±1.41[e]	-	10.00±0.00[e]	-	10.00±0.00[f]	-	-	-	-
	+Ve	39.00±1.41[a]	37.50±0.71[a]	40.00±0.00	40.00±0.00[a]	39.00±1.41	39.00±1.41	29.00±1.41	27.00±1.41	29.00±1.41	28.00±0.00
	-Ve	-	-	-	-	-	-	-	-	-	-

SA = *Staphylococcus aureus;* BS[b] = *Bacillus subtilis*; KB = *Klebsiellae pneumonia;* EC = *Escherichia coli*; ST = *Salmonella typhi*; PA = *Pseudomonas aeruginosa;* CA = *Candida albicans*; AN = *Aspergillus niger*; RS = *Rhizopus stolonifer*; PN[f] = *Penicillum notatum*; +Ve = Gentamicin (5 g/mL) (bacterial) and tioconazole (70 %) (Fungal); -Ve = Dimethylsulfoxide (DMSO); (-) = No activity; MC: *Momordica charantia;* MT: *Monodora tenuifolia*

Moreover, the positive controls (Gentamicin) have zones of inhibition ranging from 37.50±0.71-40.50±0.71 mm against all tested bacterial. Tioconazole, positive control for all tested fungal, displayed the inhibitory zone of 28.00±0.00 mm against the most susceptibility fungal (*C. albicans*) and inhibition zone of 25.50±0.71 mm against the least susceptible fungal (*R. stolonifer*). In contrast, no inhibitory activity was observed in DMSO (negative control). It is an implication that DMSO, the solvent for the reconstitution of the crude extracts did not involve in the susceptibility of all the tested bacterial and fungal to the corresponding extracts. Generally, Gram-negative bacterial were considered to be the most resistant to all the plant seed extracts in comparison to Gram-positive bacterial with the exception to the *E. coli* (a negative bacterial) that was found to be among the susceptible bacterial to all the tested seed extracts.

The difference in susceptibility among the negative bacterial could be as a result of distinct in their cell structures. In addition, the inhibitory effects of all the seed extracts on the growth of the clinical fungal showed that *C. albicans* was the most susceptible among the tested fungal with the maximum zones of inhibition. Although, the variations between the antimicrobial sensitivity can be due to difference in chemical composition of the seed extracts, culture medium, inoculums size, and emulsifier choice. This study was supported by some earlier studies that *S. aureus* was the most inhibited by the plant extracts. The resistance of some Gram-negative bacterial to tested seed extracts has also been reported by Essawi and Srour (2000). The multi-layered and complexity of the Gram-negative structure, the outer membrane do act as a barrier to different environmental substances such as antibiotics. It could be observed that some of the previously reported studies are in agreement with the results obtained from this study. Inhibitory activity of the tannins against microorganisms has been reported due to its microbial precipitation pattern and ability to make proteins unavailable for them to grow. Also, saponins a special kind of glycosides have also been reported as an antifungal agent. The development of potential antimicrobials from plant materials seems rewarding due to its possibility of leading to development of phytomedicine to act against microbes.

4.4.1 Minimum inhibitory concentration (MIC) of the seed extracts

The seed extracts minimum inhibitory concentrations (MICs) can be defined as the lowest concentration of the seed extract that inhibited the growth of the tested microorganisms up to 48 h compared to the positive control. Table 4.9 contains the MIC values of all the seed extracts against the entire tested Gram positive, Gram negative bacterial and fungal. The MIC value of PN seed extract against *S. aureus* and *E. coli* was found to be 0.625 mg/mL. MIC value of 2.50 mg/mL was obtained against *B. subtilis*, *S. typhi* and *P. aeruginosa*. *K. pneumonia* was found to be the most resistant to seed extract of PN among the tested bacterial with MIC value of 5.0 µg/mL. On the other hand, the antifungal activity of PN seed extract against *C. albicans* was recorded at a MIC value of 5.0 mg/mL. *A. niger*, *R. stolonifer* and *P. notatum* were the least susceptible fungi to the seed extract of PN with MIC value of 10.0 mg/mL.

The CP seed extract MIC value against *S. aureus* was 1.25 mg/mL while the value of 2.50 mg/mL was obtained for *B. subtilis*, *E. coli* and *S. typhi*. *K. pneumonia* and *P. aeruginosa* were resistant to CP seed extract at concentration of 5.0 mg/mL. The MIC of CP seed extract against *C. albicans* and *A. niger* was found to be 5.0 mg/mL. *P. notatum* and *R. stolonifer* were the most susceptible fungal against seed extract of CP with MIC value of 0.625 mg/mL. The MIC values obtained for MM seed extract against bacterial was in the range of 2.50-10.0 mg/mL. *S. aureus* and *E. coli* were found to be having the same MIC value of 2.50 mg/mL as the most susceptible; followed by *B. subtilis* (5.0 mg/mL). MIC value of 10.0 mg/mL was obtained *for K. pneumonia*, *S. typhi* and *P. aeruginosa* respectively. All the tested fungi were found to be resistant to MM seed extract with the MIC value of 10.0 mg/mL. Seed extract of CE was highly active against *S. aureus* with a recorded MIC value of 1.25 mg/mL, being the most susceptible bacterial.

Table 4.9: Minimum inhibitory concentration (MIC) of the seed extracts against indicated organisms

	Minimum inhibitory concentration (mg/mL)									
Sample	**SA**	**BS[b]**	**KP**	**EC**	**ST**	**PA**	**CA**	**AN**	**PN[f]**	**RS**
BP	2.500	2.500	5.000	1.250	2.500	5.000	5.000	5.000	5.000	5.000
BS	2.500	2.500	ND	2.500	ND	5.000	2.500	5.000	10.000	10.000
CE	1.250	5.000	10.000	2.500	5.000	10.000	5.000	10.000	5.000	5.000
CP	1.250	2.500	5.000	2.500	2.500	5.000	5.000	5.000	0.625	0.625
EA	2.500	5.000	1.250	5.000	1.250	5.000	5.000	10.000	5.000	10.000
MC	2.500	2.500	10.000	1.250	5.000	5.000	5.00	5.000	ND	ND
MM	2.500	5.000	10.000	2.500	10.000	10.000	10.000	10.000	10.000	10.000
MT	2.500	5.000	5.000	1.250	2.500	5.000	5.000	10.000	10.000	10.000
PB	1.250	2.500	5.000	1.250	5.000	5.000	5.000	ND	ND	ND
PN	0.625	2.500	5.000	0.625	2.500	2.500	5.000	10.000	10.000	10.0

SA = *Staphylococcus aureus;* BS = *Bacillus subtilis*; KB = *Klebsiellae pneumonia;* EC = *Escherichia coli*; ST = *Salmonella typhi*; PA = *Pseudomonas aeruginosa;* CA = *Candida albicans*; AN = *Aspergillus niger*; RS = *Rhizopus stolonifer*; PN[f] = *Penicillum notatum*; ND = Not determined; BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida*

CE seed extract displayed MIC value of 2.5 mg/mL against *E. coli*. MIC of the CE seed extract against *B. subtilis*, *E. coli* and *K. pneumonia* was 5.00.0 mg/mL. Contrary to the bacteria, CE seed extract against the fungi was in the range of 5.00-10.0 mg/mL; where *A. niger* and *R. stolonifer* (10.0 mg/mL) were the least susceptible fungi to CE seed extract. MIC value of 1.25 mg/mL was obtained for *S. aureus* and *E. coli* as the most susceptible bacteria to the seed extract of PB, followed by *B. subtilis* and *P. aeruginosa* with minimum inhibitory concentration of 2.5 mg/mL. *K. pneumonia* and *R. stolonifer* was found to have the same MIC value of 5.00 mg/mL being the least susceptible among the bacteria. Unlike other seed extracts, *A. niger*, *P. notatum* and *R. stolonifer* were resistant to the seed extract of PB; *C. albicans* was the only susceptible fungal to PB seed extract with MIC value of 5.00.0 mg/mL.

BS seed extract was found to be the most potent against *S. aureus*, *B. subtilis* and *E. coli* with the minimum inhibitory concentration of 2.5.0 mg/mL. MIC value of 5.00 mg/mL was obtained for *P. aeruginosa* while *K. pneumonia* and *S. typhi* found were resistant to BS seed extract. In contrast, fungi was highly sensitive to BS seed extract and the MIC values were in the range of 2.5-10.0 mg/mL, *C. albicans* was the most susceptible with MIC value of 2.5 mg/mL while *R. stolonifer* was the least susceptible having MIC value of 10.0 mg/mL. EA seed extract was the most potent against *S. aureus*, *K. pneumonia* and *S. typhi* with the same MIC (1.25.0 mg/mL) while *B. subtilis*, *E. coli* and *P. aeruginosa* have moderate sensitivity with MIC value of 5.00 mg/mL. The seed extract of EA activity against the tested fungal was mild at the concentration range of 5.00-10.0 mg/mL. Fungicidal activity of EA seed extract against *R. stolonifer* was recorded at the minimum concentration of 10.0.0 mg/mL. MIC values obtained for BP seed extract against all the tested organisms were in the range of 1.25-10.0 mg/mL. *S. aureus*, *B. subtilis* and *E. coli* were the most sensitive to BP seed extract with the same MIC value of 1.25 mg/mL. The MIC value for *C. albicans*, *A. niger*, *P. notatum* and *R. stolonifer* was 5.00 mg/mL.

MC seed extract exhibited strong and broad inhibitory activity against both positive and negative Gram bacterial. MIC values obtained for MC seed extract were in the range of 1.25-5.00 µg/mL. *S. aureus*, *B. subtilis* and *E. coli* were the most sensitive to the seed extract of MC with inhibitory concentration of 1.25 µg/mL. The least sensitive was *K. pneumonia* with MIC value of 10.0 mg/mL. Antimicrobial activity of MC seed extract against *C. albicans* and *A. niger* was found to be mild, with inhibitory

concentrations of 5.00.0 mg/mL and 10.0.0 mg/mL respectively. *P. notatum* and *R. stolonifer* were found to be resistant to MC seed extract at all concentrations. Lastly, the MIC values obtained for MT seed extract for the inhibition of the microorganisms showed that seed was more active against bacterial than fungal. MIC values of MT seed extract against all tested bacteria were in the range of 2.5-5.00 mg/mL while the least susceptible fungal (*P. notatum*) have MIC value of 10.0 mg/mL. The lowest values of MICs observed in this study may likely be due to the difference in mechanisms of action between numerous constituents in the seed extracts. Many researchers have also reported that medicinal plant effectiveness may not be as a result of one main active compound but due to various constituent mixtures of the plants (Essawi and Srour, 2000). The synergistic effect of some of the constituents in the seed extracts also enhances their potent antimicrobial activity.

4.5 Fatty acid composition of the seed extracts

The fatty acids compositions of the seed extracts detected by GCFID were myristic, palmitic acid, palmitoleic, stearic, oleic acid, linoleic, α-linoleic and arachidic acids known as functional food supplement and of high medicinal values. The most dominant fatty acids in seed extracts of PN was linoleic with the percentage composition of 36.37 %, followed by oleic acid (17.24 %) (Table 4.10). The most predominant saturated fatty acids found in PN seed extracts was arachidic acid with percentage composition of 11.06 %. In PN seed extracts, the total percentage composition of poly unsaturated fatty acids (PUFAs) was 42.7 % as the most abundant while the total saturated fatty acids composition was 23.08 %. It has been reported by several researchers that vegetable oils rich in linoleic acid could prevent coronary heart disease and atherosclerosis, therefore, the presence of high amount of linoleic acid in the PN seeds could make it nutritional valuable and also signify its potential use for medicinal purposes. Myristic acid (4.81 %), palmitic acid (5.09 %), palmitoleic (6.26 %), stearic acid (2.12 %), and α-linoleic acid (6.40 %) were also found in PN seeds. The ratio of oleic/linoleic acid and poly unsaturated/ saturated fatty acids (PUFA/SFA) were 0.47 and 1.85 respectively. These ratio measures the seeds rancidity power.

The seed extract of EA is rich in both oleic acid (30.57 %) and linoleic acid (37.87%) as the major constituents. The seed extract also contained significant amount of saturated fatty acid (SFA), the saturated fatty acids found in EA seed extracts were stearic acid (6.85%) and palmitic acid (7.99 %). Arachidic acid (0.32 %) and α-linoleic (1.22 %) fatty acids contents of the seeds were found to be low. Percentage composition of unsaturated fatty acid was 69.66 % while saturated fatty acid was 15.16 %. The ratio of PUFAs to SFA was 2.58 which indicated that seed *E. anomala* has more of PUFAs than SFAs. PUFA is an important metabolism modulator and the seed extracts may be considered for important nutritional purposes. The FA analysis in seed extract of PB indicated that linoleic acid (42.72 %) present in highest amount followed by oleic acid (30.70 %). It also has 13.46 % and 11.34 % of palmitic acid and stearic acid. Palmitoleic acid (0.27 %) and arachidic acid (0.36 %) were the least saturated fatty acids in PB seeds. The ratio of PUFA/SFA was 1.70. More so, the percentage composition of unsaturated fatty acid (73.69 %) was found higher than saturated fatty acids (25.16 %) and the oleic ratio to linoleic was 0.72.

Linoleic acid (26.37 %) and oleic acid (11.8 20 %) were the most dominant unsaturated fatty acids in CP seed extract followed by α-linoleic acids with the percentage composition of 4.48 %. On the other hand, the main saturated fatty acids in the extract were palmitic acid (5.00 %) and arachidic acid (5.18 %). Stearic acid was the least dominant among the saturated fatty acids found in CP seeds with the least percentage concentration of 1.69 %. Although, the seeds of CP have low level of unsaturated fatty acid, the ratio of PUFA/SFA (2.60) showed that unsaturated fatty acids were the main constituents, thus suggesting the seeds for both nutritional and medicinal uses. The percentage composition of unidentified component of the seed extract CP 45.46 % and the oleic to linoleic ratio was 1.15 which indicated a good shelf life of the seeds oil. The seeds of BS extract demonstrated a very low presence of FA. The most abundant FA in the seeds was oleic acid (2.2.50 %), an unsaturated fatty acid, followed by linoleic acid (2.18 %). Stearic acid content of BS seeds was 1.16 % as the dominant saturated fatty acid. A low presence of arachidic acid (0.52 %), α-linoleic (0.57 %) and stearic acid (0.38 %) were also demonstrated. Fatty acids analysis of BS seed extract revealed high concentration of unidentified components with percentage composition of 92.69 %.

Linoleic and oleic acids were the dominant unsaturated fatty acids in the seed extract of MT, the percentage compositions were 22.99% and 16.13 % respectively. Other unsaturated fatty acids content of the seeds was α-linoleic (0.71 %) and palmitoleic acid (0.49 %). The main saturated fatty acid in MT was palmitic acid with percentage content of 7.36 % followed by stearic acid (0.49 %) and arachidic acid (0.27 %). The total content of saturated fatty acids was 9.78 % while the unsaturated fatty acid was 40.32 %. The percentage composition of unidentified component of the seed extracts was 49.9 %. The ratio of PUFA to SFA was 2.42 indicating a good medicinal value of the seeds.

Generally, the results of the fatty acids analysis in the seed extracts showed that the seeds contain high percentage of oleic and linoleic acids as the most dominant unsaturated fatty acids. Fatty acids like linoleic, arachidic and linolenic acids which are known as essential fatty acids with a natural preventive function of cardiovascular disease and alleviation of other disease, due to their roles in total cholesterol and HDL cholesterol control. Fatty acid content of all the seed extracts may help in the prevention of cardiovascular disease. The presence of high content of linoleic acid, suggests that the seeds consumption or usage as a supplement will help in fight against coronary heart disease (CHD). High percentage oleic acid in plant or vegetable oils has been reported to have beneficial effect in management of diabetes and obesity and non insulin dependent diabetes mellitus (Vassiliou *et al.,* 2009). Oleic acid has also been reported to reduce blood pressure (Teres *et al.,* 2008) and LDL cholesterol levels (O'Byrne *et al.,* 1997) and high ratio of oleic to linoleic increases the seed extract shelf life.

Table 4.10: Fatty acid composition of the seed extracts

CN	Fatty acid	RT(min)	Fatty acid composition of the seeds (%)					
			BS	CP	EA	MT	PB	PN
$C_{14:0}$	Myristic	2.86	----	----	----	----	----	4.81
$C_{16:0}$	Palmitic	4.254	1.16	5.00	7.99	7.36	13.46	5.09
$C_{16:1}$	Palmitoleic	4.46	----	----	----	0.49	0.27	6.26
$C_{18:0}$	Stearic	6.322	0.57	1.69	6.85	2.15	11.34	2.12
$C_{18:1}$	Oleic	6.518	2.50	11.82	30.57	16.13	30.70	17.24
$C_{18:2}$	Linoleic	7.05	2.18	26.37	37.87	22.99	42.72	36.37
$C_{18:3}$	α-linoleic	7.639	0.38	4.48	1.22	0.71	----	6.40
$C_{20:0}$	Arachidic	8.608	0.52	5.18	0.32	0.27	0.36	11.06
Total SFA	-------	-----	2.25	11.87	15.16	9.78	25.16	23.08
Total UFA	-------	-----	5.06	42.67	69.66	40.32	73.69	60.27
Total MUFA	-------	-----	2.50	11.82	30.57	16.62	30.97	17.50
Total PUFA	------	-----	2.56	30.85	39.09	23.70	42.72	42.77
PUFA/MUFA	-------	-----	1.14	2.60	2.58	2.42	1.70	1.85

SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; UF: Unsaturated fatty acid; CN: Carbon number; RT: Retention time; BS: *Blighia sapida*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida;* Fatty acid content of the seeds extracts was determined by using fatty acid methyl ester (FAME) mix C14-C22 (Supelco cat no 18917) as the reference standard

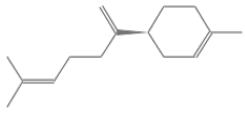
4.6 Phytocomponents of the seed extracts

The combination of separation (GC) and identification (MS) made GCMS an ideal technique for volatile and semi-volatile bioactive compounds identification, as one of the crucial techniques for active constituents like long chain hydrocarbons, alcohols, acids, amino and nitro compounds identification in plant materials. The GCMS analysis was carried out for the identification of bioactive compounds which might have contributed to the physiological functions of the seed extracts in most of the biological assays conducted in this study. Most of the bioactive constituents identified in the seed extracts have been reported to be having important chemical properties and therefore revealed various compounds with valuable biological activities.

The most abundant and common active components in all the seed extracts was 9, 12-Octadecanoic acid and hexadecanoic acid with the reported antioxidant, 5-α-reductase inhibitory and antihemolytic properties as shown in Table 4.11. Oleic acid with known antioxidant, cancer preventive and hypocholesterolemic properties was also identified in all the seed extracts. The list of compounds identified in each of the seed extract is presented in Table 4.12-4.21 in order of their elution. The structural assignments of the compounds were based on a systematic search for match of molecular ions extracted from the mass chromatograms that were compared with those data in the literature. The GC-MS spectra obtained for the seed extracts of BS, CP, EA, MM and MT showed different percentage composition of some volatile compounds such as, copaene, isoledene, cubenol, α-cubene, gamma elemene, limonene, epiglobulol and caryophyllene. 9-Octadecenoic acid ($C_{19}H_{36}O_2$), with retention time (RT) of 19.17 and percentage composition of 26.08 was the major compound identified in seed extract of BP plant. The seed extract of BS contained 8, 11-Octadecadienoic acid as the most prominent compound with percentage composition of 28.4 with RT of 19. 20. The notable compound that present in significant amount in CE seed extract is 9, 12-Octadecadienoic acid (53.68 %, RT:18.95).

The GC-MS spectrum for MC showed different peaks indicating numerous bioactive compounds with octadecanoic acid ($C_{19}H_{38}O_2$), RT 19.25, 22.69 % being the most prominent while oleyl alcohol ($C_{18}H_{36}O$), RT 19.98, 35.35 % was the most abundant compound in MM and PN respectively. In the seed extract of MT, 9-octadecenoic acid (45.13 %, RT 19. 14) was present in large quantities. The GC-MS analysis result of the chemical composition of seed extract of PB is presented in Table 4.19. The spectrum showed 12 peaks indicating 12 different bioactive compounds with Octadec-9-enoic acid ($C_{18}H_{34}O_2$), RT 20.31, 65.14 % as the major compound. These entire compounds might be the one contributing individually or synergistically for the various biological activity such as antioxidants, antidiabetic and antimicrobial activities exhibited by the seeds.

Table: 4.11 Phytocomponents identified in the seed extracts by GCMS with highest percentage composition

Sample	MF	Compound name	PC	Molecular structure
EA	$C_{15}H_{24}$	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl	17.99	
CE	$C_{19}H_{34}O_2$	9, 12-Octadecadienoic acid methyl ester	53.68	
BP	$C_{18}H_{34}O_2$	Octadec-9-enoic acid	54.80	
BS	$C_{19}H_{34}O_2$	8, 11-Octadecadienoic methyl ester	28.41	
CP	$C_{17}H_{34}O_2$	Hexadecanoic acid methyl ester	7.82	
PB	$C_{18}H_{34}O_2$	Octadec-9-enoic acid	65.14	
MC	$C_{19}H_{38}O_2$	Octadecanoic acid methyl ester	22.68	
MM	$C_{19}H_{38}O_2$	Octadecanoic acid methyl ester	34.86	
MT	$C_{15}H_{24}$	α-Cubebene	4.93	
PN	$C_{18}H_{36}O$	Oleyl alcohol	23.96	

MF: Molecular formular; PC: Percentage composition; BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida;* Mass spectra of the compounds found in the seed extract was matched with the National Institute of Standards and Technology (NIST) library

Table 4.12: Phytocomponents identified in the seed extract of BP by GC-MS

S/N	Compound name	Peak area (%)	Formula	Molecular weight (g/mole)	Retention time (min)
1.	Hexadecanoic acid, methyl ester	1.91	$C_{17}H_{34}O_2$	270	17.36
2.	n-Hexadecanoic acid	2.39	$C_{16}H_{32}O_2$	256	18.17
3.	9-Octadecenoic acid, methyl ester	26.08	$C_{19}H_{36}O_2$	296	19.17
4.	Octadecanoic acid, methyl ester	3.82	$C_{19}H_{38}O_2$	298	19.29
5.	Oleic acid	0.70	$C_{18}H_{34}O_2$	282	19.93
6.	Cyclopentadecanone, 2-hydroxyl	0.28	$C_{15}H_{28}O_2$	240	19.94
7.	14-Tricosenyl formate	0.11	$C_{24}H_{36}O_2$	366	19.97
8.	Octadec-9-enoic acid	54.8	$C_{18}H_{34}O_2$	282	20.04
9.	1, 2- benzene dicarboxylic acid, diisooctyl ester	0.42	$C_{28}H_{46}O_4$	446	22.66

Mass spectra of the compounds found in the seed extract of BP was matched with the National Institute of Standards and Technology (NIST) library; BP: *Butyrospermum paradoxum*

Table 4.13: Phytocomponents identified in the seed extract of BS by GC-MS

S/N	Compound name	Peak area (%)	Formula	Molecular weight (g/mole)	Retention time (min)
1.	Cyclohexene, 4-ethenyl-4-methyl -3- (1-methylethenyl-1-(1-methylethyl)	2.88	$C_{15}H_{24}$	204	10.78
2.	Dodecane 2, 6, 11-trimethyl	0.9	$C_{15}H_{32}$	212	9.80
3.	Copaene	0.17	$C_{15}H_{24}$	204	10.84
4.	Ylangene	0.25	$C_{15}H_{24}$	204	11.18
5.	α-Cubebene	0.37	$C_{15}H_{24}$	204	11.27
6.	Cyclohexane, 1-ethenyl-1-methyl 2-, 4- Bis (1-methyl ethenyl), (Is-1)	0.21	$C_{15}H_{24}$	204	11.44
7.	Aromadendrene	2.53	$C_{15}H_{24}$	204	11.82
8.	Gamma Elemene	0.76	$C_{15}H_{24}$	204	11.96
9.	Isoledene	0.51	$C_{15}H_{24}$	204	12.20
10.	1,6-cyclodecadiene, 1-methyl-5- methylene -8-(1-methylethyl)	4.96	$C_{15}H_{24}$	204	12.65
11.	Epiglobulol	4.94	$C_{15}H_{26}O$	222	13.20
12.	Carotol	1.82	$C_{15}H_{26}O$	222	13.26
13.	Dihydro-cis-alpha-copaene-8-ol	0.36	$C_{15}H_{26}O$	222	13.34
14.	Caryophyllene	0.76	$C_{15}H_{24}$	204	13.60
15.	Spathulenol	0.96	$C_{15}H_{24}O$	220	13.95
16.	TAU-Muurolol	2.05	$C_{15}H_{26}O$	222	14.64
17.	Cubenol	2.09	$C_{15}H_{26}O$	222	14.84
18.	Ledol	0.16	$C_{15}H_{26}O$	222	14.96
19.	Pentadecanoic acid, 14-methyl-methyl ester	5.61	$C_{17}H_{34}O_2$	270	17.50
20.	Hexadecanoic acid, ethyl ester	0.59	$C_{34}H_{66}O_4$	538	18.03
21.	n-Hexadecanoic acid	2.07	$C_{16}H_{32}O_2$	256	18.42
22.	8, 11-Octadecadienoic, methyl ester	28.41	$C_{19}H_{34}O_2$	294	19.20
23.	Heptadecanoic acid, 15-methyl-methyl ester	0.01	$C_{19}H_{38}O_2$	298	19.43
24.	Octadecanoic acid	0.51	$C_{18}H_{36}O_2$	284	20.12
25.	9-Octadecenoic acid methyl ester	0.24	$C_{19}H_{36}O_2$	296	20.75

Mass spectra of the compounds found in the seed extract of BS was matched with the National Institute of Standards and Technology (NIST) library; BS: *Blighia sapida*

Table 4.14: Phytocomponents identified in the seed extract of CE by GC-MS

S/N	Compound name	Peak area (%)	Formula	Molecular weight (g/mole)	Retention time (min)
1.	Hexadecanoic acid, methyl ester	6.52	$C_{17}H_{34}O_2$	270	17.34
2.	Eicosanoic acid	3.46	$C_{20}H_{40}O_2$	312	18.07
3.	9,12-Octadecadienoic acid, methyl ester	53.68	$C_{18}H_{32}O_2$	280	18.95
4.	9-Octadecenoic acid, methyl ester	1.83	$C_{19}H_{36}O_2$	296	19.02
5.	Octadecanoic acid, methyl ester	0.9	$C_{19}H_{38}O_2$	298	19.21
6.	Oleic acid	8.20	$C_{18}H_{34}O_2$	282	19.66
7.	9-Tricosene	2.94	$C_{23}H_{46}$	322	20.73
8.	1, 2-benzene dicarboxylic acid, diisooctyl ester	12.93	$C_{28}H_{46}O_4$	446	22.64

Mass spectra of the compounds found in the seed extract of CE was matched with the National Institute of Standards and Technology (NIST) library; CE: *Cyperus esculentus*

Table 4.15: Phytocomponents identified in the seed extract of CP by GC-MS

S/N	Compound name	Peak area (%)	Formula	Molecular weight (g/mole)	Retention time (min)
1.	3-Careen	0.50	$C_{10}H_{16}$	136	4.6
2.	β-Pinene	0.97	$C_{10}H_{16}$	136	5.6
3	Limonene	0.56	$C_{10}H_{16}$	136	7.0
4	1 ,6-Octadien-3-ol, 3, 7-dimethyl	0.97	$C_{10}H_{18}O$	154	9.26
5	α-Cubebene	0.46	$C_{15}H_{24}$	204	15.31
6.	Copaene	0.98	$C_{15}H_{24}$	204	16.33
7	Caryophyllene	4.34	$C_{15}H_{24}$	204	17.07
8	1 ,6 ,10- Dodecatriene, 7, 11-dimethyl-3- methylene-	5.86	$C_{15}H_{24}$	204	18.01
9	Cyclohexene, 3-(1, 5-dimethyl-4-hexenyl)-6-methylene-,[s-(R*, S*0]-	9.93	$C_{10}H_{18}O$	154	19.77
10	Cyclohexene, 4-(1 ,5-dimethyl-1,4-hexadienyl)-1-methyl-	0.46	$C_{15}H_{24}$	204	20.085
11	Cubenol	0.26	$C_{15}H_{26}O$	222	21.44
12	Guaiol	0.06	$C_{15}H_{26}O$	222	21.52
13	Hexadecanoic acid, methyl ester	7.82	$C_{17}H_{34}O_2$	270	28.25
14	Tetradecanoic acid 10, 13-dimethyl-, methyl ester	1.30	$C_{17}H_{34}O_2$	270	28.31
15	9, 12-Octadecadienoic acid, methyl ester	7.05	$C_{20}H_{36}O_2$	308	31.35
16	8 ,11-Octadecadienoic acid, methyl ester	5.25	$C_{19}H_{34}O_2$	294	31.415
17	Octadecanoic acid methyl ester	1.88	$C_{19}H_{38}O_2$	298	31.93
18	Linoeic acid ethyl ester	0.21	$C_{19}H_{34}O_2$	294	32.47
19	11-Eicosenoic acid, methyl ester	2.08	$C_{21}H_{40}O_2$	324	34.86
20	Eicosanoic acid, methyl ester	0.35	$C_{21}H_{42}O_2$	326	35.27

Mass spectra of the compounds found in the seed extract of CP was matched with the National Institute of Standards and Technology (NIST) library; CP: *Croton penduliflorous*

Table 4.16: Phytocomponents identified in the seed extract of EA by GC-MS

S/N	Compound name	Peak area (%)	Formula	Molecular weight (g/mole)	Retention time (min)
1.	α- Pinene	0.72	$C_{10}H_{16}$	136	4.6
2.	β- Pinene	1.02	$C_{10}H_{16}$	136	5.60
3.	α- Cubene	0.41	$C_{15}H_{24}$	204	15.29
4.	Copaene	0.03	$C_{15}H_{24}$	204	16.11
5.	Cyclohexane,1-ethenyl-1-methyl -2, 4- Bis (1-methylethenyl)-1, (1s- (1	1.12	$C_{15}H_{24}$	204	16.39
6	1 H-Cyclopro [E] azulene, 1α, 2, 3, 4, 4α, 7β-octahydro-1, 1,4,7-tetram	0.28	$C_{15}H_{24}$	204	16.78
7	Caryophyllene	5.99	$C_{15}H_{24}$	204	17.13
8	1, 6, 10-dodecatriene, 7, 11-dimethyl-3-methylene -, z	0.77	$C_{15}H_{24}$	204	18.14
9	1 H-Cycloprop [E] azulene, decahydro-1, 1, 7-trimethyl-4-methylene	1.64	$C_{15}H_{24}$	204	18.82
10	α-Cedrene	3.40	$C_{15}H_{24}$	204	19.03
11	Cyclohexene, 1-methyl-4-(5-methyl -1-methylene -4-hexenyl) -,[s]-	17.99	$C_{15}H_{24}$	204	19.37
12	α –Elemene	0.73	$C_{15}H_{24}$	204	20.48
14	Caryophyllene oxide	0.29	$C_{15}H_{24}O$	220	21.13
15	Cubenol	0.08	$C_{15}H_{26}O$	222	21.74
16	Hexadecanoic acid, methyl ester	8.26	$C_{17}H_{34}O_2$	270	28.32
17	9, 12-Octadecadienoic acid, methyl ester	7.06	$C_{20}H_{36}O_2$	308	31.44
18	Octadecanoic acid, methyl ester	1.25	$C_{19}H_{38}O_2$	298	31.94
19	11-Eicosenoic acid, methyl ester	0.97	$C_{21}H_{40}O_2$	324	34.85
20	o-Anisic acid, 2-adamantyl ester	1.92	$C_{18}H_{22}O_3$	286	42.34

Mass spectra of the compounds found in the seed extract of EA was matched with the National Institute of Standards and Technology (NIST) library; EA: *Erythrococca anomala*

Table 4.17: Phytocomponents identified in the seed extract of MC by GC-MS

S/N	Compound name	Peak area (%)	Formula	Molecular weight (g/mole)	Retention time (min)
1.	Decanoic acid, methyl ester	6.61	$C_{11}H_{22}O_2$	186	10.78
2.	Hexadecanoic acid, methyl ester	3.17	$C_{17}H_{34}O_2$	270	17.40
3.	9, 12-Octadecadienoic acid, methyl ester	20.98	$C_{20}H_{36}O_2$	308	18.97
4.	9,12, 15-Octadecxatrienoic	17.53	$C_{18}H_{30}O_2$	278	19.05
5.	Octadecanoic acid methyl ester	22.69	$C_{19}H_{38}O_2$	298	19.25
6.	4-Hexadecen-6-yne	1.01	$C_{16}H_{28}$	220	20.67
7.	11-Eicosenoic acid, methyl ester	8.03	$C_{21}H_{40}O_2$	324	20.71
8.	Eicosanoic acid methyl ester	0.53	$C_{21}H_{40}O_2$	324	20.93
9.	1, 2- benzene dicarboxylic acid, mono (2-ethylhexyl) ester	3.13	$C_{16}H_{22}O_4$	278	22.64

Mass spectra of the compounds found in the seed extract of MC was matched with the National Institute of Standards and Technology (NIST) library; MC: *Mormodica charantia*

Table 4.18: Phytocomponents identified in the seed extract of MM by GC-MS

S/N	Compound name	Peak area (%)	Formula	Molecular weight (g/mole)	Retention time (min)
1.	α-Phellandrene	5.53	$C_{10}H_{16}$	136	5. 80
2.	Copaene	0.69	$C_{15}H_{24}$	204	11.23
3.	Caryophyllene	0.16	$C_{15}H_{24}$	204	11.82
4.	Naphthalene	0.29	$C_{10}H_{8}$	128	12.51
5.	α-Muurolene	0.44	$C_{15}H_{24}$	204	12.81
6.	Isoledene	4.19	$C_{15}H_{24}$	204	13.02
7.	α-Cubebene	0.20	$C_{15}H_{24}$	204	13.22
8.	Cubenol	0.93	$C_{15}H_{26}O$	222	14.42
9.	TAU-Cadinol	0.43	$C_{15}H_{26}O$	222	14.60
10.	α-Cadinol	1.66	$C_{15}H_{26}O$	222	14.82
11.	7-Hexadecenoic acid methyl ester	0.39	$C_{17}H_{32}O_2$	268	17.22
12.	Pentadecanoic acid	4.43	$C_{15}H_{30}O_2$	242	17.46
13.	n-Hexadecanoic acid	0.04	$C_{16}H_{32}O_2$	256	18.23
14.	Octadecanoic acid, methyl ester	34.86	$C_{18}H_{38}O$	298	19.21
15.	Oleyl alcohol	35.45	$C_{18}H_{36}O$	268	19.98
16.	Octadecanoic acid	0.43	$C_{18}H_{36}O_2$	284	20.03
17.	1, 19-Eicosadiene	0.20	$C_{20}H_{38}$	278	20.73
28.	Eicosanoic acid, methyl ester	0.56	$C_{21}H_{40}O_2$	324	20.95
19.	1,2- benzene dicarboxylic acid diisooctyl ester	0.02	$C_{28}H_{46}O_4$	446	22.66

Mass spectra of the compounds found in the seed extract of MM was matched with the National Institute of Standards and Technology (NIST) library; MM: *Monodora myristica*

Table 4.19: Phytocomponents identified in the seed extract of MT by GC-MS

S/N	Compound name	Peak area (%)	Formula	Molecular weight (g/mole)	Retention time (min)
1.	Cyclohexene, 4-3thenyl – 4 – methyl-3- 91-methylethenyl)-1-(1-methyl)	0.02	$C_{15}H_{24}$	204	10.07
2.	Copaene	0.04	$C_{15}H_{24}$	204	10.84
3.	Tricyclo 2,2,1,0 (2,6) heptane,1,7-dimetghyl-7-4-methyl-3-pentenyl	0.18	$C_{15}H_{24}$	204	11.76
4.	Ylangene	0.03	$C_{15}H_{24}$	204	11.96
5.	Isoledene	0.69	$C_{15}H_{24}$	204	12.82
6.	α-Cubebene	4.93	$C_{15}H_{24}$	204	13.06
7.	Gamma Elemene	0.35	$C_{15}H_{24}$	204	13.14
8.	Epiglobulol	0.09	$C_{15}H_{26}O$	222	13.16
9.	IH-Cycloprop (azulene, decarhydro-1,1,7- Trimethyl-4 methylene	0.02	$C_{15}H_{24}$	204	13.56
10.	α-Cadinol	1.80	$C_{15}H_{26}O$	222	14.82
11.	Carotol	0.02	$C_{15}H_{26}O$	222	15.21
12.	Spathulenol	0.11	$C_{15}H_{24}O$	220	16.18
13.	n-Hexadecanoic acid	1.66	$C_{16}H_{32}O_2$	256	18.09
14.	9, 12-Octadecadienoic acid, methyl ester	0.01	$C_{20}H_{36}O_2$	308	18.99
15.	9-Octadecenoic acid methyl ester	45.13	$C_{19}H_{36}O_2$	296	19.14
16.	Octadecanoic acid methyl ester	3.71	$C_{18}H_{38}O$	298	19.25
17.	Linoleic acid ethyl ester	0.1	$C_{20}H_{36}O_2$	308	19.55
18.	9-Eicosyne	24.26	$C_{20}H_{38}$	278	19.79

Mass spectra of the compounds found in the seed extract of MT was matched with the National Institute of Standards and Technology (NIST) library; MM: *Monodora tenuifolia*

Table 4.20: Phytocomponents identified in the seed extract of PB by GC-MS

S/N	Compound name	Peak areas (%)	Formula	Molecular weight (g/mole)	Retention time (mins)
1.	Hexadecanoic acid, methyl ester	2.06	$C_{16}H_{32}O_2$	256	17.40
2.	n-Hexadecanoic acid	3.63	$C_{16}H_{32}O_2$	256	18.33
3.	9-Octadecenoic acid, methyl ester	0.04	$C_{19}H_{36}O_2$	296	18.99
4.	6-Octadecenoic acid, methyl ester	0.58	$C_{19}H_{36}O_2$	296	19.11
5.	Tetradecanoic acid 12-methyl, methyl ester	1.88	$C_{16}H_{32}O_2$	256	19.37
6.	Oleic acid	2.05	$C_{18}H_{34}O_2$	282	19.66
7.	9-Octadecenoic acid, 1,2,3 propanetriyl ester	0.03	$C_{57}H_{104}O_6$	884	20.02
8.	Octadec-9-enoic acid	65.14	$C_{18}H_{34}O_2$	282	20.30
9.	E-9-tetradecenoic acid	2.51	$C_{14}H_{26}O_2$	226	20.80
10.	15-tetracosenoic acid	0.03	$C_{24}H_{48}O2$	368	21.01
11.	1-Heptadecanol	0.02	$C_{17}H_{36}O$	256	22.15
12.	10-Octadecenoic acid, methyl ester	0.02	$C_{19}H_{36}O_2$	296	22.56

Mass spectra of the compounds found in the seed extract of PB was matched with the National Institute of Standards and Technology (NIST) library; PB: *Parkia biglobosa*

Table 4.21: Phytocomponents identified in the seed extract of PN by GC-MS

S/N	Compound name	Peak Area (%)	Formula	Molecular weight (g/mole)	Retention time (min)
1	2-Butanone, 4-(acetyloxy)-	0.05	$C_6H_{10}O_3$	130	4.06
2	Undecane, 5, 7-dimethyl-	0.39	$C_{13}H_{28}$	184	13.12
3	Octadecane, 3, ethyl-5 (-2-(ethybutyl)-	0.64	$C_{26}H_{54}$	366	14.94
4	Pentadecanoic acid, 14-methyl-, methyl ester	2.64	$C_{17}H_{34}O_2$	270	17.48
5	Oleic acid	4.54	$C_{18}H_{34}O_2$	282	18.17
6	9, 12-Octadecanoic acid	20.01	$C_{18}H_{32}O_2$	280	18.95
7	10-Octadecanoic acid, methyl ester	1.27	$C_{19}H_{36}O_2$	294	19.00
8	15-Tetracosenoic acid, methyl ester	0.04	$C_{25}H_{48}O_2$	380	19.21
9	Oleyl alcohol	23.96	$C_{18}H_{36}O$	268	19.70
10	Cyclopropaneoctadecanoic acid, 2-hexyl-, methyl ester	12.16	$C_{18}H_{34}O_2$	282	20.71
11	9-Hexadecanoic acid	1.45	$C_{16}H_{30}O_2$	254	20.97

Mass spectra of the compounds found in the seed extract of PN was matched with the National Institute of Standards and Technology (NIST) library; PN: *Picralima nitida*

4.7 Antioxidant activity of the seed extracts

Plants are invaluable source of natural antioxidant which have been scientifically proved for their synergistic and protective roles against numerous degenerative disorders such as diabetes, cardiovascular disease, stroke, cancer, Alzheimer's and Parkinson's diseases. Analysis of the antioxidant activity of the selected plant seeds revealed that PN seed extracts had highest DPPH radical scavenging potential that was displayed at 86.29±0.9 %, followed by PB (77.00±4.35 %) and BP (71.83±7.99 %), hence classified as high antioxidant plant seeds. The percentage inhibition antioxidant activities of seed extracts of MM, BS and MT were recorded at 66.29±0.9 %, 51.23±0.37 % and 67.80±3.98 % respectively making them to belong to class of plant seeds with moderate antioxidant activity. The antioxidant activity of seed extracts of CP (29.42±2.59 %), EA (21.88±5.67 %) and MC (19.9±1.66 %) were grouped to the class of plant seeds with low antioxidant activity. The antioxidant percentage activities of the seed extracts were also within the values obtained for ascorbic acid (87.80±1.10 %) and quercetin (89.95±0.63 %) used as positive controls. The seed extracts antioxidant potentials were classified as high, if the activity is ≥ 70 %, moderate (≥ 50 %) and low (< 50 %) (Rufino *et al.,* 2010).

The seed extracts with the percentage antioxidant activity above 50 % were further evaluated for determination of IC_{50} values. It is defined as the minimum concentration at which the seed extracts exhibit percentage inhibition activity against the DPPH radical above 50 % (Huang *et al.,* 2005). More so, the lower the IC_{50} value, the higher the antioxidant power of the tested seed extracts and the higher the pIC_{50}. Among the seed extracts evaluated for IC_{50}; PN had lowest IC_{50} value (12.20±0.25 µg/mL) as the most potent seed extract followed by PB (14.20±0.74 µg/mL). BS seed extract exhibited IC_{50} value of 20.36±0.96 µg/mL while the seed extracts of MT showed the IC_{50} value of 23.67±0.37 µg/mL. The lowest antioxidant activity was displayed by MM seed extract having the highest IC_{50} value of 31.14±0.81 µg/mL. Statistically, the antioxidant activity of each of the seed extracts was significantly different at $P<0.05$ as showed on Table 4.22. IC_{50} value of 22.38±0.37 µg/mL was obtained for ascorbic acid used as positive control. On the other hand, DPPH radical scavenging potential of the seed extracts and the positive control (ascorbic acid) were within the same range (4.51–4.91) using the P^{IC}_{50} = ($-\log_{10}$ IC_{50} value) as a measure of radical scavenging strength of the seed extract against DPPH. The results suggest variation in the types

and amounts of bioactive compounds in each of the seed extracts. Antioxidants protect us from various diseases by fighting against free radicals. They can act either by scavenging the reactive oxygen species or protecting the antioxidant mechanisms. The results of this study showed that most of the seed extracts contain photochemical constituents that are capable of donating hydrogen to a free radical to savage the potential damage. The finding agrees with some studies that reported antioxidant contents in different plants which could be influenced by the geographic region where they were grown and climatic variables such as solar radiation and temperature as determinant factors. All these plant seeds could be regarded as having high nutraceutical important on the account of numerous health benefits and their use as lipid peroxidation inhibitors in the food industry. The two generally accepted mechanisms of phenolic antioxidants action of the seed extracts against DPPH stable radical are hydrogen atom transfer (HAT) and sequential proton loss electron transfer (SPLET) (Gülçin and Beydemir, 2013) as shown in the figure above. The hydroxyl (OH) groups in flavonoids play an important role as the site of ionization and electron transfer according to the mechanism discovered recently by Litwinienko and Ingold, (2003). Also, a SPLET mechanism was proposed by Foti *et al.* (2004). SET based methods detect the ability of a potential antioxidant by the transfer of one electron to reduce any compound, including metals, carbonyls, and radicals (Huang *et al.*, 2005).

Table 4.22: DPPH antioxidant activity of the seed extracts

Sample code	Inhibition (%)	IC_{50}±SD (μg/ml)	P^{IC}_{50}
BP	71.83±7.99^{de}	15.46±0.45^{e}	4.81
BS	51.23±0.37^{f}	20.36±0.96^{d}	4.69
CE	21.80±1.65^{h}	ND	ND
CP	29.42±2.59^{g}	ND	ND
EA	21.88±5.67^{h}	ND	ND
MC	19.94±1.66^{h}	ND	ND
MM	66.29±0.90^{de}	31.14±0.81^{a}	4.51
MT	67.80±3.98^{de}	23.67±0.37^{b}	4.62
PB	77.00±4.35^{cd}	14.20±0.74^{f}	4.85
PN	86.29±0.90^{ab}	12.20±0.75^{g}	4.91
Ascorbic	87.80±1.10^{a}	22.38±0.37^{c}	4.65
Quercetin	89.95±0.63^{a}	ND	ND

Values are expressed as mean±SD of triplicate experiments for both the percentage inhibition and IC_{50}; values sharing a common superscript in the column are not significant different at *p<0.05;* ND: Not determined; P^{IC}_{50} = (-$\log_{10}$ IC_{50} value): Measure of radical scavenging strength of the seed extracts; BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida

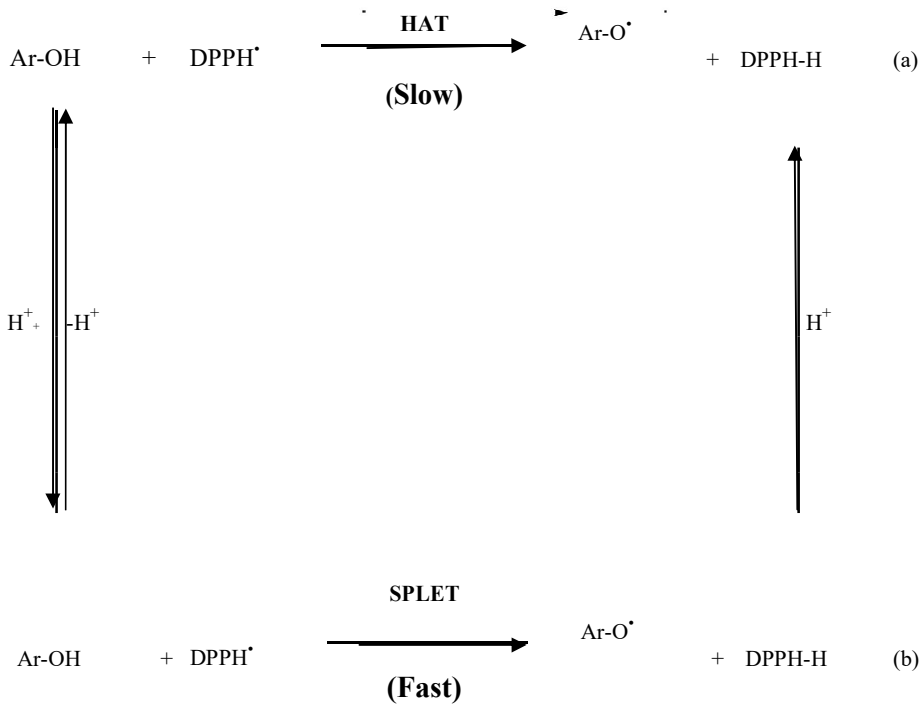


Figure 4.1: Conventional hydrogen atom transfer (HAT) and sequential proton loss electron transfer (SPLET) process of a phenolic compound (Gülçin and Beydemir, 2013)

4.7.1 Ferric reducing power activity of the seed extracts

The seed extracts reducing capability of Fe^{3+}-Fe^{2+} was determined in the reducing power assay in which the tested seed extracts changed yellow colouration of the overall solution to green as functions of their reducing powers. The reductant i.e. the bioactive compounds in the seed extracts are always responsible for the reduction of Fe^{3+}/ferricyanide complex reduction to ferrous form. The bioactive compounds present in the seed extracts shown to be potent reductant and cause the reduction of Fe^{3+}/ferricyanide complex to ferrous; and this proof of the reducing power of the seed extracts. Ascorbic acid was used as the positive control in this assay and the reducing power of the positive control and the seed extracts is shown on Table 4.22. The result showed that the seed extracts and the ascorbic acid (positive control) percentage reducing powers were not significantly different at $P<0.05$. The values obtained were in the range of 72.11±1.10-87.33±8.21 % (Table 4.23). But the dose response curves (Table 4.22) used for determination of IC_{50} values of the seed extracts with reducing power above 50 % displayed significant differences among the seed extracts.

The ranking order of the seed extracts and the positive control reducing powers based on the IC_{50} values were ascorbic acid, EA. BP, PN > MC > BS, PB, MM > CP > MT. The P^{IC}_{50} values also reflected that ascorbic acid has highest reducing power (6.16) followed by PN (5.98), BP (5.97) and EA (5.93). MC, BS, PB and MM exhibited moderate reducing powers in the range of 5.19-5.60 calculated by pIC_{50} when compared with the ascorbic acid pIC_{50} value of 6.16 as a measurement of the reducing strength of the seed extracts. The seed extracts of CP and MT exerted low reducing powers with pIC_{50} values of 4.73 and 4.64 respectively. Overall results of the ferric reducing powers of the seed extracts indicated that the seed extracts exhibited high reducing power when compared with the ascorbic acid and have high tendency of scavenging reactive oxygen species which could damage tissues out of the body systems of diabetic patients or during diabetic conditions. Reducing power of bioactive compounds in the seed extracts reflect their electron donating capacity and their associated antioxidant activity.

Bioactive compounds with antioxidant effects can be reductants and inactivate oxidants (Köksal, and Gülçin, 2008). The reducing capacity of the bioactive compounds in the seed extracts is a direct reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$. The bioactive compounds role in free (Fe^{3+}) to the reduced product leads to the formation of an intense Perl's Prussian blue complex, $Fe_4[Fe(CN^-)_6]_3$, which has a strong absorbance at 700 nm (Bursal, and Gülçin, 2011). The proposed mechanisms for the transformation of the complex are shown below with the following chemical equations.

$$Fe(CN)_6^{3-} \xrightarrow{\text{Reductant}} Fe(CN)_6^{4-}$$

$$Fe(CN)_6^{4-} + Fe^{3+} \xrightarrow{\text{Reductant}} Fe_4[Fe(CN)_6]_3$$

The ferric ions (Fe^{3+}) reducing antioxidant power of the seed extracts takes the advantage of an electron transfer in which a ferric salt (Fe^{3+}) is transformed to Fe^{2+}, this method measures the antioxidant properties of pure compounds that better reflect their potential protective effects. Bioactive compounds which are responsible for the antioxidant activity of the seed extracts reduced Fe^{3+}-ferricyanide complexes to the ferrous (Fe^{2+}) form. The Prussian blue colored complex is formed by the addition of $FeCl_3$ and then, ferric form (Fe^{2+}) is converted to ferrous form (Fe^{2+}). Therefore, the amount of the seed extracts reduction power is determined by the measurement of Perl's Prussian blue formed at 700 nm (Chung *et al.,* 2002). An increase in absorbance of the reaction mixture indicates an increase in the reducing capacity due to an increase in the formation of the complex. In the reaction mixture, a yellow colour of the test solution changes to green or blue depending on the reducing power of the antioxidant. A higher absorbance indicates higher ferric reducing power of the seed extracts (Gülçin *et al.*, 2002).

Table 4.23: Ferric reducing power (FRAP) activity of the seed extracts

Sample code	Inhibition (%)	IC_{50}±SD (μg/ml)	P^{IC}_{50}
BP	76.25±6.08[a]	1.07±0.01[h]	5.97
BS	80.88±7.52[a]	6.10±0.02f	5.21
CE	72.59±1.51[a]	9.53±0.01[c]	5.02
CP	87.83±8.21[a]	18.6±0.05[b]	4.73
EA	84.35±0.69[a]	1.17±0.07[h]	5.93
MM	85.22±8.89[a]	6.38±0.01[e]	5.19
MC	79.44±5.42[a]	2.49±0.12[g]	5.60
MT	80.88±7.52[a]	22.82±0.01[a]	4.64
PB	72.11±1.10[a]	6.67±0.06[d]	5.18
PN	79.50±1.77[a]	1.04±0.03[h]	5.98
Ascorbic acid	74.47±2.91[a]	0.69±0.01[i]	6.16

***Values are expressed as mean±SD of triplicate experiments for percentage inhibition and IC_{50}**
Values sharing a common superscript in the same column are not significant different at *p<0.05
*** P^{IC}_{50} = (-$\log_{10}$ IC_{50} value): Measure of ferric reducing power of the seed extracts**
BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida*

4.8 Total phenolic and flavonoids contents of the seed extracts

4.8.1 Total phenolic content of the seed extracts

Total phenolic contents of the seed extracts is another mechanism of determine the plants antioxidant potential. This is based on phosphomolybdic and phosphotungstic acid complexes reduction to blue chromogens in the presence of phenolic compounds present in the plant seed extracts. The phenolics content of the seed extracts obtained from gallic acid calibration curve presented on Table 4.23 and Fig. 4.5 were classified as low (<10 mg GAE/g), medium (10-50 mg GAE/g) and high (> 50 mg GAE/g) by using Rufino *et al.* (2010) classification. All the plant seed extracts remarkably had high amount of phenolic contents (Table 4.24). Among the seed extracts examined, PN seed extract displayed the highest total phenol content of 146.61±3.81mg GAE/g, which was 3.04 fold greater than the CE seed extracts with least total phenol content of 48.27±1.95 mg GAE/g. This also justified the highest radical scavenging potential of PN seed extract against DPPH i.e. highest antioxidant activity against DPPH radicals.

PB and BP seed extracts content of phenols were the same (124.61±0.25 mg GAE/g) as the second most abundant; followed by MC (106.36±3.08 mg GAE/g), BS (103.11±0.79 mg GAE/g) and MM (97.35±0.01 mg GAE/g) respectively. The phenolic content of MT (78.80±0.89 mg GAE/g) and EA (72.66±0.37 mg GAE/g) were also higher than that of CE (48.27±1.95 mg GAE/g) being the least abundant (Figure 4.6).

Phenolic antioxidants form an important class of compounds which serve to inhibit the oxidation of materials of both commercial and biological importance. The main role of antioxidants is to intercept and react with free radicals at a rate faster than the substrate, and since free radicals are able to attack a variety of molecules in the tissue cells such as lipids, fats, and proteins, it is believed that they are implicated in a number of important degenerative diseases including diabetes and oxidative stress (Beckman and Ames, 1998). There are two pathways for oxidation in which antioxidants can play a preventive role. The first is H-atom transfer, illustrated below for the important case of lipid peroxidation:

$$RH \longrightarrow R^{\bullet} \quad \text{(initiation)} \quad (1)$$

$$R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet} \quad \text{(Addition of } O_2\text{)} \quad (2)$$

$$RO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet} \quad \text{(H-atom exchange)} \quad (3)$$

Once a free radical $R^{\bullet}$ has been generated, then reactions (2) and (3) form a chain reaction. As the chain cycles through (2) and many lipid molecules (R–H) are converted into lipid hydroperoxide (ROOH), resulting in oxidation and rancidity of fats. Reaction (2) is very fast whereas (3) is much slower (Burton, 1986). For the phenolic antioxidant, the generic term (ArOH,) will be used since by definition it contains at least one hydroxyl group attached to a benzene ring. The role of the antioxidant ArOH is to interrupt the chain reaction according to equation (4)

$$RO^{\bullet} + ArOH \longrightarrow ROOH + ArO^{\bullet} \quad (4)$$

To be effective $ArO^{\bullet}$ must be a relatively stable free radical, so that it reacts slowly with substrate RH but rapidly with $RO^{\bullet}$, hence the term “chain-breaking antioxidant”. It is known that the most effective lipid-soluble chain-breaking antioxidant in human blood plasma is R-tocopherol (R-TOH), the most active component of Vitamin E (Burton, 1986). *In vivo*, the R-tocopheroxyl radical ($R\text{-}TO^{\bullet}$) is regenerated by reaction with Vitamin C, so that the chain reaction causing lipid peroxidation is broken, (Burton, 1986).

Table 4.24: Total phenols and flavonoids content of the seed extracts

Sample	Total phenols	Total flavonoids
BP	124.61±0.25^{b}	7.31±0.13^{cd}
BS	103.11±3.79^{c}	6.29±0.26^{d}
CE	48.27±1.95^{f}	10.96±1.85^{c}
CP	60.25±3.90^{ef}	8.90±0.00^{cd}
EA	72.66±9.37^{de}	19.92±0.00^{b}
MC	106.36±3.08^{c}	7.50±0.03^{cd}
MM	97.35±8.01^{c}	26.64±2.11^{a}
MT	78.80±8.98^{d}	29.54±0.40^{a}
PB	124.65±5.86^{b}	7.781±0.01^{cd}
PN	146.61±3.81^{a}	9.36±0.40^{cd}

Values are expressed as mean±SD of triplicate experiments; BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida*

4.8.2 Total flavonoids contents of the seed extracts

The seed extracts flavonoids contents measurement is also an important instrument in determine plants antioxidant potential. The quantity of flavonoids present in each of the plant seeds used for this study was presented in Table 4.24. The quantity of the flavonoids in each of the seed extract was measured from qucertin calibration curve. The content of flavonoids in the seed extracts was in the range of 7.31±0.13-29.54±0.40 mg QE/g. Among the seed extracts, MT and MM displayed highest total flavonoid content of 29.54±0.40 mg QE/g and 26.64±2.11 mg QE/g respectively; followed by EA (19.92±0.00 mg QE/g). The seed extracts of BS, BP and MC were had lowest content of flavonoids of 6.29±0.26 mg QE/g; 7.31±0.13 mg QE/g and 7.50±0.03 mg QE/g respectively. The variation observed in the values obtained for the flavonoids content of the plant seeds could be as result of some factors like the location of the plants, age and climatic condition. It is also an indication that the bioactive compounds present in all these seed extracts have keto group of C-4 along with either the C-5 or C-3 hydroxyl group of flavanols or flavones that can easily form acid-stable complexes with aluminum. In addition, aluminum chloride can also develop acid-liable complexes with A or B-ring of flavonoids ortho-dihydroxyl groups followed by the production of chromogens, which is an indicator of availability of flavonoids compounds or derivatives in all the seed extracts.

4.9 Advance glycation endproducts (AGEs) activity of the seed extracts

Glycation is a reaction between the sugars carbonyl group and the proteins amino groups (mostly, the lysine amino group and the guanidine group of Arginine), with the final formation of advanced glycation endproducts (AGEs). Due to contribution of many traditional medicinal plants which have been used practically for the control of many chronic ailments like diabetes. The antiglycating activities of all the seed extracts under this study were investigated. All the seed extracts reduced the formation of advance glycation end product determined fluorometericaly as shown in Table 4.25. Nine out of the seed extracts inhibited the production of fluorescent AGEs above 50% and their IC_{50} values were determined through concentration dose responses curves with the standard inhibitor (Quercetin) known as AGEs inhibitor, was used as the positive control.

The seed extract of BP decreased AGE fluorescence by 34.26±0.60 % as the weakest inhibitor at a single dose concentration of 1.0 mg/mL. The seed extracts of BS, EA and PN were the strongest inhibitors of AGEs with the IC_{50} values 0.46±0.0 µg/mL, 1.38±0.0 µg/mL and 1.74±0.13 µg/mL respectively in a dose-dependent manner when compared to the quercetin (IC_{50}, 1.59±0.14 µg/mL). MT seed extract was also strong inhibitor of AGE with IC_{50} value of 3.90±1.12 µg/mL. The seed extracts of CP, CE and PB showed moderate inhibitions with no significance activity against AGEs having IC_{50} values in the range of 5.15±0.14-5.71±0.02 µg/mL. On the contrary, the seed extracts of MM and MC exhibited weak inhibitory activities against AGE where the values of IC_{50} were 4.6 and 6.14 folds lower than the positive control (IC_{50}, 1.59±0.15 µg/mL). The results revealed that majority of the seed extracts could be employed as AGEs inhibitor to prevent several pathologies such as chronic renal insufficiency, nephropathy, neuropathy, arthritis, cataract and Alzheimer's disease (Morena *et al.,* 2002). Since the role of AGEs in the promotion of diabetic complication have been shown in many studies, inhibition of AGEs formation is considered as a tool in preventing progressions of diabetic complications as demonstrated by all the seed extracts.

Table 4.25: Advance glycation endproducts (AGEs) activity of the seed extracts

Sample code	Inhibition (%)	IC_{50}±SD (μg/ml)	P^{IC}_{50}
BP	34.26 ± 0.60^{h}	ND	ND
BS	68.54 ± 4.0^{cd}	0.46 ± 0.00^{e}	6.34
CE	83.67 ± 4.60^{b}	5.71 ± 0.02^{bc}	5.24
CP	92.39 ± 1.83^{a}	5.15 ± 0.14^{bc}	5.30
EA	55.06 ± 2.50^{e}	1.38 ± 0.0^{e}	5.86
MC	85.24 ± 2.24^{b}	7.32 ± 0.34^{b}	5.14
MM	55.72 ± 0.65^{df}	9.77 ± 1.33^{a}	5.02
MT	67.66 ± 3.14^{de}	3.90 ± 1.12^{cd}	5.57
PB	74.92 ± 3.81^{c}	5.47 ± 0.00^{bc}	5.26
PN	61.72 ± 3.23^{ef}	1.74 ± 0.13^{de}	5.76
Quercetin	68.70 ± 3.26^{cd}	1.59 ± 0.15^{de}	5.87

***Values were expressed as mean±SD of triplicate experiments for percentage inhibition and IC_{50}**
Values sharing a common superscript in the same column are not significantly different at *p<0.05;* ND: Not `determined; P^{IC}_{50} = ($-\log_{10}$ IC_{50} value): The measure of strength of the seed extract as potent inhibitors of advance glycation endproducts formation; BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida

4.10 ALR1 and ALR2 enzymes protein concentration

The protein contents of crude enzymes of ALR1 and ALR2 extracted from bovine's kidney and eyes was measured to be able to determine their efficiency by using BSA calibration curve. The quantity of protein in both ALR1 and ALR2 enzymes were estimated to be 0.144 mg/mL and 0.094 mg/mL respectively. From the values of protein obtained for both enzymes; an average reaction rates of 0.190±0.02 U/min and 0.227±0.01 U/min were gotten for ALR1 and ALR2 enzymes respectively. The reaction rate was measured by the enzymes consumption of the substrate (NADPH) in the absence of inhibitor that can prevent the enzymes catalysis reaction. The decrease in the amount of the substrate concentration determined by taking the reaction absorbance showed that the amount of protein in the ALR1 and ALR2 are enough for the reaction to take place at the required time and temperature.

4.11 Optimization of ALR1 and ALR2 enzymes, cofactor and substrate

4.11.1 Optimization of ALR1 and ALR2 enzymes

ALR1 and ALR2 enzymes optimization is one of the essential requirements in any enzyme assay before carrying out the experimental assay. Both volume and concentration of enzyme to be used in the inhibition assay with either substrate or the inhibitor to be tested must be optimized experimentally by varying the volume of the enzyme. The volume of ALR1 enzyme to be used in the inhibition assay was determined by varying the volume from 30.0 µL to 100.0 µL in the reaction mixture. As the volume of the enzyme was varied, it was observed that increase in the volume of enzyme, increases NADPH consumption and this better the product formation. The change in absorbance was also recorded. At 70 µL of the ALR1 enzyme, it was observed that amount of the enzyme in the reaction medium has saturated the active pocket of the substrate and the reaction tend to deviate from normal pattern as showed (Figure 4.2). And it is an indication that at that volume (70 µL) the amount of the enzyme in the reaction medium is sufficient to perform the enzyme inhibition assay by the seed extracts (Table 4.26). One unit of the enzyme is defined as an amount required catalyzing the oxidation of 1.0 µM of NADPH per mins. Given results suggested that 70 μL of the crude extract is sufficient to perform the enzymatic reaction.

Table 4.26: Experimental data for the optimization of aldehyde reductase enzyme (ALR1)

Enzyme Conc. (μL)	Experiment1	Experiment 2	Experiment 3
0	0	0	0
30	0.051	0.056	0.061
40	0.069	0.072	0.075
50	0.088	0.085	0.091
60	0.115	0.12	0.125
70	0.141	0.138	0.139
80	0.141	0.146	0.144
90	0.152	0.146	0.149
100	0.149	0.155	0.151

Values are single data of individual experiment

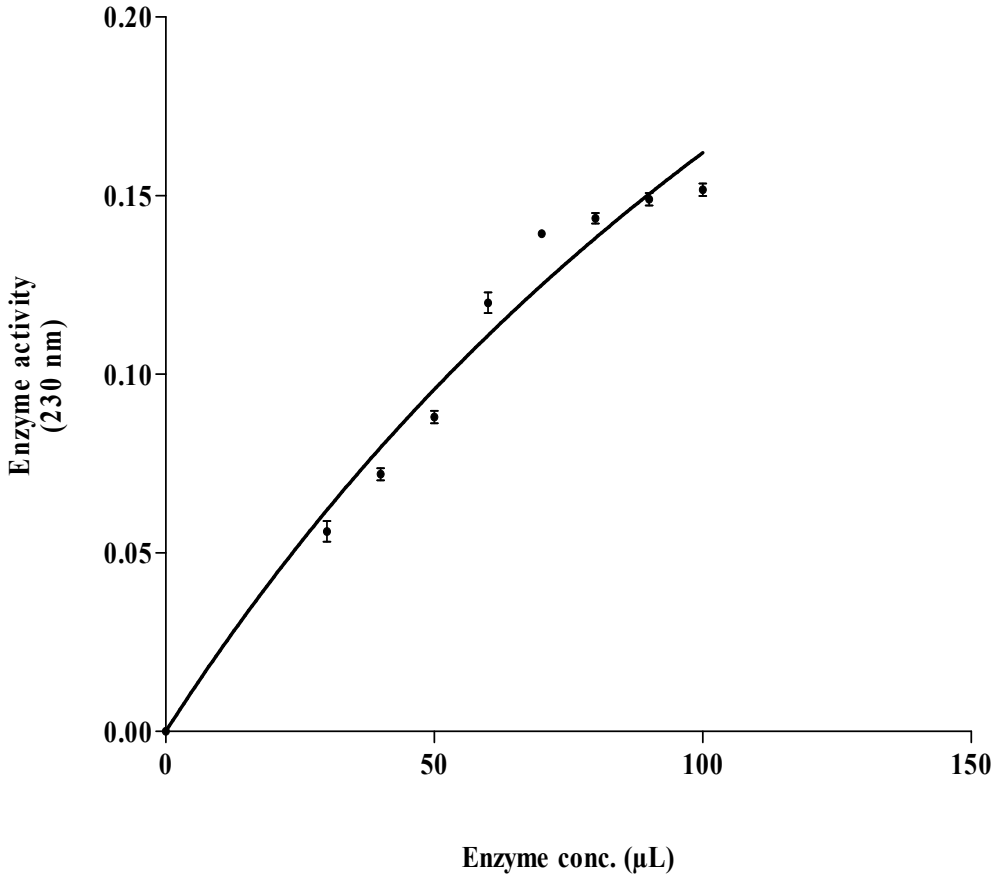


Figure 4.2 **Michealis Menten plot for ALR1 enzyme, at varying concentrations of ALR1 and at saturation concentration of D, L-glyceraldehyde and NADPH**

4.11.2 Substrate (D, L-glyceraldehyde) optimization

As optimization of enzyme is important, it is also necessary to make sure the substrate to be used in assay with the respective enzyme is also optimized. The substrate optimization was performed by using 70 µL of enzyme in the inhibition assay and varying the volume of 50 mM of D, L-glyceraldehyde in the range of 10-70 µL at the fixed concentrations and volumes of co-factor. After the optimization, data obtained were subject to Michaelis-Menten plot and a curve was obtained from which volume of the D, L-glyceraldehyde (substrate) to be used in the assay was determined. GraphPad prism software (version 5.0) was used to obtain the Michaelis-Menten plot (Figure 4.3). The reaction rate and maximum velocity for the enzyme reaction at the particular temperature and time were determined to be Km (0.165±0.02 mM) and Vmax (2.76±0.13 mins/mM) respectively. Enzyme velocity for the reaction was 1.381 mins/mM determined mathematically from the Vmax obtained from the curve.

Table 4.27: Experimental data for the optimization of Substrate

Substrate conc. (μL)	Experiment 1	Experiment 2	Experiment 3
0	0	0	0
10	0.987	1.098	0.898
20	1.788	1.456	1.389
30	1.89	1.567	1.99
40	2.098	1.788	1.987
50	2.123	1.877	2.234
60	2.198	1.988	2.298
70	2.234	2.311	2.098

Values are single data of individual experiment

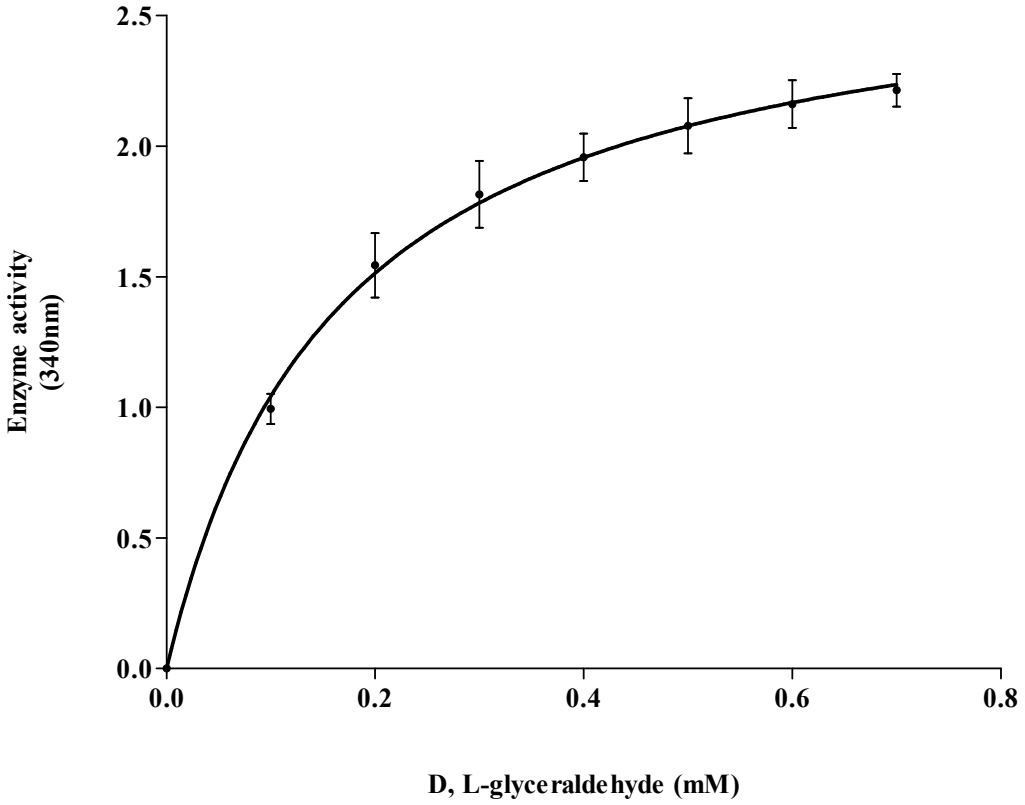


Figure 4.3 **Michaelis-Menten plot of D, L-glyceraldehyde, at varying concentrations of D, L-glyceraldehyde, saturation concentration of NADPH and optimized (ALR1) enzyme**

4.11.3 Optimization of cofactor (NADPH)

The same method used to determine the enzyme volume and substrate concentration and volume was also repeated for the cofactor. The volume of the cofactor was varied while those of NADPH and enzyme were kept constant in the reaction mixture. The change in absorbance measured is presented on Table 4.28. The plot of Michealis-Menton curve was also carried out by the use of data on Table 4.27 as shown in Fig. 4.4. The volume of NADPH to be used in the enzyme experimental assay was determined from GraphPad prison software (version 5.0) to be 50 μL. From the Michealis-Menton curve in Figure 4.4, values of 8.16±0.49 mins/mM and 0.303±0.04 mM were obtained for Vmax and Km respectively. Further, mathematical expression of Vmax from the graph yielded 4.08 mins/mM as the reaction velocity. The graph also indicated that the substrate to be used in the subsequent experiment obeys Michealis-Menton law.

Table 4.28: Experimental data for the optimization of cofactor

Cofactor conc. (*μ*L)	Experiment 1	Experiment 2	Experiment 3
0	0	0	0
10	2.134	2.456	2.366
20	3.122	3.345	3.566
30	3.567	4.098	3.811
40	4.123	4.876	4.267
50	4.789	5.566	4.688
60	5.378	5.898	5.411
70	5.898	6.134	5.567

Values are single data of individual experiment

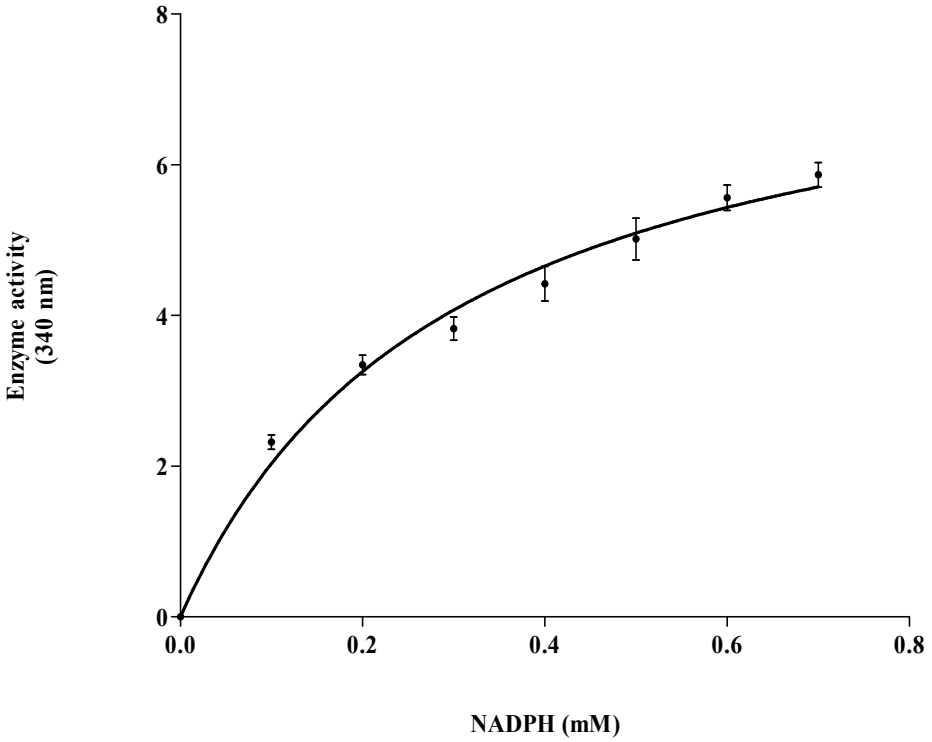


Figure 4.4: **Michaelis-Menten plot of cofactor at varying concentrations of NADPH, at saturation concentration of D, L-glyceraldehyde and optimised (ALR1) enzyme**

4.12 Seed extracts enzymatic screening

4.12.1 Aldose reductase (ALR2) inhibitory activity of the seed extracts

It has been demonstrated that human and bovine aldose reductase enzyme sequences are 81% of identical in characteristic and 89% of homology with the identical proposed active sites (Gui *et al.*, 1995). Evaluation of the seed extracts potency was basically based on the consumption of NADPH (oxidation) by ALR2 monitored spectrophotometrically (Del Corso *et al.*, 2000). A dose relationship of ALR2 inhibition was established for all the seed extracts, and among the studied seed extracts, BS seeds (IC_{50} 0.57±0.05 µg/mL) exhibited highest ALR2 inhibitory activity followed by PB and MM seed extracts with IC_{50} values of 1.31±0.17 µg/mL respectively. The seed extracts of PN (IC_{50} 1.02±0.13 µg/mL), MT (IC_{50} 2.96±0.75 µg/mL) and BP (IC_{50} 4.29±0.14 µg/mL) showed moderate inhibitory activity compared to quercetin, a known standard inhibitor of ALR2 (Table 4.18).

The lowest inhibition of ALR2 was found in the seed extract of MC (IC_{50} 14.87±0.47 µg/mL). Seed extracts of EA and CE were weak inhibitors of bovine aldose reductase with the IC_{50} values of 7.51±0.13 µg/mL and 11.05±0.04 µg/mL as presented in Table 4.29. The seed extract of BS proved to be the most potent inhibitor as displayed by the obtained data of IC_{50} the P^{IC}_{50} values. The P^{IC}_{50} presents the strength of the inhibitors with respect to their minimum inhibitory concentrations required to inhibit the enzyme by 50%. The P^{IC}_{50} values obtained for all the seed extracts and the reference inhibitor showed their power against the tested enzyme (ALR2). The variation in the seed extracts potency could be as a result of difference in their phytochemicals compositions, such as polyphenols and flavonoids compounds which have been reported as the potent inhibitors of aldose reductase.

Several studies have revealed that ALR2 is an important pharmacological enzyme target and this enzyme inhibition could be a major way for the diabetes complications treatment. Thus there is a need for new and effective inhibitors of ALR2. A large number of aldose reductase inhibitors (ARI) have been developed and some of them have been evaluated up to preclinical and clinical trials. However, pharmacokinetic drawback, low *in vivo* efficacy and adverse side effects have always hampered their development.

Presently, epalrestat is the only commercially available ARI approved for diabetic neuropathy management and is currently used in few Asian countries such as Japan and India. Sorbinil was withdrawn from clinical trials because of its hypersensitivity reaction (Lipinski *et al.,* 1992). Imirestat, a fluorine derivative of sorbinil was also stopped due to its toxicity. Tolrestat is a carboxylic acid derivative which was launched in 1989 into the market but latter withdrawn throughout the world in 1996 as a result of its chronic liver toxicity and poor *in vivo* efficacy. On the other hand, fidarestat exhibited higher selectivity and efficacy than the sorbinil (the parent compound), it provides beneficial clinical effects in the diabetic neuropathy with no signal adverse effects, it is now the current most promising drug candidates among ARIs (Hotta *et al.,* 2004).

Table 4.29: Aldose reductase activity (ALR2) of the seed extracts

Sample code	Inhibition (%)	IC_{50}±SD (µg/ml)	P^{IC}_{50}
BP	79.99±1.87^{bc}	4.29±0.04^{e}	5.37
BS	82.23±1.48^{bc}	0.57±0.05^{i}	6.24
CE	83.73±0.60^{a}	11.05±0.04^{c}	4.96
CP	80.32±2.08^{bc}	12.18±0.08^{b}	4.91
EA	65.53±0.83^{d}	7.51±0.03^{d}	5.12
MC	78.47±0.43^{abc}	14.87±0.02^{a}	4.83
MM	81.35±1.76^{bc}	1.70±0.01^{gh}	5.77
MT	72.68±0.56^{bc}	2.96±0.05^{f}	5.53
PB	81.95±4.62^{bc}	1.31±0.01^{h}	5.88
PN	83.23±1.34^{a}	2.02±0.03^{g}	5.69
Quercetin	75.19±5.57^{bc}	1.51±0.03^{h}	5.82

***Values are expressed as mean±SD of triplicate experiments for percentage inhibition and IC_{50}**
Values sharing a common superscript in a column are not significantly different at *p<0.05
*** P^{IC}_{50} = (-$\log_{10}$ IC_{50} value): Measure of the seed extracts potency against ALR2 enzyme; BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida***

4.12.2 Aldehyde reductase (ALR1) inhibitory activity of the seed extracts

Aldehyde reductase (ALR1) is a member of aldo-keto family, and it was assumed that unsuccessful profile of many inhibitors of aldose reductase (ALR2) in the clinical trials was due to their concurrent inhibition of the closely related ALR1 (EL-kabbani *et al.,* 2005). The seed extracts selected for this study were tested for their ability to inhibit bovine kidney aldehyde reductase (ALR1), and the obtained data for their IC_{50} values were presented on Table 4.30. The physiological role of ALR1 in the body include reduction of many aldehydes and metabolizes of compounds such as methylglyoxal and 3-deoxyglucosone regarded as the intermediates in advanced glycation end products formation. Thus, the development of adverse side effects of many inhibitors of ALR2 enzyme may be as a result of their concurrent inhibition of ALR1 (Vander *et al.,* 1996). To understand the inhibitory activity of the seed extracts against ALR2 enzyme, the role of inhibitor under study in inhibiting ALR1 enzyme has to be carried out and nature of selectivity has to be determined too.

The ALR1 inhibitory potential of the seed extracts expressed as µg/mL of the IC_{50} values were compared to the valproic acid, a reference inhibitor. In the series of the seed extracts studied, some of the seed extracts which were potent inhibitors of the ALR2 were weak inhibitors of ALR1. The strongest inhibitor of ALR1 was the seed extracts of PB, PN and CP with IC_{50} values of 1.86±0.05 µg/mL, 1.94±0.02 µg/mL and 1.98±0.15 µg/mL respectively. For the seed extracts of BP and MM, a profound moderate inhibition was observed with IC_{50} values of 3.70±0.69 µg/mL and 3.95±0.76 µg/mL. In comparison with valproic acid (IC_{50} 4.57±0.13 µg/mL), the seed extracts of MT and MC have IC_{50} values of 4.40±0.38 µg/mL and 4.81±0.15 µg/mL similar to the value obtained for the reference inhibitor (valproic acid). On the other hand, the least inhibition of ALR1 was noticed in BS, EA and CE with the following IC_{50} values: 5.82±0.05 µg/mL, 6.57±0.07 µg/mL and 6.99±0.17 µg/mL presented in Table 4.19. In this study, most of the strong inhibitors of ALR2 like BS, MT and MM seed extracts were weak inhibitors of ALR1. In diabetes, one of the primary roles of ALR1 is detoxification of reactive α-oxoaldehyde glycating agents from the tissues (Shinohara *et al.,* 1998). And the selectivity degree of any AKR inhibitor toward ALR2 is important for any successful aldose reductase inhibitor (AR1), the selectively of these seed extracts were further studied (Table 4.31).

Table 4.30: Aldehyde reductase (ALR1) activity of the seed extracts

Sample code	Inhibition (%)	IC_{50}±SD (μg/ml)	$P^{IC_{50}}$
BP	69.01±1.18^{b}	3.70±0.69^{c}	5.43
BS	69.77±4.10^{bc}	5.82±0.05^{ab}	5.24
CE	74.93±3.48^{bc}	6.99±0.17^{a}	5.16
CP	76.98±4.85^{bc}	1.98±0.15^{d}	5.70
EA	74.42±5.38^{bc}	6.57±0.07^{a}	5.18
MC	70.60±4.04^{bc}	4.81±0.15^{bc}	5.32
MM	72.95±7.48^{bc}	3.95±0.76^{c}	5.40
MT	76.15±2.00^{ab}	4.40±0.38^{bc}	5.36
PB	78.65±1.90^{bc}	1.86±0.05^{d}	5.73
PN	81.52±4.85^{a}	1.94±0.21^{d}	5.71
Valporate acid	84.00±2.51^{a}	4.57±0.13^{bc}	5.34

***Values are expressed as mean±SD of triplicate experiments for percentage inhibition and IC_{50}**
Values sharing a common superscript in a column are not significantly different at *p<0.05
$P^{IC_{50}}$ = (-$\log_{10}$ IC_{50} value): Measure of the seed extracts potency against ALR1 enzyme; BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida

4.12.3 Selectivity of the seed extract on ALR2 over ALR1

The most important feature of aldose reductase inhibitors (ARIs) in pharmacological application is their selectivity (El-Kabbani *et al.,* 1998). Selectivity index of any inhibitor against ALR2 enzyme is defined as the ratio of ALR1 to ALR2 enzyme. The co-inhibition of ALR2 with relevant oxido-reductases that share structure and physiological functions like ALR1 may result into adverse side effects and total failure of such inhibitor or any drug designed for the management of ALR2 or as its inhibitor. To ensure the inhibitory role of the seed extracts and their selectivity towards ALR2, the seed extracts selectivity index were evaluated (Table 4.31) by studied a close related aldehyde reductase (ALR1). Among the most efficient AR1s, the selective indices were found to be highest in BS seed extract. A selective index of 10.21 was recorded for BS seed extract, the most potent inhibitor of ALR2. In the series of the seed extracts obtained as the moderate inhibitors of ALR2, a selective index in the range of 0.68-1.48 was recorded for PN, PB and MT respectively. A selectively index of 2.32 was obtained for MM seed extract as the second most potent inhibitor of ALR2 among the tested seed extracts (Table 4.20).

Table 4.31: The seed extracts selectivity towards ALR2 enzyme

Sample	ALR2 (µg/mL)	ALR1 (µg/mL)	Selectivity (ALR1/ALR2)	Antioxidant (µg/mL)
BP	4.29±0.14^{e}	3.70±0.69^{c}	0.86	15.46 ±0.45
BS	0.57±0.05^{i}	5.52±0.05^{ab}	10.21	20.36±0.96
CE	11.05±0.04^{c}	6.99±0.17^{a}	0.63	21.80 ±0.00 (%)
CP	12.1 ±0.08^{b}	1.98±0.15^{d}	0.16	29.42±0.81 (%)
EA	7.51±0.03^{d}	6.57±0.07^{a}	0.87	21.88±5.67 (%)
MC	14.87±0.02^{a}	4.81±0.15^{bc}	0.32	19.94±1.66 (%)
MM	1.70±0.01^{gh}	3.95±0.76^{c}	2.32	31.14±0.81
MT	2.96±0.05^{f}	4.40±0.38^{bc}	1.48	23.67±0.37
PB	1.31±0.07^{h}	1.86±0.05^{d}	1.42	14.20 ±0.74
PN	2.02±0.03^{g}	1.94±0.21^{d}	0.96	12.2 ±0.75
PC	1.51±0.13^{h}	4.57±0.13^{bc}	3.03	ND

***Values are expressed as mean±SD of triplicate experiments for IC_{50} and antioxidant activity**

Values sharing a common superscript in a column are not significantly different at *p<0.05

***PC: positive controls (Valporate acids for ALR1 and quercetin for ALR2); ND: determined**

Selectivity: IC_{50} (ALR1)/IC_{50} (ALR2); BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida

4.12.4 Determination of Kinematic parameter (K_{max} and V_{max})

4.12.4.1 Determination of K_{max} and V_{max} for α-glucosidase enzymes

The kinetic parameters (K_m and V_{max}) of α and β-glucosidase enzymes are measure of the enzyme maximum velocity, reaction rates, concentrations and volumes of the enzymes and the substrate to be used in the reaction medium. The parameters were calculated using the data on Table 4.32. The data were and which were fitted into Michealis-Menton equation using GraphPad PRISM software. From the graph, 0.130±0.13 mM was obtained for substrate concentration (Km) while 0.0994±0.05 mM/sec was obtained for the enzyme maximum velocity (Vmax). It can be observed from Michealis-Menton curve (Fig.4.25) that enzyme velocity varies linearly with substrate concentration for small concentration of substrate. Increase in substrate concentration, the velocity "plateaus" which was indicated that velocity becomes independent of substrate concentration at large values of substrate concentration. This followed the simplest model that accounted for the enzyme behaviour at high concentration of substrate. Km which is the substrate concentration when the reaction is half maximal i.e. Km= Vmax/2. Substrate concentration was also calculated theoretical to be 0.0125 mM which compared favourably with the value obtained through Michealis-Menton equation (0.130 mM). It implies that the enzyme, substrate and experimental condition are all in order and the reaction obeys Michealis-Menton law.

Table 4.32: Experimental data for the determination of Kinetic parameters of α-glucosidase enzyme

Substrate (mM)	Experiment 1	Experiment 2	Experiment 3
0.003900	0.007	0.006	0.010
0.007800	0.009	0.006	0.008
0.015625	0.015	0.017	0.014
0.031250	0.019	0.021	0.017
0.062500	0.030	0.033	0.035
0.125000	0.049	0.047	0.047
0.250000	0.061	0.062	0.061
0.500000	0.082	0.084	0.083
1.000000	0.087	0.088	0.098

Substrate concentration was varied from 1.0-0.0039 mM at a fixed concentration of enzyme (2.5 U)

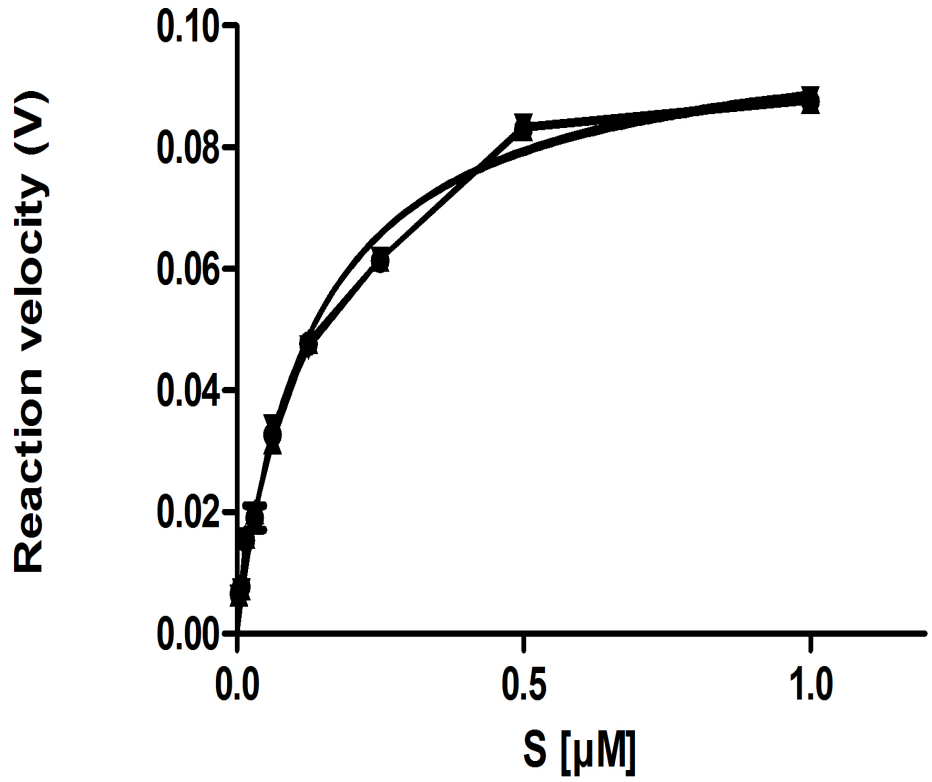


Figure 4.5: **Michealis Menten plot of α-glucosidase enzyme at varying concentration of α-D-glucopyranoside (substrate)**

4.12.4.2 Determination of K_{max} and V_{max} for β-glucosidase enzymes

The kinetic parameter values obtained for K_m and V_{max} for β-glucosidase enzyme from the Michealis-Menton curve presented in Figure 4.6 were 1.59±0.21 mM and 1.91±0.17 mM/sec respectively. It can be observed that at small concentration of substrate (s); enzyme velocity varies linearly with substrate concentration. Increase in substrate concentration, the velocity "plateaus" which was indicated that enzyme velocity (v) is independent of substrate concentration at large values of substrate concentration. It also showed that the β-Glucosidase enzyme also obeyed the law.

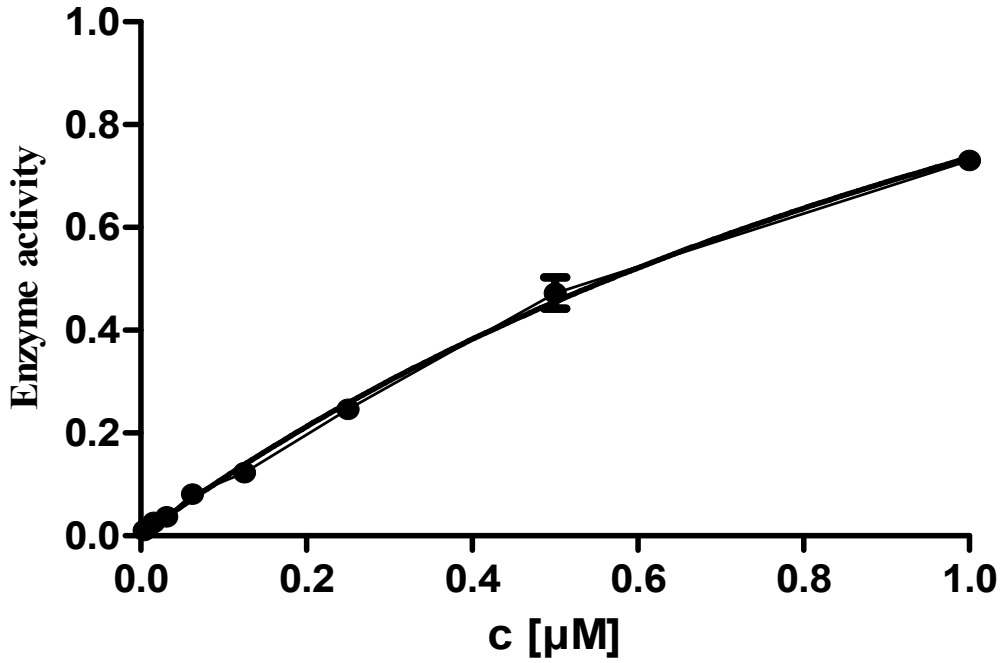


Figure 4.6: **Michealis Menten plot of β-glucosidase enzyme at varying concentration of β-D-glucopyranoside**

4.12.5 α-Glucosidase activity of the seed extracts

In type 2 diabetes, uncontrollable rises in post-prandial blood glucose level result in hyperglycemic conditions causing derangements of metabolism in the body. In diabetes patients, the breaking down of starches, oligosaccharides and disaccharide can be prevented by the inhibition of α-glucosidase before being absorbed from the upper jejunum and duodenum of the intestinal. The potency of the selected plant seeds against α-glucosidase was evaluated and the result is being shown in Table 4.33. The inhibitory activity of seed extract of CP and acarbose against α-glucosidase were recorded at IC_{50} values of 1.33±0.07 µg/mL and 3.84±0.02 µg/mL respectively. CP seed was found to be the most potent seeds among the tested plant seeds. The seed extract of MT (IC_{50} 2.84±0.00 µg/mL) strongly inhibited α-glucosidase and closely followed by the seed extract of PB (IC_{50} 3.73±0.03 µg/mL). The seed extracts of PN, CE, BS and EA exhibited inhibitory activity against α-glucosidase with IC_{50} values in the range of 4.38±0.01-4.75±0.01 µg/mL, the IC_{50} indicated that the seed extracts are strong inhibitors of α-glucosidase. An IC_{50} value of 5.93±0.08 µg/mL was obtained for BP as a moderate inhibitor of α-glucosidase (Table 4.33). The seed extract of MC showed inhibitory activity against α-glucosidase with IC_{50} value of 17.93±0.02 µg/mL being the least effective seed extract of 4.67 folds lower than the positive control (3.84±0.02 µg/mL).

Table 4.33 shows the effectiveness of all the seed extracts in inhibiting α-glucosidase enzyme, and most of the seed extracts were seen as better inhibitor of α-glucosidase than the positive control (acarbose) on the basis of their resulting IC_{50} values. For better understanding of the IC_{50} values and the potency of the seed extracts, pIC_{50} was determined. The higher the IC_{50} value of any inhibitor, the lower the potency against the tested enzyme and vice visa. The P^{IC}_{50} values also reflect the strength of each of the seed extract. The higher the P^{IC}_{50} the more potent the seed extract and the lower the P^{IC}_{50} the weaker the inhibitor. In fact, the seed extract of CP displayed highest inhibitory activity against α-glucosidase as the most potent inhibitor, even more effective than a acarbose (the control).

The enzymatic inhibition of the α-glucosidase has been linked to the presence of phenolic compounds in the plants (Kwon *et al.*, 2006). In addition, the presence of flavonoids and tannins in the seed extracts has also been reported to be responsible for their inhibitory roles against α-glucosidase activity. Major drawback of α-glucosidase inhibitors are their side effects like flatulence and diarrhea, distention of abdominal and liver toxicity. Hence, there is a need for the exploitation of the plants, spices, fruits and seeds as α-glucosidase inhibitors for possible lesser effects. Therefore, the selected plant seeds could serve as plus to the already available ones.

Table 4.33: α-Glucosidase activity of the seed extracts

Sample code	Inhibition (%)	IC_{50}±SD (μg/ml)	P^{IC}_{50}
BP	86.29±1.14^{ab}	5.93±0.08^{c}	5.23
BS	78.38±11.43^{ab}	4.75±0.01^{cd}	5.32
CE	85.07±13.12^{ab}	4.79±0.02^{cd}	5.32
CP	87.37±3.00^{ab}	1.33±0.07^{f}	5.88
EA	84.65±9.93^{ab}	4.62±0.01^{cd}	5.33
MC	74.09±9.37^{b}	17.93±0.02^{a}	4.74
MM	88.69±0.14^{ab}	8.67±0.02^{b}	5.06
MT	79.79±4.20^{ab}	2.84±0.00^{e}	5.55
PB	91.91±4.68^{a}	3.73±0.03^{de}	5.43
PN	90.26±8.19^{a}	4.38±0.01^{d}	5.36
Acarbose	88.81±10.36^{ab}	3.84±0.02^{de}	5.42

***Values are expressed as mean±SD of triplicate experiments for percentage inhibition and IC_{50}**
Values sharing a common superscript in a column are not significant different at *p<0.05
*** P^{IC}_{50} = (-$\log_{10}$ IC_{50} value): Measure of the seed extracts potency against α-glucosidae enzyme**
ND: Not determined; BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida*

4.12.6 β-Glucosidase activity of the seed extracts

β-Glucosidase is another key enzyme present in cellulase that involves in final step during hydrolysis of cellulose by the conversion of the cellobiose to glucose (Krisch *et al*., 2010). This reaction is under control by the inhibition of its product (glucose) (Singhania *et al*., 2013). Although, the conversion is uncontrollable under metabolic disorder and can lead to high blood glucose called diabetes. Digestive enzymes like β-glucosidase have been targeted as strong mechanism for the control of blood glucose concentration via the inhibition of complex carbohydrates breakdown to glucose enzymatically (Abeysekera *et al*., 2007). In the present study, different seed extracts were evaluated for their β-Glucosidase inhibitory activity, in order to find out new natural source of antidiabetic drugs. It was clearly revealed that CP and MM seed extracts were the most potent inhibitors of β-glucosidase with IC_{50} values of 2.34±0.40µg/mL and 2.60±0.22µg/mL respectively.

The seed extracts of BS, MC, BP and MT showed inhibitory activity with IC_{50} ranged of 3.14±0.03 µg/mL to 4.13±0.10 µg/mL as moderate inhibitor of β-glucosidase enzyme (Table 4.34), followed by the seed extracts of PN (5.89±0.18µ g/mL) and PB (5.36±0.40 µg/mL). Lastly, β-glucosidase was poorly inhibited by the seed extract of CE with IC_{50} of 10.11±0.70 µg/mL. The difference in the seed extracts inhibitory activity against β-glucosidase could be due to the variation in the phenolic contents of the seed extracts which have been reported by Karunaratne *et al*. (2014). There is less report on the inhibitory activity of the herbal plants against β-Glucosidase despite the role of the enzymes in the management of diabetes mellitus. This study revealed the important features of these plants seeds as source of antidiabetic agent with beta glycoside enzyme inhibitory activity. Therefore, the use of these plant seeds should be encouraged as effective therapy for postprandial hyperglycemia management with little or no drawback.

Table 4.34: β-glucosidase activity of the seed extracts

Sample code	Inhibition (%)	IC_{50}±SD (μg/ml)	P^{IC}_{50}
BP	68.62±1.20[a]	4.97±0.42[e]	5.30
BS	60.63±5.66[a]	3.22±0.80[f]	5.49
CE	70.31±0.88[a]	10.11±0.70[a]	4.99
CP	79.40±0.12[a]	2.34±0.40[g]	5.63
EA	67.67±7.84[a]	7.23±0.80[b]	5.14
MC	63.08±6.44[a]	3.14±0.03[g]	5.50
MT	70.96±2.28[a]	4.13±0.10[f]	5.38
MM	62.33±0.22[a]	2.60±0.22[g]	5.58
PB	55.16±0.19[a]	5.36±0.40[d]	5.25
PN	58.62±7.82[a]	5.89±0.18[c]	5.3
PC	13.48±0.62[b]	ND	ND

***Values are expressed as mean±SD of triplicate experiments for percentage inhibition and IC_{50}**
Values sharing a common superscript in a column are not significant different at *p<0.05
*** P^{IC}_{50} = (-$\log_{10}$ IC_{50} value): Measure of the seed extracts potency against β-glucosidase; ND: Not determined and PC: positive control (acarbose); BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida***

4.12.7 15- Lipoxygenase activity of the seed extracts

Table 4.35 shows LOX inhibitory activity of all the seed extracts selected for this study. Among the seed extracts, MC and PB extracts showed strongest anti-LOX activity with IC_{50} values of 0.81±0.07 mg/mL and 0.96±0.29 mg/mL in comparison to 0.062±0.03mg/mL obtained for positive control (quercetin). MT, EA and CE seed extracts recorded strong LOX inhibition with IC_{50} values of 1.26±0.14mg/mL, 1.63±0.39 mg/mL and 1.15 mg/mL respectively. LOX inhibition of CP, BP and BS seed extracts was moderately weak inhibitor of LOX with the percentages inhibition of 39.12±0.46 %, 38.26±0.98% and 36.80±1.93% respectively, the values are less than 50 % required for determination of their IC_{50} values. Anti-lipoxygenase inhibitory activity of PN (34.45±5.2%) and MM (30.36±1.45%) seed extracts was found to be the least. The seed extract lipoxygenase inhibitory activity have decreased order of MC > PB > CE > MT > CP > BP > BS > PN > MM (Table 4.35). Higher inhibitory activity is associated with a lower value of IC_{50} and higher pIC_{50} value. MC extract was the most potent on the other hand, MM (30.36%) was the least LOX inhibitory activity. The variation observed in lipoxygenase activity of the seed extracts on 15-LOX inhibition could be as a result of difference in their phytoconstituents composition as reported by Djeussi *et al.* (2013).

More so, LOXs and their metabolites have also been linked to numerous human cancers such as lung, breast, colon and prostrate (Samuelsson, 1987). Also, many of LOX inhibitors like Zilenton and Minocyclines available in the market have side effects and many of them have been bannned (Charlier and Michaux, 2003). Antioxidants like polyphenolic and flavonoids from plant could serve as good substituent for the synthetic drugs as inhibitor of lipoxygenase enzymes to prevent inflammatory. The development of new inhibitors of LOX with fewer side effects is essential, due to side effects of the available ones (Tomy *et al*., 2014). Inhibition of LOX may influence the inflammation processes and thus be of interest for modulation of the lipoxygenase pathway. Therefore, many of the plants could be employed in the management of oxidative stress, since inhibitors LOX have been considered as therapeutically useful in the treatment or management of many diabetic related diseases.

Table 4.35: Activity of the seed extract against 15-Lipoxygenase

Sample code	Inhibition (%)	IC_{50}±SD (µg/ml)	P^{IC}_{50}
BP	38.26±0.98^{d}	ND	ND
BS	36.80±1.93^{d}	ND	ND
CE	66.27±2.92^{bc}	1.15±0.04^{b}	5.94
CP	39.12±0.46^{d}	ND	ND
EA	67.06±6.20^{abc}	1.63±0.39^{a}	5.79
MC	65.62±1.78^{c}	0.81±0.07^{d}	6.09
MM	30.36±1.45^{e}	ND	ND
MT	72.56±2.05^{a}	1.26±0.14^{b}	5.90
PB	71.83±3.31^{ab}	0.96±0.29^{c}	6.02
PN	34.45 ±5.45^{de}	ND	ND
PC	66.26±2.41^{bc}	0.62±0.03^{e}	6.21

***Values were expressed as mean±SD of triplicate experiments for percentage inhibition and IC_{50}**
***Values sharing a common superscript in a column are not significantly different at *p<0.05,* ND:**
*** P^{IC}_{50} = ($-\log_{10}$ IC_{50} value): Measure of the seed extracts strength as 15-LOX inhibitor; Not determined; PC: positive control (quercetin); BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida***

4.12.8 Pancreatic lipase porcine inhibitory activity of the seed extracts

Hyperlipidemia prevalence is also disorder of metabolism characterized by the elevation of blood triglycerides and cholesterol, which is presently on increase across the globe. Presently, orlistat is the most available conventional drugs for the treatment of obesity but it is peculiar with certain gastro intestinal side effects which are unpleasant. Therefore, necessitating for the search for newer ones from medicinal plants, the pancreatic lipase porcine inhibitions by the seed extracts were shown in Table (4.36). The seed extracts inhibitory activity against the porcine pancreatic lipase was expressed in both percentage and IC_{50} values. Among the screened seed extracts against pancreatic lipase porcine enzyme, four out of the seed extracts shown high antilipase activity more above 50%. The seed extracts of CP, EA, and BP showed a significant inhibition against porcine lipase enzyme with percentage inhibition of 14.68±4.30%, 25.12±2.23% and 30.36±1.13%. Besides, MC (41.48±0.40%) and BS (43.22±3.25%) exhibited moderate antilipase activity less than 50% (Table 4.56). On the other hand, the seed extracts of PN, CE, PB and MT with the percentage inhibition greater than 50 % were further evaluated for IC_{50} values determination using dose-dependent curves. PN seed extract showed inhibitory activity against porcine pancreatic lipase with IC_{50} value of 23.59±3.09 µg/mL which was 6.55 folds lower than the orlistat (3.60±0.05 µg/mL) known standard drug of the enzyme.

Remarkably, PB seed extract displayed an inhibitory activity with the IC_{50} value of 1.34±0.0 µg/mL greater than the orlistat (3.60±0.05 µg/mL). IC_{50} values of 2.58±0.15 µg/mL and 3.17±0.04 µg/mL were obtained for MT and CE seed extracts against porcine pancreatic lipase respectively. Most of the previous studies have suggested that a direct relationship exist between phenolic compound, flavonoids, and condensed tannin composition of the seed extracts and pancreatic lipase inhibition activity. Phenolic compound has the ability to inhibit cholesterol micelles formation. Therefore, variation in the chemical composition of the seed extracts could be the one responsible for the different potency displayed against the enzyme. Among applied strategies for anti-obesity agents are energy expenditure increase (blockage of adipogenise or lipolysis induction followed by fat oxidation and energy intake reduction and absorption of nutrition inhibition).

Table 4.36: Activity of the seed extracts against pancreatic lipase porcine

Sample code	Inhibition (%)	IC_{50}±SD (μg/ml)	P^{IC}_{50}
BP	30.36±1.13^{e}	ND	ND
BS	43.22±3.25^{d}	ND	ND
CE	66.25±2.47^{bc}	3.17±0.04^{b}	5.50
CP	14.68±4.30^{f}	ND	ND
EA	25.12±2.23^{e}	ND	ND
MC	41.48±0.4^{d}	ND	ND
MM	39.79±3.15^{d}	ND	ND
MT	70.11±3.32^{b}	2.58±0.15^{b}	5.59
PB	66.42±2.47^{bc}	1.34±0.00^{b}	5.87
PN	64.34±3.21^{c}	23.59±3.09^{a}	4.63
Orlistat	81.48±0.35^{a}	3.60±0.05^{b}	5.44

***Values are expressed as mean±SD of triplicate experiments for percentage inhibition and IC_{50}**

Values sharing a common superscript in a column are not significantly different at *p<0.05.

*** P^{IC}_{50} = (-$\log_{10}$ IC_{50} value): Seed extract strength as pancreatic lipase porcine enzyme; ND: Not determined; BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida***

4.12.9 Cytotoxicity effect of the seed extracts on cancer cell lines (HeLa)

In Nigeria, most of the plant seeds have been taken orally without cytotoxicity evaluation on Hela cell lines. Therefore, to ensure safety of these medicinal plant seeds and their further standardization their cytotoxicity effect was performed on the HeLa cell lines. The result of the toxic potential of all the seed extracts on the cell lines was presented on Table: 4.37. Based on the screening program of the National Cancer Institute (NC1), a crude extract is assumed to have cytotoxicity effect *in vitro*, if the IC_{50} value is less than 20.0 µg/mL or less than 4.0 µg/mL for pure compound. The result percentage inhibitions and the IC_{50} values of the seed extracts screened against HeLa cell lines are shown on Table 4.36. The cytotoxic potencies of the seed extracts was in the order of CP> MC> BS> BP> EA> MT> MM> CE> PB> PN. For the seed extracts with percentage inhibition above 50% against HeLa cell lines, a dose-response curve was produced for each seed extract for IC_{50} values determination. The IC_{50} values on Table 4.37 indicated that the seed extract of CP was the most potent against HeLa cell lines. CP exhibited cytotoxic activity with IC_{50} value of 1.26±0.02 µg/mL. The seed extracts of MC and BS exhibited potent cytotoxicity with IC_{50} values of 2.93±0.0 µg/mL and 3.85±0.03 µg/mL respectively.

The seed extracts of BP, EA and MT showed a relatively high cytotoxic activity against HeLa cell lines with IC_{50} values ranging from 7.15±1.87 µg/mL to 7.90±0.05 µg/mL. MM seed extract possessed mild cytotoxic activity against HeLa cell lines with IC_{50} value of 10.97±1.86 µg/mL. Cytotoxicity activity of the PN seed extract relatively low with the percentage inhibition of 43.71±4.13%. PB seed extract cytotoxicity activity (54.86%) higher than seed extract of PN. The two seeds were the least active against HeLa cell lines among all the tested seed extracts. The cytotoxic activity of these extracts might be as results of the presence of phytochemical compounds such as monoterpene, triterpenes and flavones which have been reported in some plant extracts to possess antitumor activity with inhibition of cancer progression (Crowell and Gould, 1994). Triterpenoids also possess anticacinogenic and inflammatory properties (Manez *et al.,* 1994). Phenolic compounds (De Souza *et al.*, 2002), flavonoids, glycosides and tannins have also been reported with cytotoxic activities (Rajeshkumar *et al.,* 2002).

Table 4.37: Cytotoxicity effect of the seed extracts on HeLa cancer cell lines

Sample codes	Inhibition (%)	IC_{50}±SD (μg/ml)	P^{IC}_{50}
BP	69.34±0.17^{bc}	7.15±1.87^{b}	5.15
BS	71.91±3.45^{f}	3.85±0.03^{c}	5.41
CE	67.92±0.34^{bcd}	10.97±1.86^{a}	4.96
CP	71.91±3.45^{b}	1.26±0.02^{d}	5.90
EA	63.83±1.65^{d}	7.67±1.93^{b}	5.11
MC	65.41±1.89^{cd}	2.93±0.00^{d}	5.53
MM	58.14±3.83^{e}	ND	ND
MT	69.89±0.53^{bc}	7.9±0.05^{b}	5.10
PB	54.86±3.82^{e}	ND	ND
PN	43.71±4.13^{f}	ND	ND
Vincristine	80.82±2.05^{a}	ND	ND

***Values are expressed as mean±SD of triplicate experiments for percentage inhibition and IC_{50}**
***Values sharing a common superscript in a column are not significantly different at *p<0.05.* ND:**
***P^{IC}_{50} = (-$\log_{10}$ IC_{50} value): Measure of cytotoxic strength of the seed extracts; Not determined**
BP: *Butyrospermum paradoxum*; BS: *Blighia sapida*; CE: *Cyperus esculentus*; CP: *Croton penduliflorous*; EA: *Erythrococca anomalo*; MC: *Momordica chrantia*; MM: *Monodora myristica*; MT: *Monodora tenuifolia*; PB: *Parkia biglobosa*; PN: *Picralima nitida*

4.13 Effect of PN, BS and PB seed extracts on oral glucose tolerance test in albino rats loaded with glucose

The best approach for diabetes treatment is decreasing in the elevated fasting blood glucose. This could be achieved by decreasing or delaying glucose absorption via the inhibition of enzymes responsible for the starch hydrolysis such as α-amylase and α-glucosidase in the digestive tract. The known inhibitors of these enzymes always in carbohydrate digestion delay and the time required for the digested glucose to be absorbed into the blood stream, thus leading to a reduction in glucose absorption time and blockage of postprandial plasma glucose increment (Rhabasa-Lhoret *et al.,* 2004) . The *in vivo* α-glucosidase activity of PN, PB and BS seed extracts were study in normal rats. The result of oral glucose tolerance test of the seed extracts examined at doses of 500 mg/kg and 100 mg/kg on normal rats loaded with glucose at 2 g/kg was presented on Table 4.38. The plasma levels of glucose of the normal rats reached maximum peak 30 mins after the glucose administration orally. A steady decreased was later observed as showed on Table 4.38. All the seed extracts significantly lowered ($P<0.05$) the blood glucose at both doses but it was highly significant at the higher dose (1000 mg/kg) of the seed extracts administered.

Table 4.38: Effects of PN, BS and PB seed extracts on oral glucose tolerance in albino rats

Treatments	Fasting blood glucose level			
	0 min	**30 min**	**60 min**	**120 min**
BS (500 mg/kg)	75.33 ± 4.33^{ab}	128.3 ± 7.86^{bcd}	130.00 ± 13.01^{ab}	131.67 ± 8.62^{ab}
BS (1000 mg/kg)	81.67 ± 2.60^{b}	107 ± 11.59^{cd}	127.00 ± 7.00^{ab}	112.00 ± 14.00^{bc}
PB (500 mg/kg)	59.33 ± 4.33^{c}	182.67 ± 11.33^{a}	160.00 ± 6.00^{a}	155.33 ± 8.41^{a}
PB (1000 mg/kg)	66.0 ± 5.26^{ab}	128.67 ± 10.65^{bcd}	132.67 ± 11.26^{ab}	125.67 ± 9.39^{b}
PN (500 mg/kg)	78.00 ± 7.21^{ab}	154 ± 9.29^{abc}	127.33 ± 6.69^{ab}	109.67 ± 8.95^{bcd}
PN (1000 mg/kg)	76.33 ± 3.84^{ab}	109.33 ± 5.25^{cd}	116.3 ± 12.99^{ab}	81.33 ± 12.17^{de}
Glibenclamide	110.0 ± 5.86^{a}	162.00 ± 3.5^{ab}	120.67 ± 3.42^{ab}	87.00 ± 10.79^{cde}
Vehicle	80.0 ± 4.16^{b}	79.33 ± 4.81^{d}	76.60 ± 5.00^{b}	70.00 ± 3.00^{e}

Values are mean±SD (n=5); values with the same superscript are not significantly different at *P<0.05* when compared to controls; BS: *Blighia sapida*; PB: *Parkia biglobosa*; PN: *Picralima nitida

4.13.1 Effect of PN, BS and PB seed extracts on oral sucrose tolerance test in albino rats loaded with sucrose

Oral sucrose tolerance test was carried on sucrose primed model rats treated with 500 mg/kg and 1000 mg/kg of PN, PB and BS seed extracts along with the glibenclamide (standard drug) as a positive control. There was increase in blood glucose levels of the animals 30 mins after the sucrose loading into the animals. The plant extracts at dose of 1000 mg/kg produced a significant reduction in plasma glucose levels of the normal rats when compared to the controls (normal and glibenclamide) at 60 mins and 120 mins after the loading of sucrose. The antihyperglycemic effects of the seed extracts with reference to the standard drug (glibenclamide) in normoglycemia rats are the complement evident of their antidiabetic roles that was showed through the enzymatic screening of the seed extracts on the enzymes that hydrolyses carbohydrate to monosaccharide. The blood glucose lowering effects of the seed extracts started 30 mins after the loading of sucrose and was monitored for 2 h. All the extracts were significantly lowered the blood glucose levels in studied animals and their activities may be due to their ability to enhance the insulin release from the rats' pancreas (Beck-Nielsen *et al*., 1988) or increased in the glucose utilization by the tissues and reduction in glucose absorption (Patel *et al*., 2011). The seed extracts effect on the glucose levels were shown on Table 4.39.

Table 4.39: Effects of PN, BS and PB seed extracts on oral sucrose tolerance test in albino rats

Treatment	Fasting blood glucose level			
	0 min	**30 mins**	**60 mins**	**120 mins**
BS (500 mg/kg)	88.67±14.99^{ab}	104.67±4.63^{bc}	88.67 ± 3.33^{bc}	84.67±4.37^{cd}
BS (1000 mg/kg	76.33±9.87^{ab}	128.33±12.60^{abc}	99.33 ± 9.49^{abc}	82.00±4.93^{cd}
PB (500 mg/kg)	66.33±3.71^{b}	162.33±13.33^{ab}	144.00±13.89^{a}	144±4.33^{a}
PB (1000 mg/kg)	77.67±4.50^{ab}	167.33±12.28^{a}	137.67±14.62^{ab}	113.67±12.73^{b}
PN (500 mg/kg)	72.33±7.30^{ab}	119.33±12.81^{abc}	105.67±8.57^{abc}	99.67±7.22$_{bc}$
PN (1000 mg/kg)	70.00±1.73^{b}	153.00±15.10^{ab}	131.33±16.33^{ab}	102±12.00^{bc}
Glibenclamide	110.00±10.5^{a}	162.00±6.70^{ab}	120.67±5.35^{abc}	87.00±18.68^{bcd}
Vehicle (10 mL/kg)	80.00±4.16^{ab}	79.33±4.81^{c}	74.00±3.055^{c}	66.67±3.75^{c}

Values are expressed as mean±SD (n=5); values having the same superscript are not significantly different at *P<0.05* when compared to the positive control; BS: *Blighia sapida*; PB: *Parkia biglobosa*; PN: *Picralima nitida

4. 13.2 PN and PB fasting blood glucose effect on diabetic mice

PN and glibenclamide significantly (*P<0.05)* lowered the blood glucose levels of diabetic mice during the period of the treatments as presented on Table 4.40. On daily basis (7, 14 and 21), the lowering effects of PN on FBG levels of the diabetic mice were significantly noticeable when compared to the controls (diabetic mice vehicle treated and normal mice vehicle treated groups). The reduction in FBG levels of diabetic mice treated with PN seed extract was as follows: PN at a dose of 100 mg/kg lowered the FBG from 245.67±3.7 mg/dL to 162.67±2.52 mg/dL after 21 days of the treatment, PN at a dose of 200 mg/kg lowered the FBG levels from 227.17±7.63 mg/dL to 133.33±1.53 mg/dL and glibenclamide at 10 mg/kg have FBG level reduction from 318.50±8.5 mg/dL to 153.67±5.93 mg/dL. The FBG level of diabetic mice treated with vehicle solution was increased from 380.75±6.24 mg/dL to 456.75±3.14 mg/dL. It was observed that FBG levels reduction in diabetic mice by PN seed extract was dose dependent and found to be significant at 200 mg/kg dose of PN seed extract in this study. Thus supports the use PN of the plant seeds in diabetes mellitus management. Further increase in the doses beyond 200 mg/kg in mice may cause hypoglycemia and death of the animals.

More so, PB seed extract antihyperglycemic activity on alloxan diabetic mice studied under the same condition with PN seed extract was also found to be dose dependent. FBG levels lowering effects of PB in diabetic mice was highly significant (*P<0.05*) at a dose of 200 mg/kg when compared to control diabetic mice treated with standard drugs (glibenclamide) (Table 4.40). The trends of FBG levels reduction in diabetic mice by PB seed extract at doses of 100 mg/kg and 200 mg/kg and glibenclamide at dose of 10 mg/kg are also represented on Table 4.40. After 21 days of the experiment, PB seed extract at a dose of 200 mg/kg significantly (P<*0.05*) lowered FBG levels of diabetic mice from 304.33±7.18 to 144±1.0 mg/dL while the diabetic mice treated with 100 mg/kg of PB was reduced from 327.83±6.07 to 193.33±0.58 mg/dL. Although, the diabetic mice treated with PB seed extract were highly hyperglycemic but a significant antihyperglycemic activity was recorded by the seed extract which confirmed its ethnomedicinal information as an antidiabetic agent.

The antihyperglycemic activity of PN and PB seed extracts may be due to their significant *in vitro* enzymatic inhibitory activities against the enzymes and the presence of some chemical constituents like saponins and tannins which are known as antihyperglycemic agents. The antihyperglycemic activity of PN and PB seed extracts could also be as a result of their glucose uptake enhancement by tannins and insulin stimulation from pancreatic β cells by flavonoids. FBG levels normalization could also be attributed to optimum utilization of glucose in diabetic mice.

Table 4.40: Effects of PN and PB seed extracts on the fasting blood glucose level of diabetic mice

	Fasting blood glucose level (mg/dL)				
Treatments	**B. IND.**	**A. IND.**	**DAY 7**	**DAY 14**	**DAY 21**
PB (100 mg/kg)	127.50 ± 5.26^{ab}	327.83 ± 6.01^{a}	306.20 ± 5.72^{ab}	235.67 ± 1.23^{b}	193.33 ± 0.58^{b}
PB (200 mg/kg)	129.83 ± 5.98^{a}	304.33 ± 7.18^{a}	269.40 ± 6.8^{abc}	188.0 ± 5.10^{b}	144.00 ± 1.0^{bc}
PN (100 mg/kg)	117.83 ± 10.14^{ab}	245.67 ± 3.73^{ab}	227.75 ± 4.7^{abc}	171.67 ± 4.7^{b}	162.67 ± 2.52^{bc}
PN (200 mg/kg)	128.83 ± 7.36^{a}	227.17 ± 7.63^{ab}	199.80 ± 3.41^{bc}	139.67 ± 2.5^{b}	133.33 ± 1.53^{c}
Non diabetic	102.17 ± 7.98^{b}	102.83 ± 7.39^{b}	107.17 ± 4.5^{c}	138.50 ± 10.0^{b}	130.75 ± 5.93^{c}
Diabetic (Vehicle)	138.50 ± 12.29^{a}	380.75 ± 6.24^{a}	391.75 ± 3.0^{a}	427.75 ± 8.36^{a}	456.75 ± 3.14^{a}
Glibenclamide (10 mg/kg)	102.33 ± 8.160^{b}	318.50 ± 8.5^{a}	289.40 ± 8.94^{ab}	217.40 ± 5.9^{b}	153.67 ± 5.93^{bc}

***Values are mean±SD for mice in each group (n=7)**

Values sharing the same superscript in the same column are not significant ($p<0.05$). B. IND.: Fasting blood glucose level before induction of diabetes, A. IND.: Fasting blood glucose level after induction of diabetes; PB: *Parkia biglobosa*; PN: *Picralima nitida

4.13.3 Effect of PN and PB on the body weight of the diabetic mice

Effect of PN extract on the mean body weight of diabetic mice is presented on Table 4.41. On days 7 and 14, a significant ($P<0.05$) loss in body weight of the diabetic mice treated with PN seed extract, glibenclamide and diabetic mice treated with vehicle solution was observed when compared to day 0 (acclimatization period) (Table 4.41). On day 21, an improvement in mean body weight of all diabetic mice treated with PN and glibenclamide was observed which could be as a result of significant reduction in blood glucose levels and optimum utilization of glucose by diabetic mice. In addition, the weights of diabetic mice treated with PB seed extract were also differing at the end of the 21 days of the experiment. This effect was profound in diabetic mice treated with PB at a dose of 100 mg/kg which could be as a result of the chronic hyperglycemic condition or the various sizes of the animal at the beginning of the experiment. This is a clear indication of imbalance of metabolism process in diabetic mice treated with PB seed extract.

Table 4.41: PN and PB seed extracts effect on the mean body weight of diabetic mice

Treatments	Body weight (g)			
	Acclimatization	Week 1	Week 2	Week 3
PB (100 mg/kg)	37.67 ± 5.85^{bc}	43.67 ± 3.93^{a}	38.80 ± 3.89^{abc}	31.33 ± 2.91^{ab}
PB (200 mg/kg)	34.67 ± 2.73^{c}	35.33 ± 2.86^{ab}	38.80 ± 1.74^{a}	36.80 ± 3.72^{ab}
PN (100 mg/kg)	43.67 ± 3.91^{ab}	43.00 ± 2.45^{a}	36.00 ± 2.44^{ab}	40.00 ± 5.03^{a}
PN (200 mg/kg)	38.33 ± 4.63^{bc}	37.33 ± 3.93^{a}	30.00 ± 5.22^{bc}	27.00 ± 4.93^{b}
Normal (Vehicle)	18.67 ± 1.63^{d}	27.00 ± 4.27^{b}	27.33 ± 2.4^{c}	29.67 ± 2.94^{ab}
Diabetic (Vehicle)	47.00 ± 4.36^{a}	39.50 ± 2.06^{a}	35.50 ± 1.71^{abc}	29.00 ± 2.62^{ab}
Glibenclamide (10 mg/kg)	36.70 ± 3.72^{bc}	39.00 ± 2.35^{a}	32.80 ± 4.15^{abc}	34.80 ± 3.83^{ab}

Values mean±SD for mice in each group (n=7); values with the same superscript in the same column are not significant (*p<0.05*); PB: *Parkia biglobosa*; PN: *Picralima nitida

4.13.4 Effect of BS and MM seed extracts on the fasting blood glucose of diabetic mice

The effect of the two seed extracts on blood glucose lowering was found to be dose dependent for MM seed extract. BS seed extract significantly lowered the FBG levels of the diabetic mice at doses of 100 mg/kg and 200 mg/kg respectively (Table.4.42). BS treated group at a dose of 100 mg/kg significantly ($p<0.05$) lowered the fasting blood glucose level of the treated group from 357.40±6.3 mg/dL to 168.00±2.37 mg/dL, diabetic mice treated with 200 mg/kg of BS seed extract also displayed FBG reduction from 404.80±6.00 mg/dL to 165.00±1.49 mg/dL. Antihyperglycemic of BS at dose of 200 mg/kg was observed that could lead to hypoglycemic, if the dose is increased above 200 mg/kg or the period of the study is extended beyond 21 days.

More so, MM seed extract was found to be highly significant in reducing the blood glucose of the treated diabetic mice. MM extract at dose of 100 mg/kg lowered the fasting blood glucose from 437.00±6.52 mg/dL to 122.00 ± 5.15 mg/dL. The effect of the two seed extracts in lowering the fasting blood glucose of the mice was significant when compared to that of the diabetic mice treated with standard drugs (glibenclamide) which lowered the diabetic mice FBG from 449.0±6.56 mg/dL to 119.67±2.89 mg/dL. Antihyperglycemic activity of the seed extracts might be as a result of their inhibitory activity on some enzymes linked to diabetes and diabetes complications and the presence of some phytochemical compounds which have been reported to be hypoglycemic agents.

Table 4.42: BS and MM seed extracts effect on the fasting blood glucose levels of diabetic mice

	Blood glucose (mg/dL)				
Treatments	**B. IND.**	**A. IND.**	**Week 1**	**Week 2**	**Week 3**
BS (100 mg/kg)	124.20±7.813[a]	357.40±6.3[a]	311.69±7.12[bc]	238.50±1.70[b]	168.00 ±2.37[b]
BS (200 mg/kg)	125.00±4.79[a]	404.80±6.00[a]	303.0±3.82[bc]	204.00±2.29[bc]	165.0 ±1.49[b]
MM (100 mg/kg)	108.60±8.05[a]	437.00±6.52[a]	335.75±9.93[bc]	235.33±1.04[b]	122.00 ±5.15[b]
MM (200 mg/kg)	125.20±7.14[a]	540.75±4.49[a]	474.60±4.29[b]	202.60±2.52[bc]	118.33±9.62[b]
Vehicle solution	108.33±10.27[a]	123.25±7.39[b]	119.67±6.43[e]	115.20 ±1.42[c]	123.75±1.49[b]
Diabetic (Vehicle)	120.18±3.10[a]	480.25±8.58[a]	518.75±4.20[a]	488.30±2.30[a]	490.25±3.96[a]
Glibenclamide (10 mg/kg)	120.00±12.33[a]	449.00±6.56[a]	224.25±3.8[d]	183.33±1.13[bc]	119.67±2.89[b]

***Values are expressed as mean±SD for mice in each group (n=7)**

Values sharing a common superscript in the same column are not significant (*p<0.05*). B. IND.: Fasting blood glucose level before induction of diabetes, A. IND.: Fasting blood glucose level after induction of diabetes; BS: *Blighia sapida*; MM: *Monodora myristica

4.13.5 Effect of BS and MM seed extracts on mean body weight of diabetic mice

The mean body weight of diabetic mice before and during the experiment is presented on Table 4.43. A significant ($P<0.05$) loss in body weight of the diabetic mice treated with the seed extracts of BS and MM was observed within the first 7 day of the experiment. From the day 14 till the end of the experiment, an improvement in mean body weight of all diabetic mice treated with both BS and MM was observed which could be as a result of significant reduction in blood glucose levels and optimum utilization of absorbed glucose from small intestine by diabetic mice.

Table 4.43: BS and MM seed extracts effect on the mean body weight of diabetic mice

Treatments	Body weight (g) Acclimatization	Week 1	Week 2	Week 3
BS (100 mg/kg)	34.00±1.79^{ab}	28.40±2.04^{b}	30.00±1.55^{b}	29.00±2.00^{b}
BS (200 mg/kg)	32.00±3.35^{b}	32.80±2.06^{ab}	34.40±2.19^{ab}	32.80±4.15^{ab}
MM (100 mg/kg)	35.67±3.204^{ab}	29.60±3.58^{b}	32.00±0.00^{ab}	34.67±5.03^{ab}
MM (200 mg/kg)	35.00±6.54^{ab}	32.00 ± 2.45^{ab}	33.20±3.01^{ab}	34.67±3.712^{ab}
Normal (Vehicle)	40.00±3.46^{a}	38.25±4.502^{a}	378.80±3.63^{a}	37.00 ±3.46^{a}
Diabetic (Vehicle)	37.20±5.02^{ab}	33.50±1.92^{ab}	33.00±2.58^{ab}	29.00±0.00^{ab}
Glibenclamide	34.33±4.80^{ab}	35.75±2.02^{ab}	34.00±2.00^{ab}	36.00±0.00^{ab}

Values are mean±SD (n=7) for mice in each group, values sharing the same superscript in the same column are not significant ($p<0.05$); BS: *Blighia sapida*; MM: *Monodora myristica*

CHAPTER FIVE

SUMMARY AND CONCLUSION

Table 5.1: Summary of the biological assays, activity and possible application of the selected plant seeds for management of diabetes and diabetes complications

Biological activity/ Property	Plant seeds	Proposed medicinal application
Antioxidant	PN, PB, MT, MM, BS and BP	Good antioxidant agents (fight against free radical and disease, inhibitor of protein glycation and peroxidation inhibitor in food).
Antimicrobial	BP, BS, CE, CP, EA, MC, MM, MT, PN and PB	They can be used for the treatment of infections from bacterial and antifungal.
Aldose reductase (ALR2)	BS, PB, PB, MM, MT and PN	They can be employed as inhibitors of ALR2 to prevent accumulation of sorbitol during diabetic condition.
Pancreatic lipase porcine	CE, MT, PB and PN	They are good inhibitor of pancreatic lipase porcine enzymes; they can be useful for the delay of fat digestions, lowering of fat and better management of obesity.
AGEs formation	BS, EA, PN and MT	Source of bioactive compounds for the prevention of AGEs (Management of diabetic complication and progression).
α-Glucosidase	CP, MT, PN, EA, BS and PB	They are useful inhibitors of α-glucosidase and they can be used to delay carbohydrate hydrolysis in diabetic patients
FRAP	PN, BP, EA, MC, PB, BS, MM, CE and MT	The plant seeds could be use to scavenge radicals from the tissue system (oxidative stress).
15-Lipoxygenase	PB, MC, MT, EA and CE	They can be used as anti-inflammatory agents.

BP: ***Butyrospermum paradoxum*****; BS:** ***Blighia sapida*****; CE:** ***Cyperus esculentus*****; CP:** ***Croton penduliflorous*****; EA:** ***Erythrococca anomalo*****; MC:** ***Momordica chrantia*****; MM:** ***Monodora myristica*****; MT:** ***Monodora tenuifolia*****; PB:** ***Parkia biglobosa*****; PN:** ***Picralima nitida***

5.1 Summary

An investigation pertaining to the antidiabetic quality and value addition to some underultilised plant seeds was undertaken with the objective to document their phytochemical constituents, metal contents, antimicrobial properties, fatty acid components, antioxidant activities, phenol and flavonoids contents, antidiabetic activities, inflammatory and cytotoxicity properties. The seed extracts phytochemical analysis showed that they contained phytochemical constituents such as flavonoids, tannins, alkaloids, phenols, terpenoids and carbohydrates which reveal that the seeds are of high medicinal values that can exert different biological activities such as antibacterial, antioxidant, analgesic and antioxidant. Mineral element composition of the plant seeds also showed that they are rich in potassium, calcium, magnesium and iron that are essential elements necessary for growth, prevention of insulin resistance and optimal performance of enzyme if used as food supplements for management of diabetes and its complication. They have low level of Pb, Ni, Cd and Cr that can cause glucose disruption and reduction in insulin secretion. Antimicrobial activity of the seed extracts showed that all the plant seeds have high antimicrobial activities; and they are highly valuable in fighting infections in human.

Fatty acid analysis of the seed extracts revealed that CP, EA, MT, PB and PN are rich in oleic and linoleic acids which are essential fatty acids, hence the seed supplements in diabetic patient meal can increase insulin sensitivity, reduce blood pressure, and level of LDL cholesterol. GCMS analysis of chemical components in all the seed extracts revealed highly valuable chemical constituents profile with reported important chemical properties and valuable biological activities. The seed extracts are also rich in phenols and flavonoids responsible for their high antioxidant activities. These are well known antioxidants essential for the prevention of oxidative stress and lipid peroxidation. The result of AGEs analysis displayed high antiglycation activities of all the seeds extracts and hence, they can be useful as AGEs inhibitor to prevent diabetic complications. The seed extracts of BS, MM, MT, PB and PN were found to have high inhibitory activities against ALR2 enzymes so; they could be used as good inhibitor of ALR2 enzymes for the prevention of sorbitol formation in diabetic patients. CP, MT and PB seed extracts display high inhibitory activities against α-glucosidase enzymes, therefore, the seeds can be used to delay carbohydrate hydrolysis for better

management of diabetes. The result of the cytotoxicity also revealed that the plant seeds have moderate toxic effect on the cancer cell lines.

Seed extracts of MC, PB, EA and CE were found to be good inhibitors of 15-lipoxygenase; an enzyme responsible for inflammatory while PB, MT, CE and PN have high inhibitory activities against pancreatic lipase porcine. The seeds are suitable for the management of obesity and prevention of hyperlipidemia. All the seed extracts except CP and MC were found to have low toxicity; therefore the seeds seem to be edible and safe for human consumption. The results obtained for PN, BS, and PB on oral glucose and sucrose tolerance shows that the seeds significantly lowered the blood glucose of the experimental rats on time interval. The seed extracts of BS, MM, PN and PB significantly lowered the fasting blood glucose of the diabetic mice; this shows that the four seeds have high antidiabetic properties and they can be used in the management of diabetes.

5.2 Conclusion

Many uncommon or conventional edible seeds have not been fully investigated with respect to their chemical constituents, antimicrobial properties, antioxidant activities and antidiabetic activities. The seeds that could be of tremendous use to man and pharmaceutical industries are lying fallow in various locations of our environment due to fear of toxicity. The plant seeds for this study were found to contain numerous bioactive compounds, nutritional elements, phenol and flavonoids. The seeds are also rich in linoleic and oleic acids which are essential fatty acids that could be employed for the treatment of cardiovascular and coronary disease when incorporated into the food. They are good inhibitor of all the enzymes that were tested and significantly lowered the fasting blood glucose in diabetic mice. I hereby suggest that we concentrate on their medicinal values and add them as supplements to our feeding plan for the management of diabetes and it complications.

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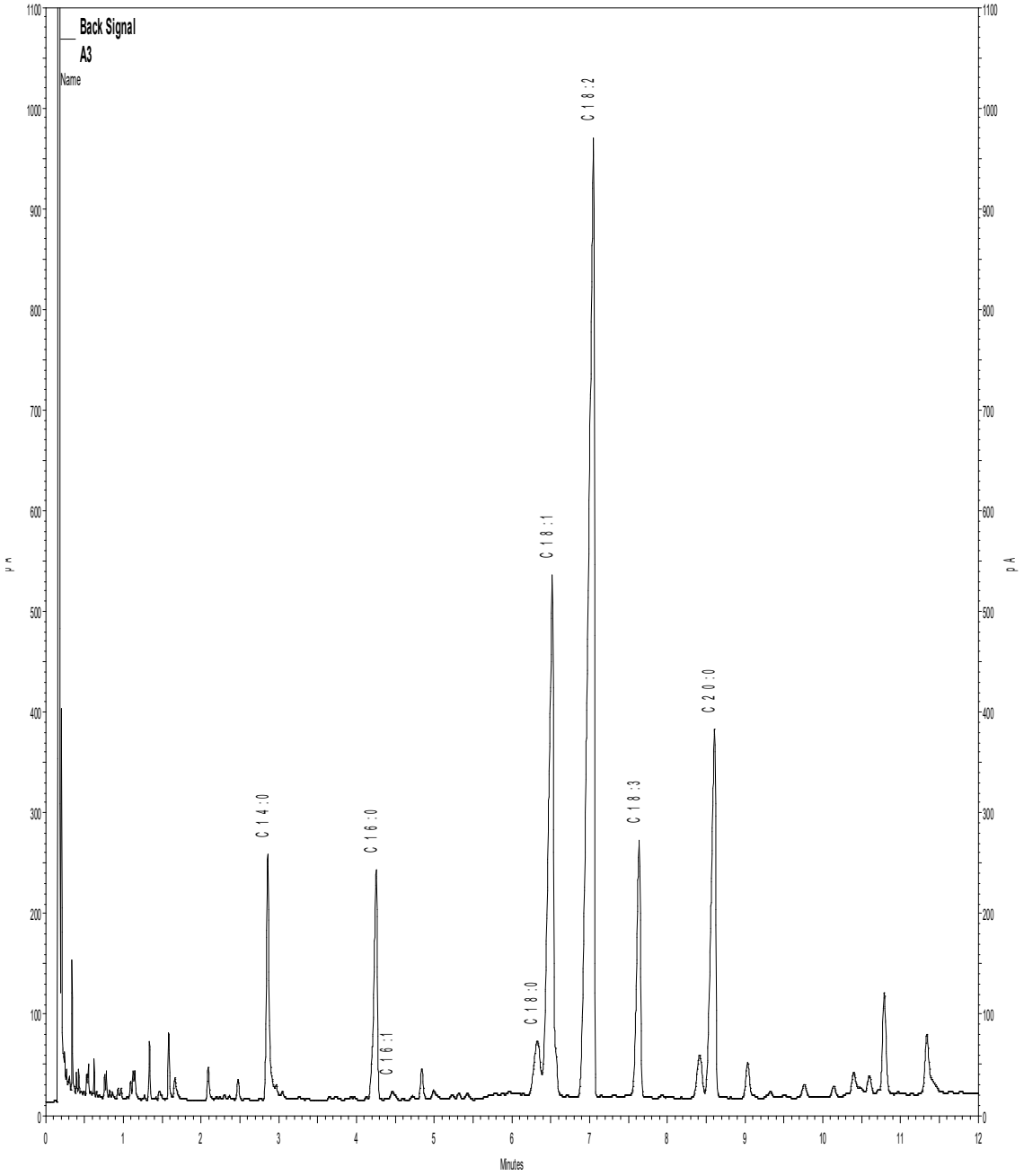
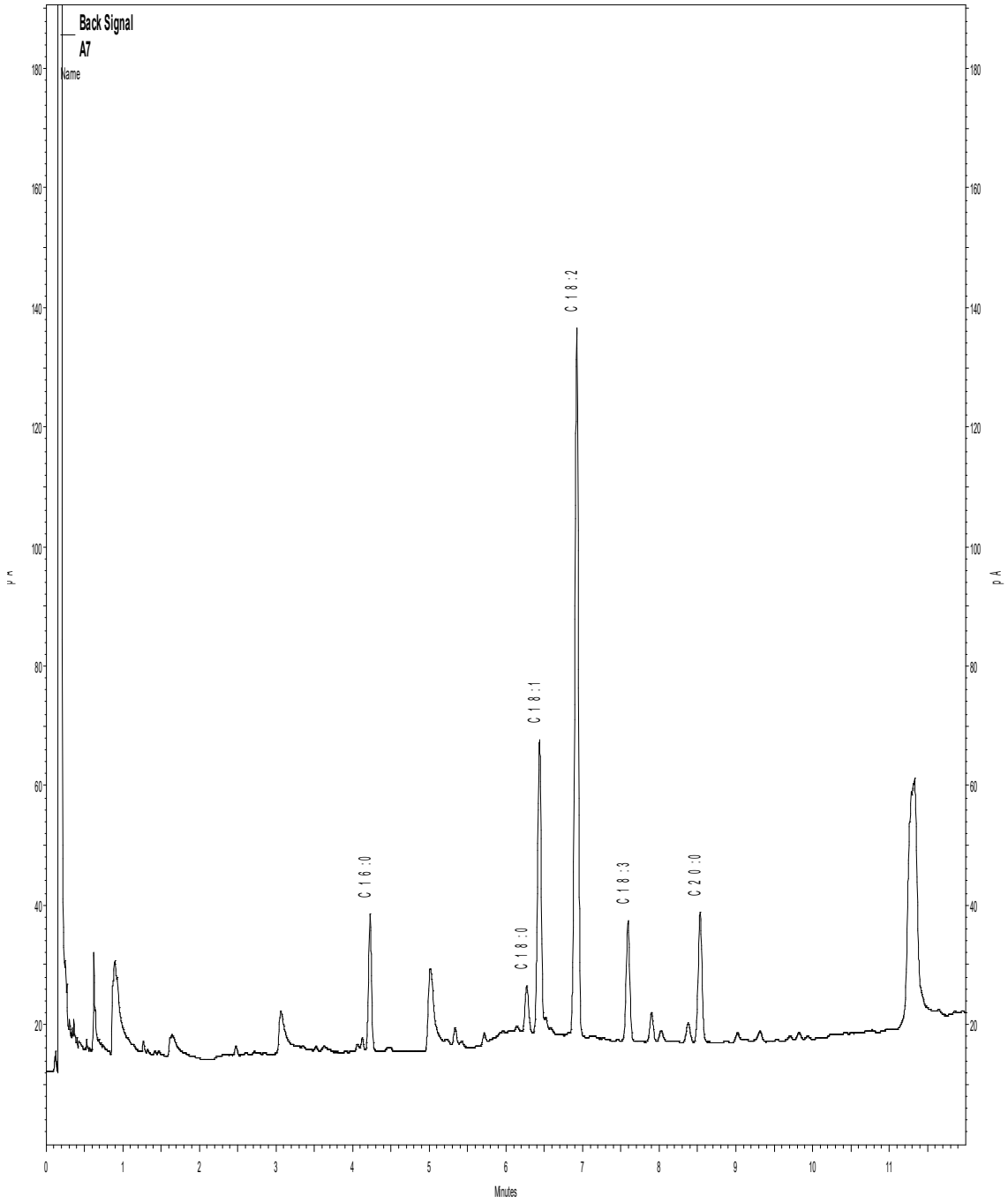
APPENDIX 1

GC-FID chromatogram of PN seed extract for fatty acid profile



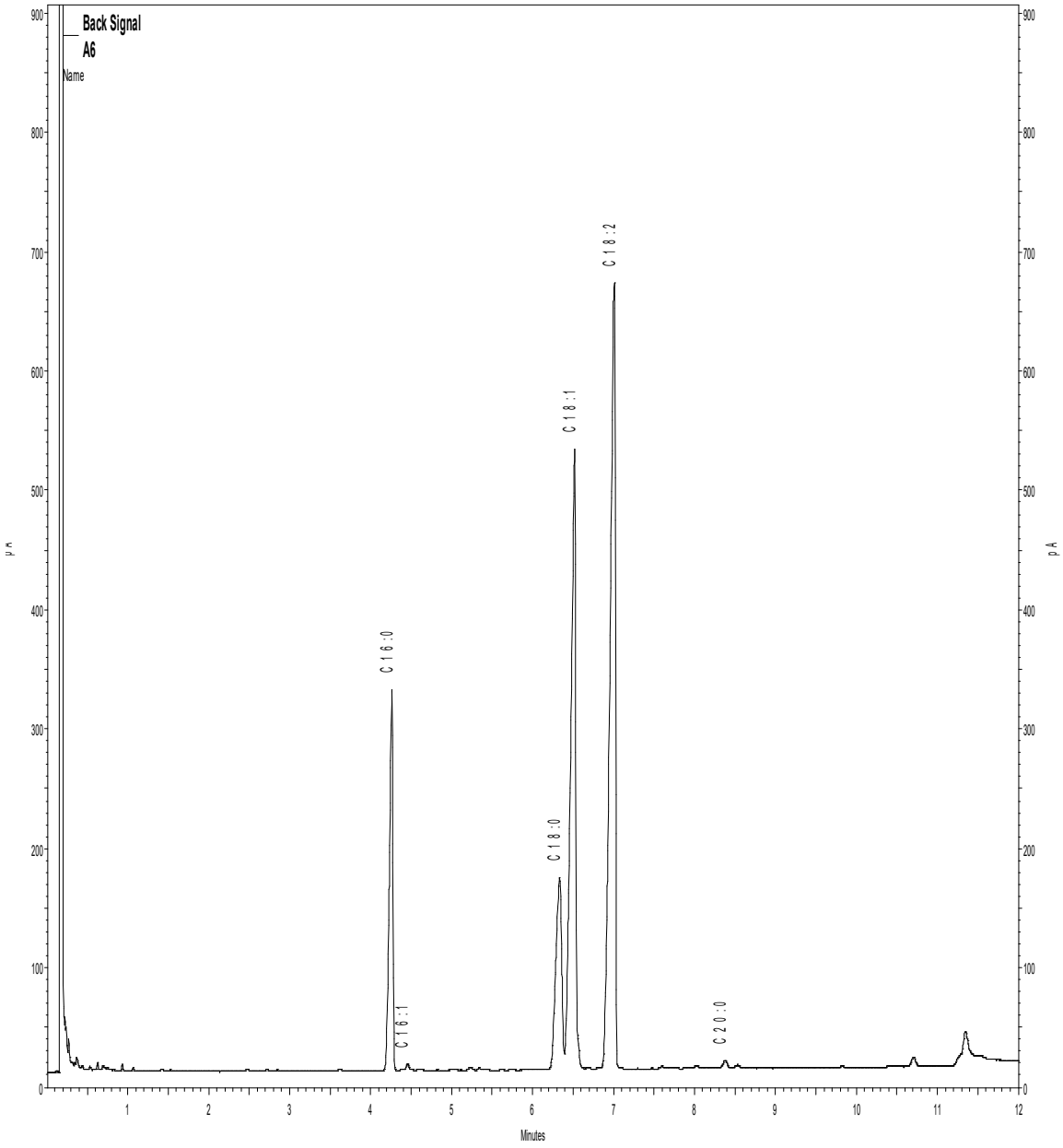
APPENDIX 1I

GC-FID chromatogram of CP seed extract for fatty acid profile



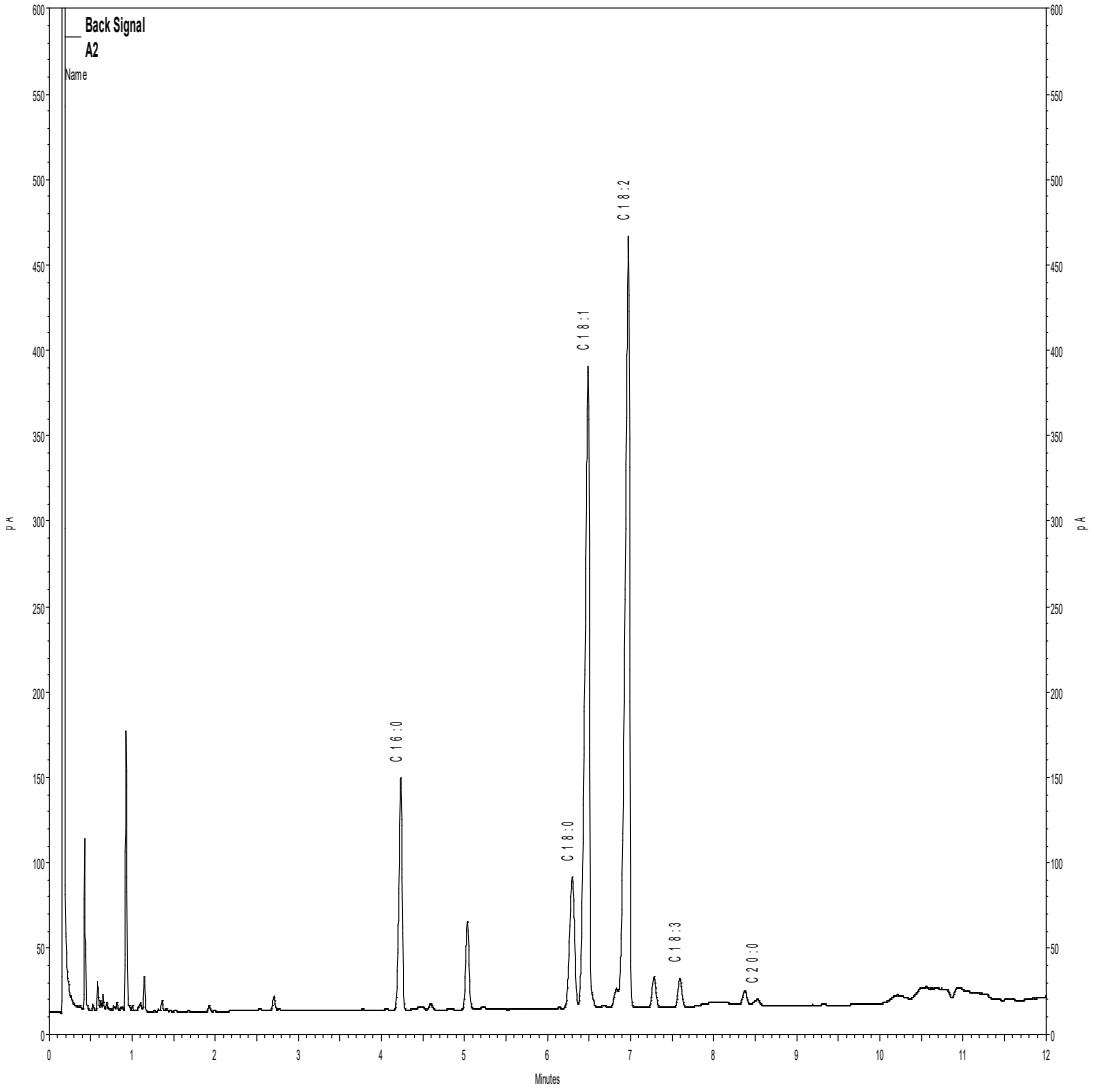
APPENDIX III

GC-FID chromatogram of PB seed extract for fatty acid profile



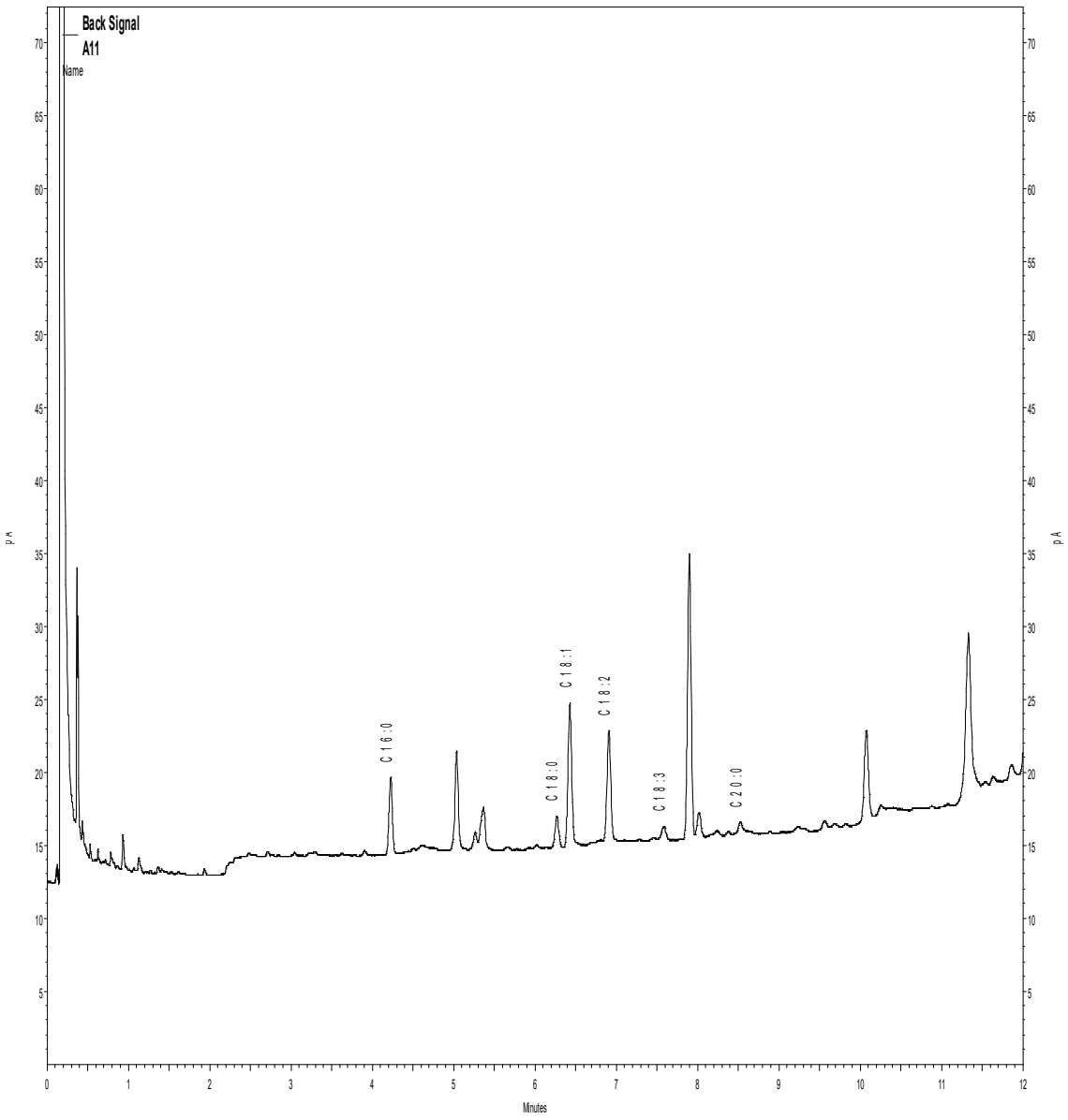
APPENDIX IV

GC-FID chromatogram of EA seed extract for fatty acid profile



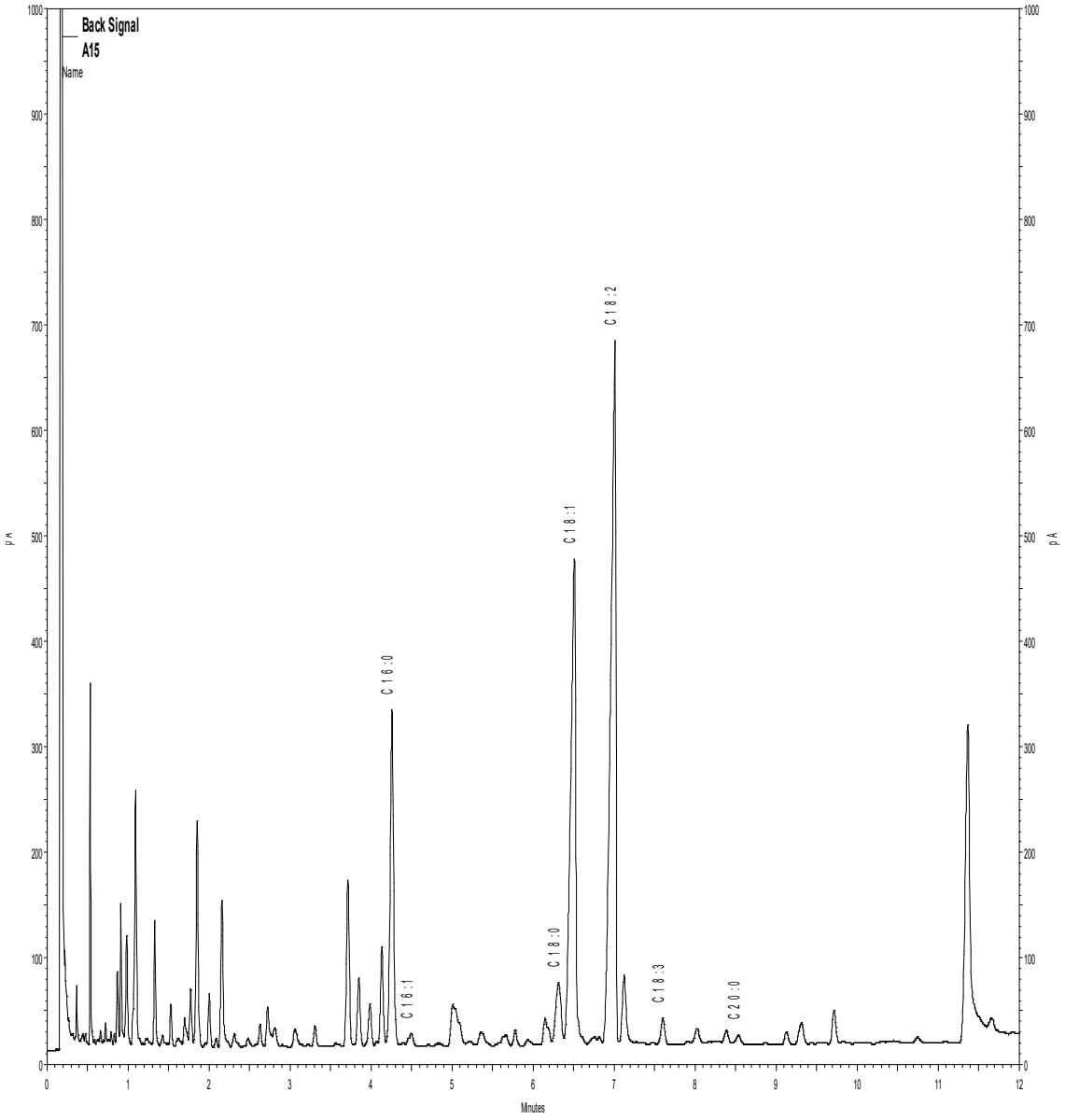
APPENDIX V

GC-FID chromatogram of BS seed extract for fatty acid profle



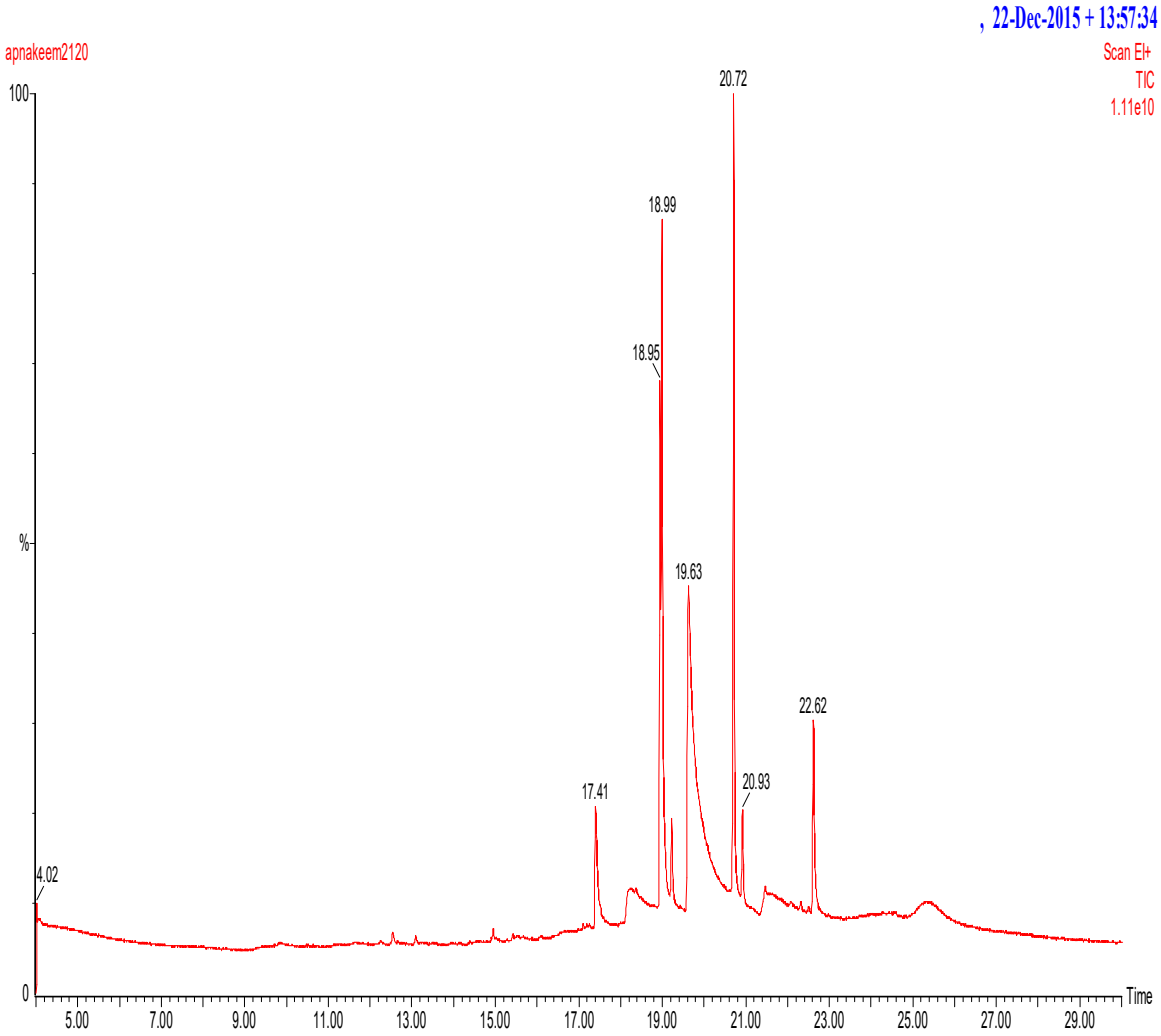
APPENDIX VI

GC-FID chromatogram of MT seed extract for fatty acid profile



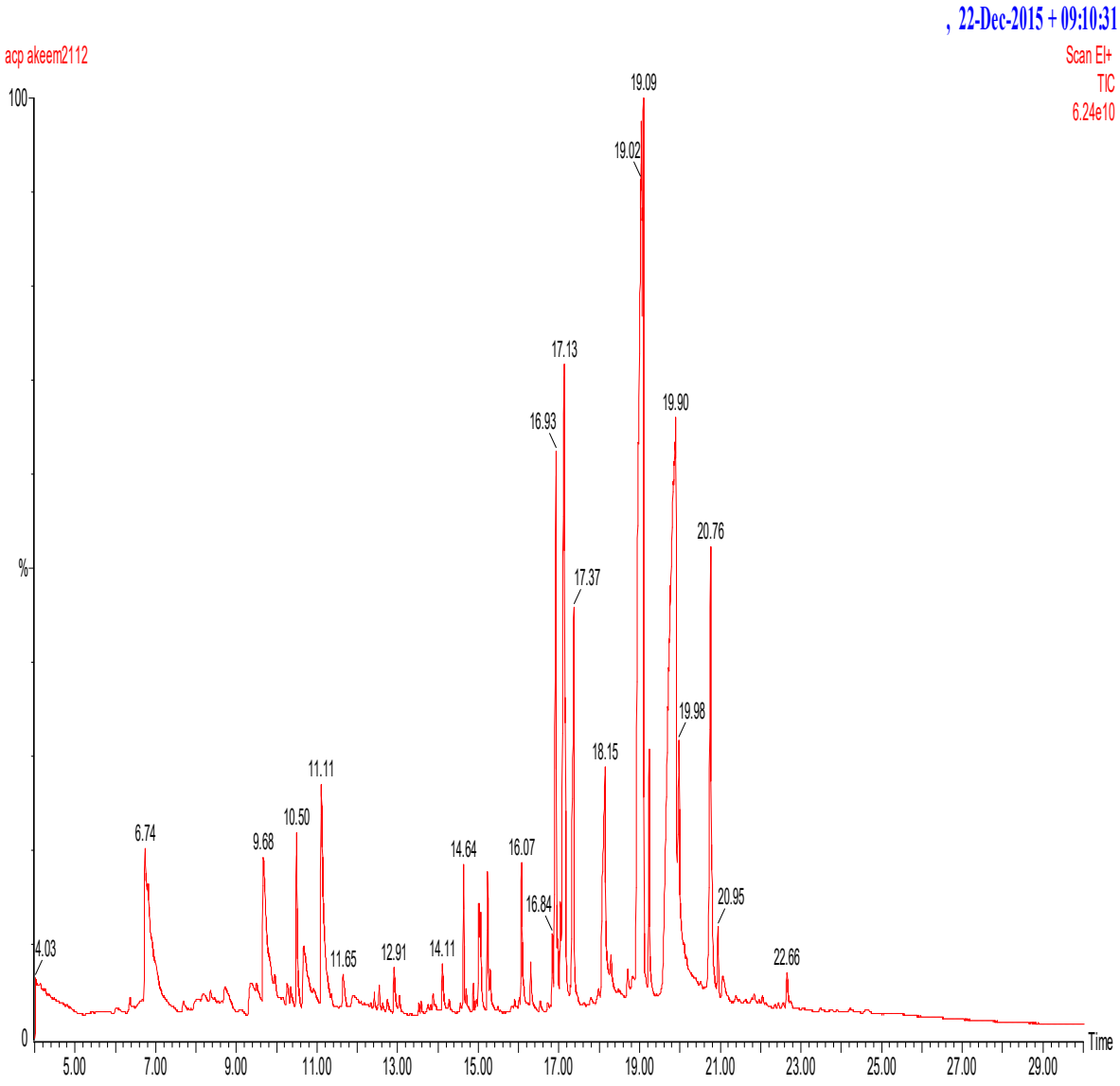
APPENDIX VII

GC-MS chromatogram of PN seed extract for determination of phytocomponents



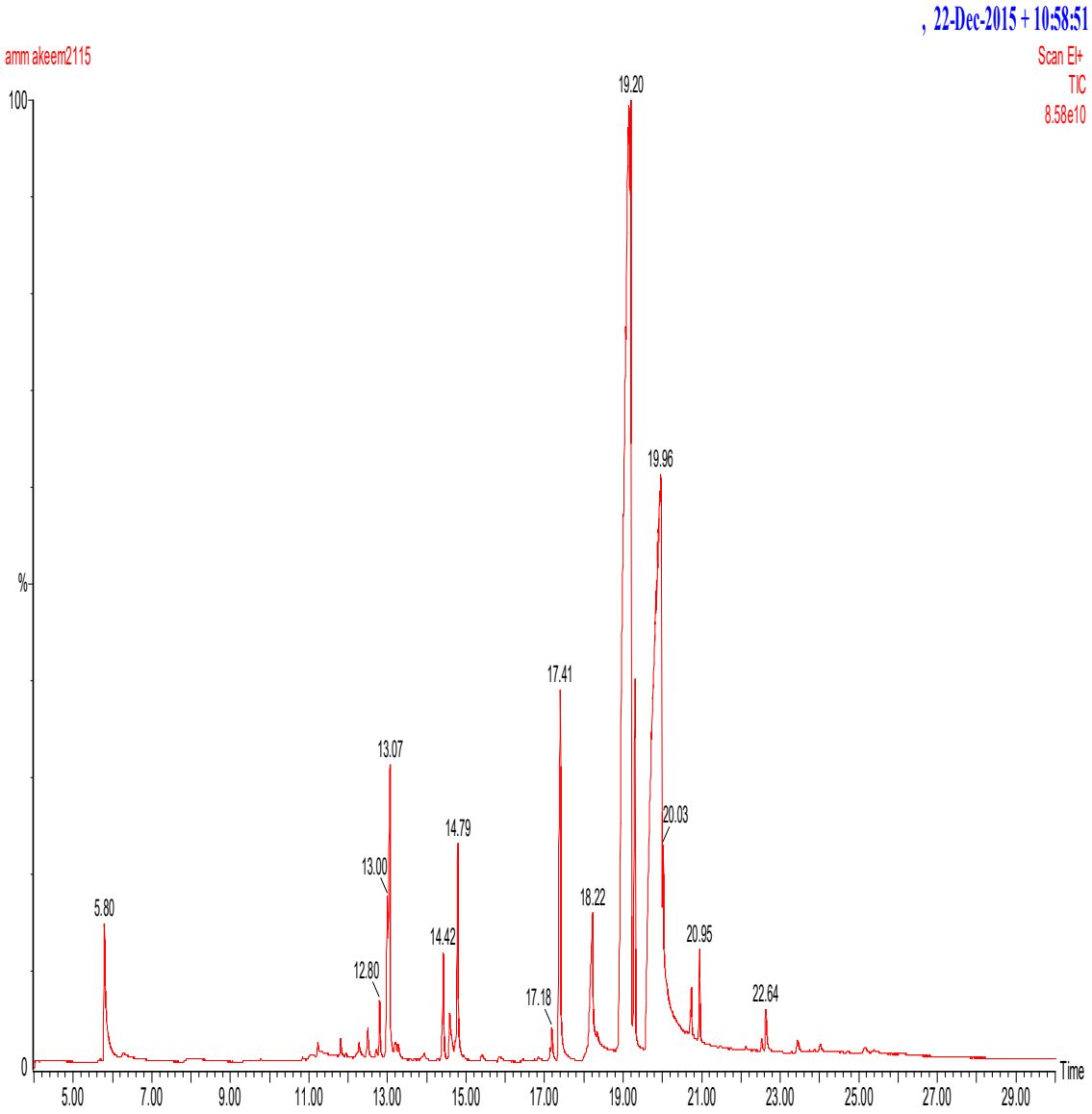
APPENDIX VIII

GC-MS chromatogram of CP seed extract for determination of phytocomponents



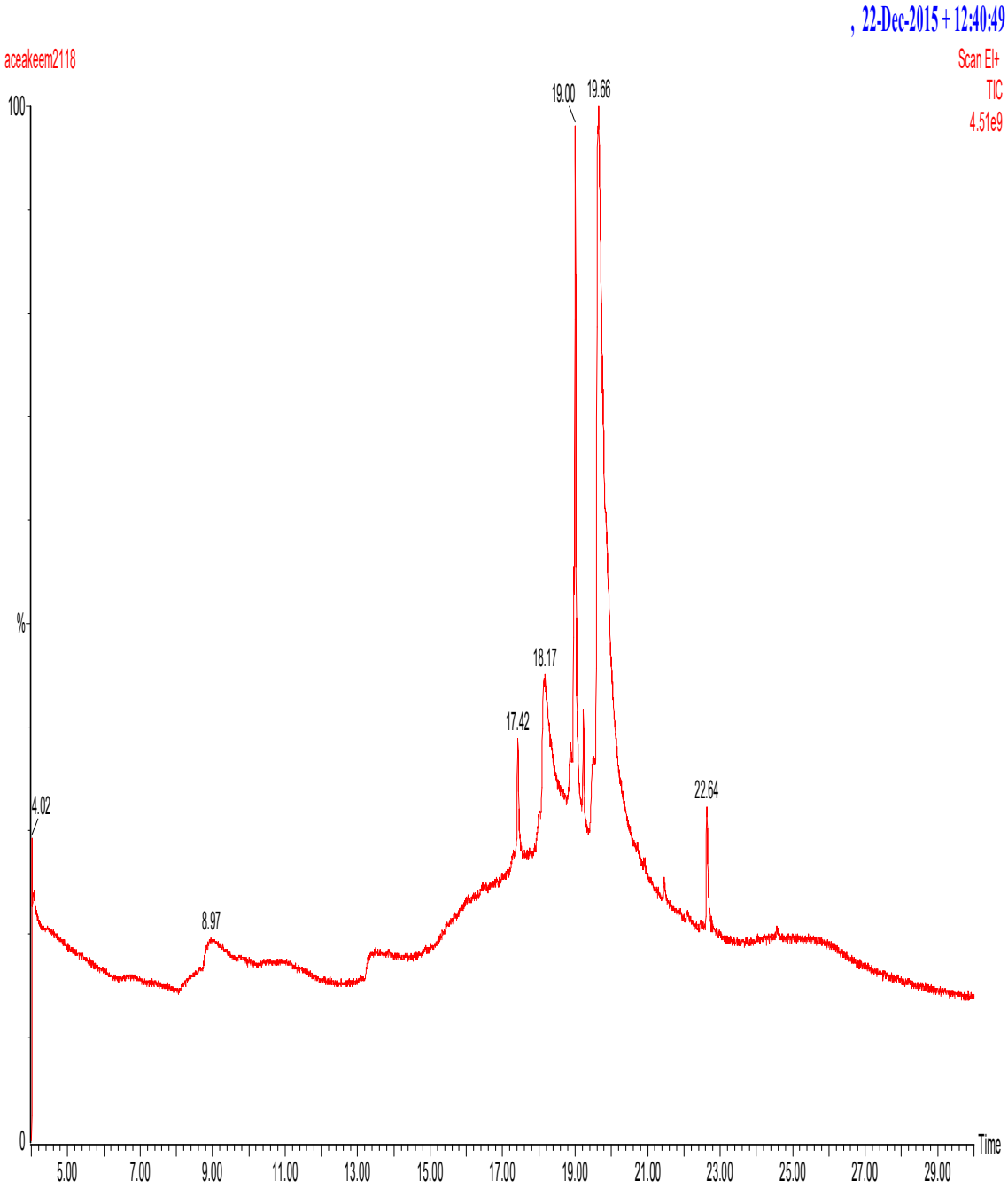
APPENDIX IX

GC-MS chromatogram of MM seed extract for determination of phytocomponents



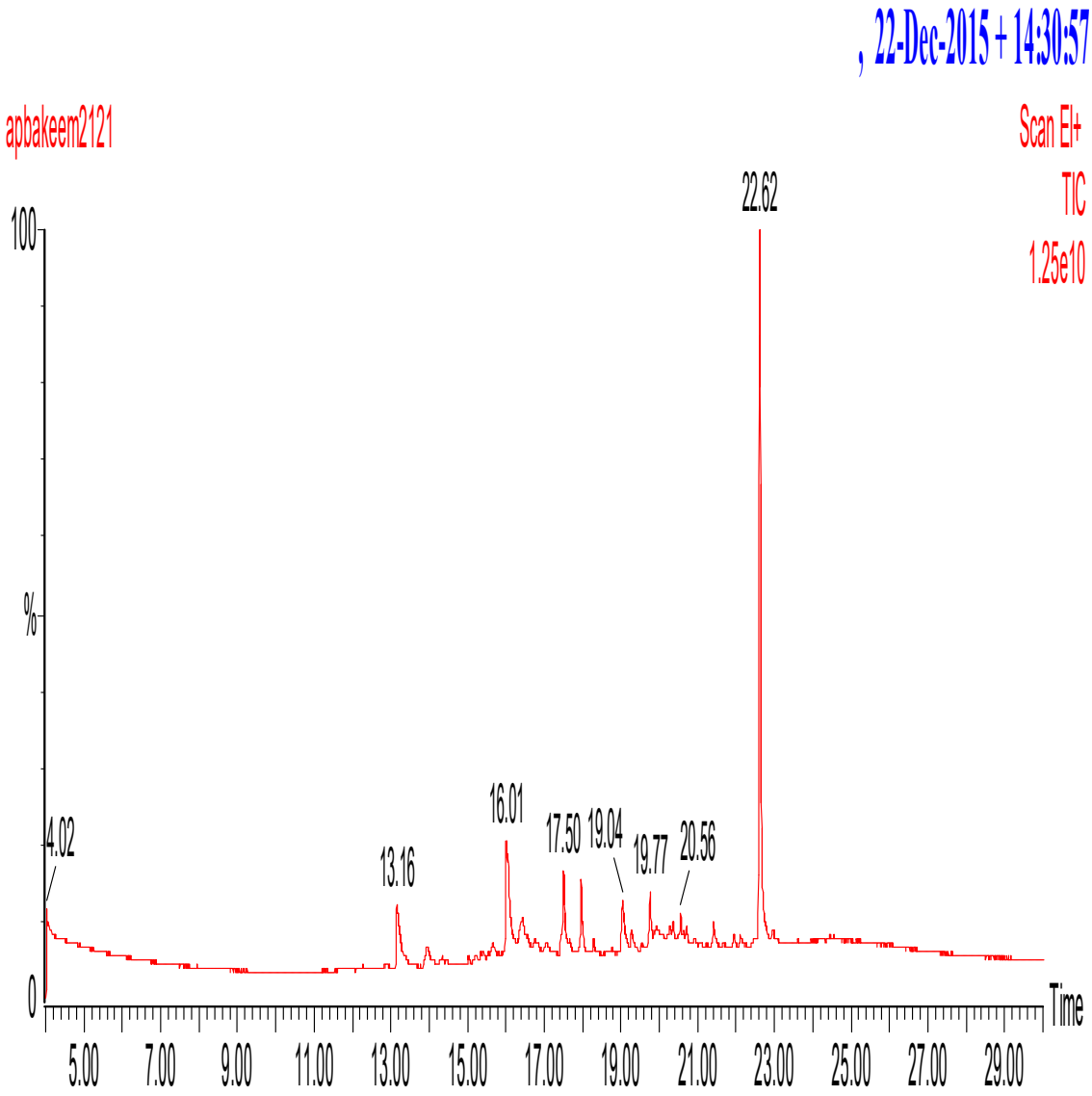
APPENDIX X

GC-MS chromatogram of CE seed extract for determination of phytocomponents



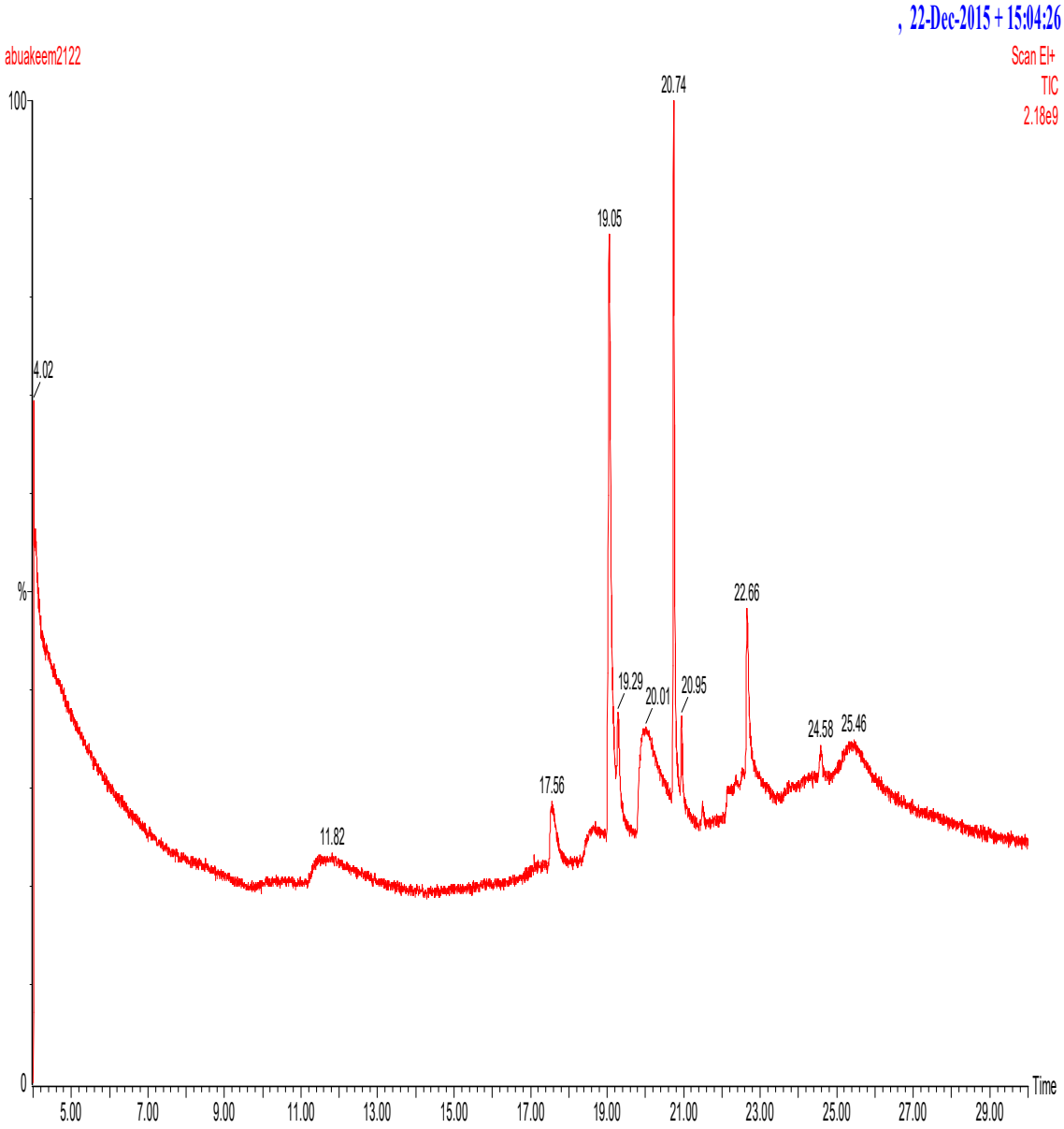
APPENDIX XI

GC-MS chromatogram of PB seed extract for determination of phytocomponents



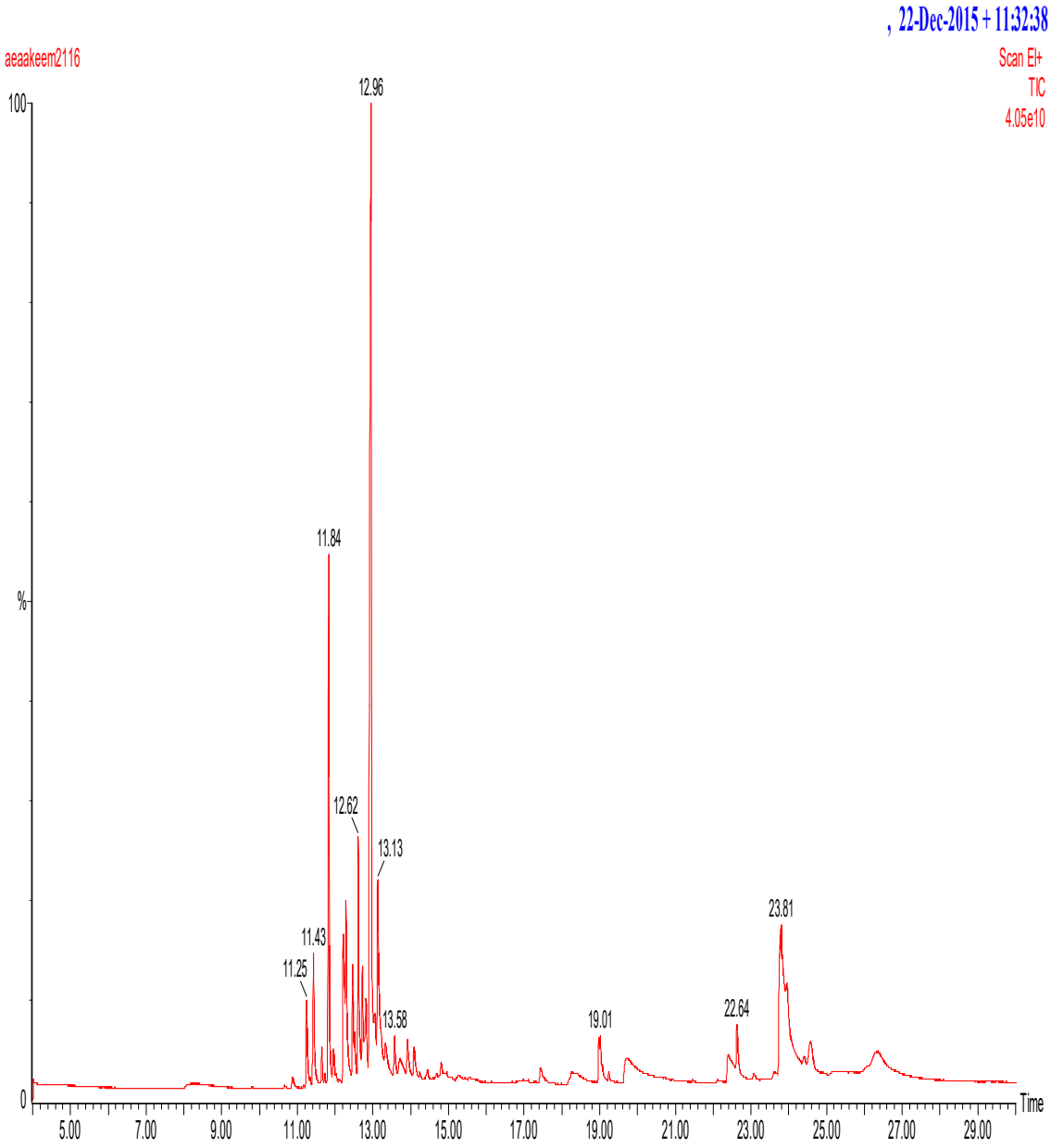
APPENDIX XII

GC-MS chromatogram of BS seed extract for determination of phytocomponents



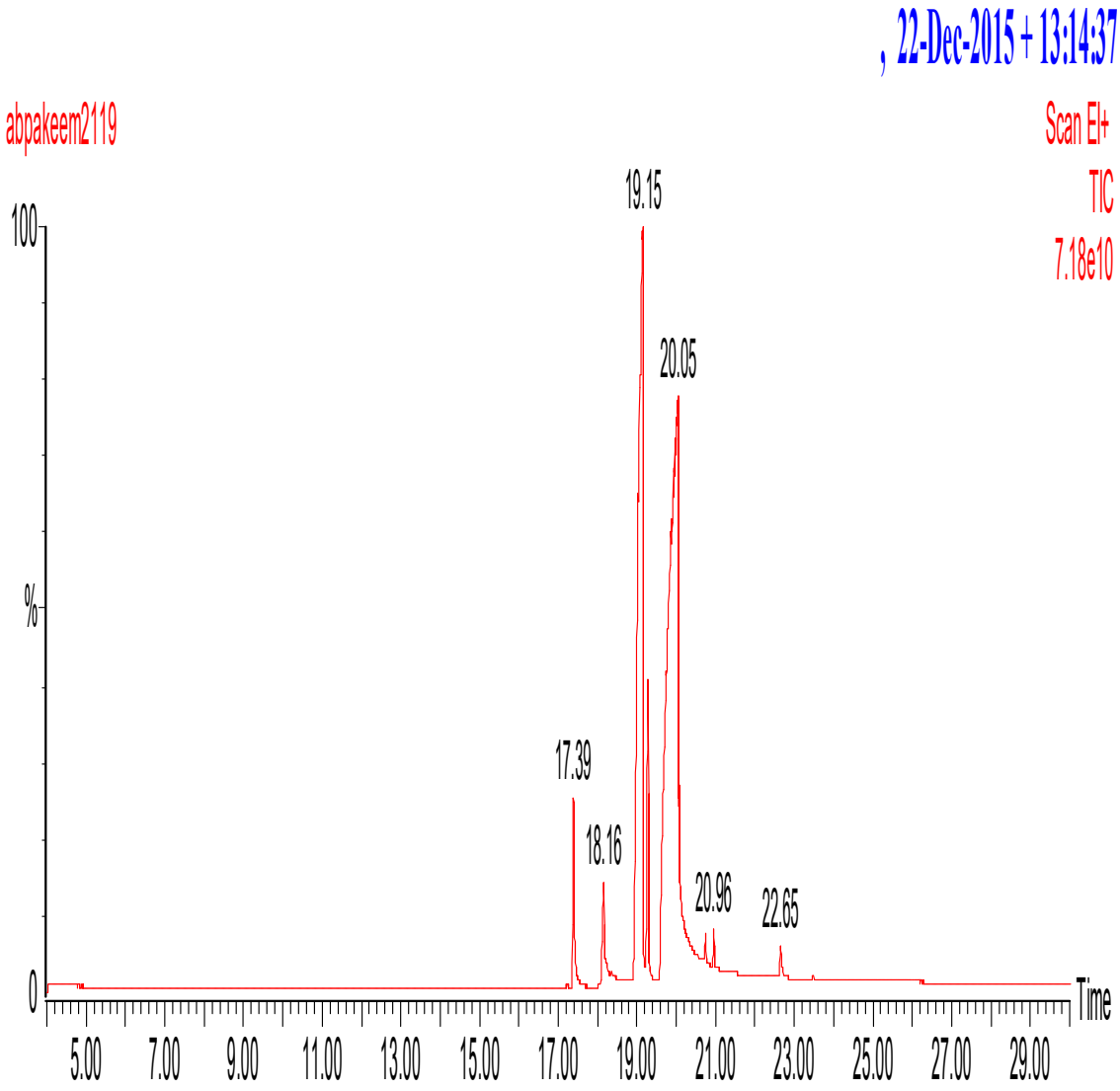
APPENDIX XIII

GC-MS chromatogram of EA seed extract for determination of phytocomponents



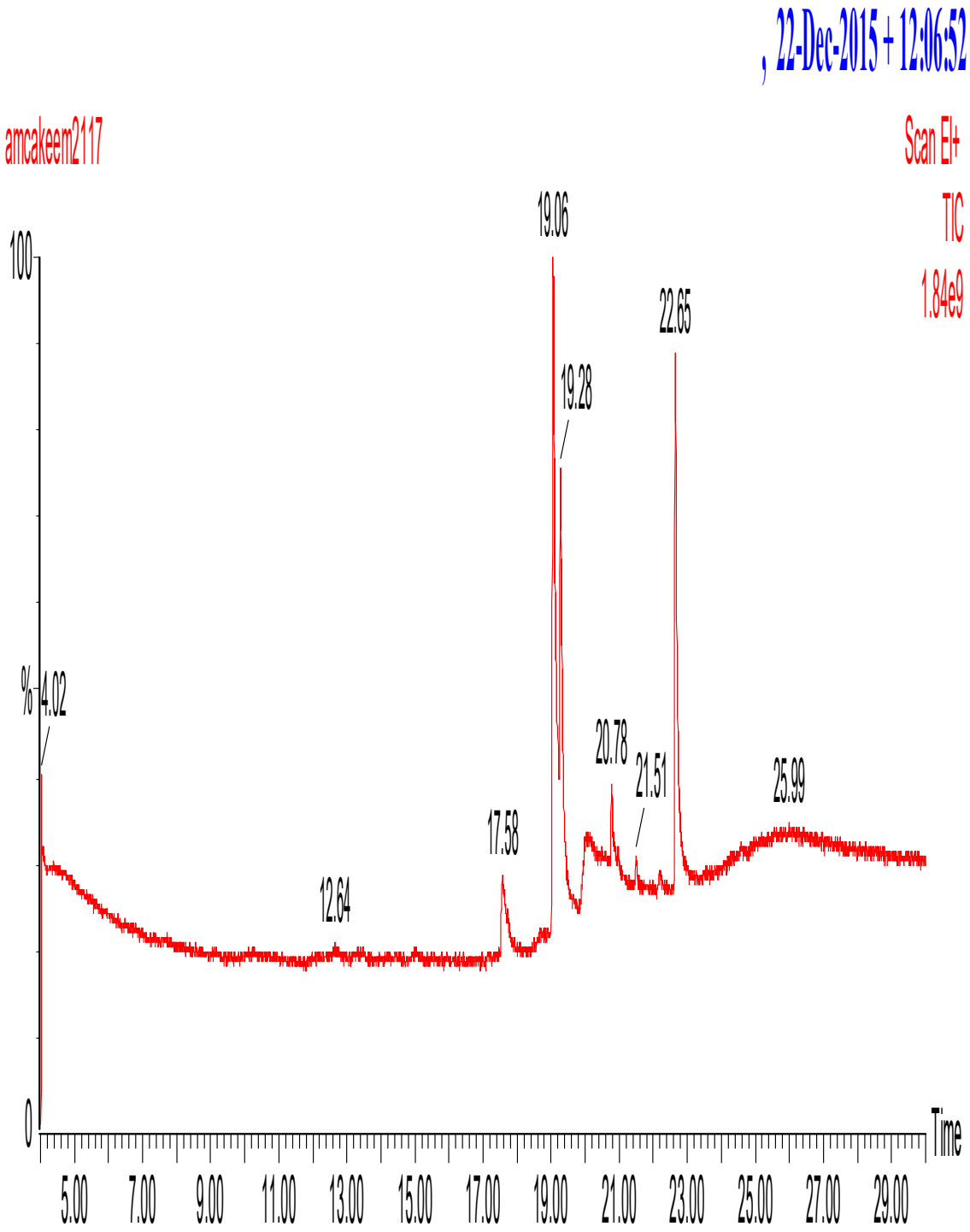
APPENDIX XIV

GC-MS chromatogram of BP seed extract for determination of phytocomponents



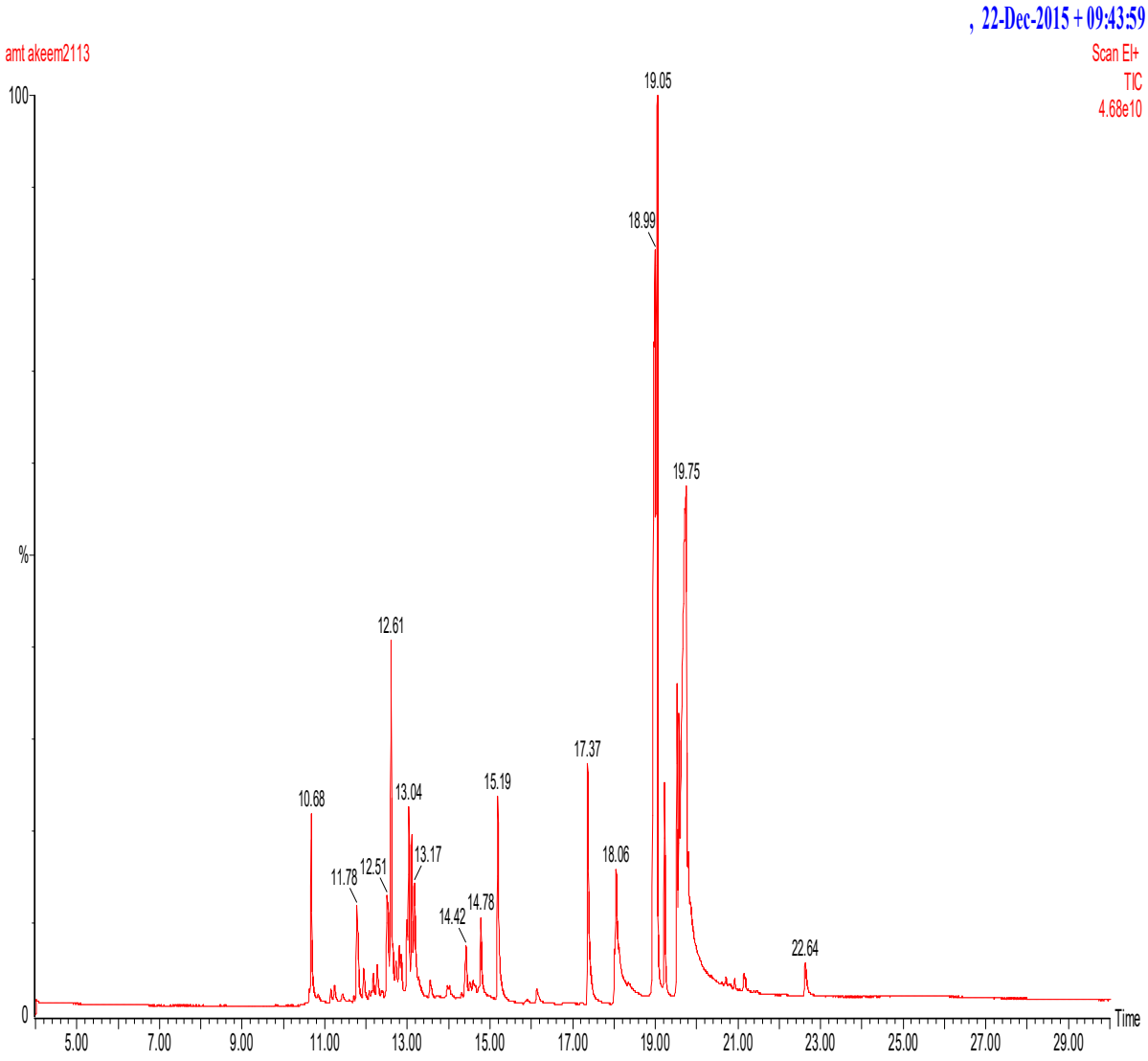
APPENDIX XV

GC-MS chromatogram of MC seed extract for determination of phytocomponents



APPENDIX XVI

GC-MS chromatogram of MT seed extract for determination of phytocomponents



APPENDIX XVII

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UI/UCH EC Registration Number: **NHREC/05/01/2008a**

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Evaluation of the Pharmacological Activities of some Nigeria Seeds

UI/UCH Ethics Committee assigned number: UI/EC/14/0059

Name of Principal Investigator: **Akeem A. Raji**

Address of Principal Investigator: Department of Chemistry,
University of Ibadan, Ibadan

Date of receipt of valid application: 13/02/2014

Date of meeting when final determination on ethical approval was made: **17/07/2014**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

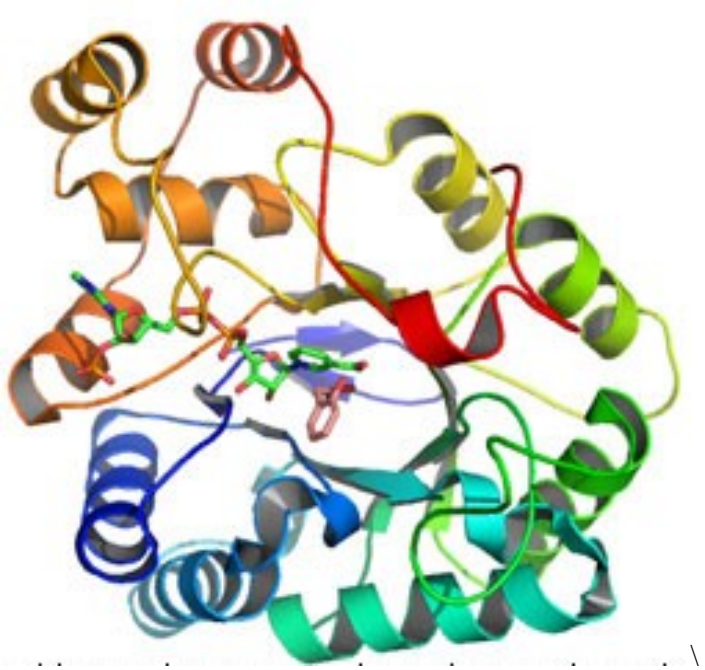
This approval dates from 17/07/2014 to 16/07/2015. If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC early in order to obtain renewal of your approval to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*

UI/UCH EC
17 JUL 2014
APPROVED
COLLEGE OF MEDICINE

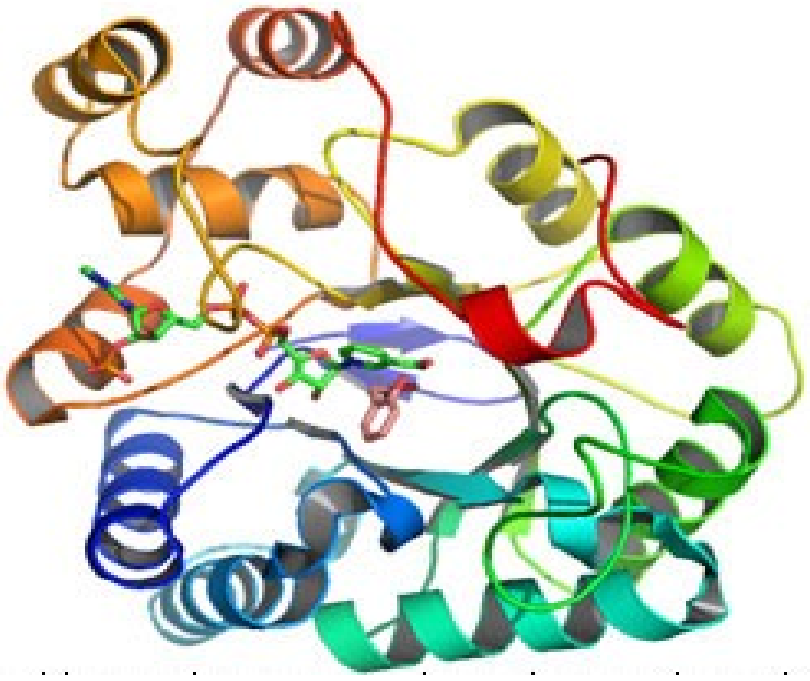
Professor A. Ogunniyi
Director, IAMRAT
Chairman, UI/UCH Ethics Committee
E-mail: uiuchirc@yahoo.com

APPENDIX XVIII



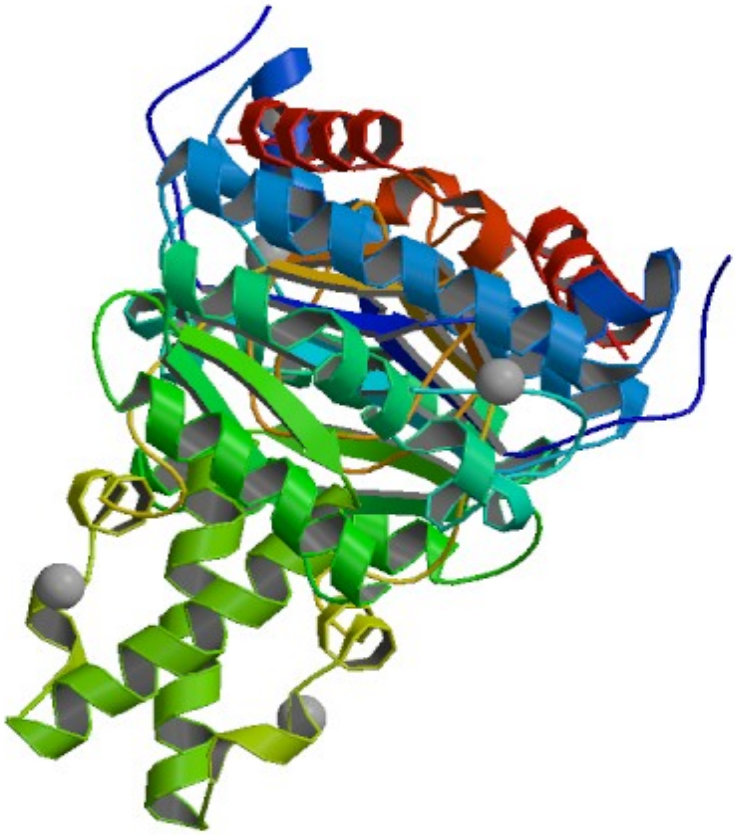
D structure of aldose reductase (ALR2) complex with NADP (green)

APPENDIX XIX



3 D structure of porcine aldehyde reductase in ternary complex with inhibitor protein chains colored from the N-terminal to the C-terminal using a rainbow (spectral) color gradient (PDB)

APPENDIX XX



3 D structure of α-glucosidase enzyme (PDB)

APPENDIX XXI

Bovine kidney

APPENDIX XXII

Isolated ALRI enzyme

APPENDIX XXIII

Group I: Animals loaded with glucose treated with500 mg/kg/b.wt of BS

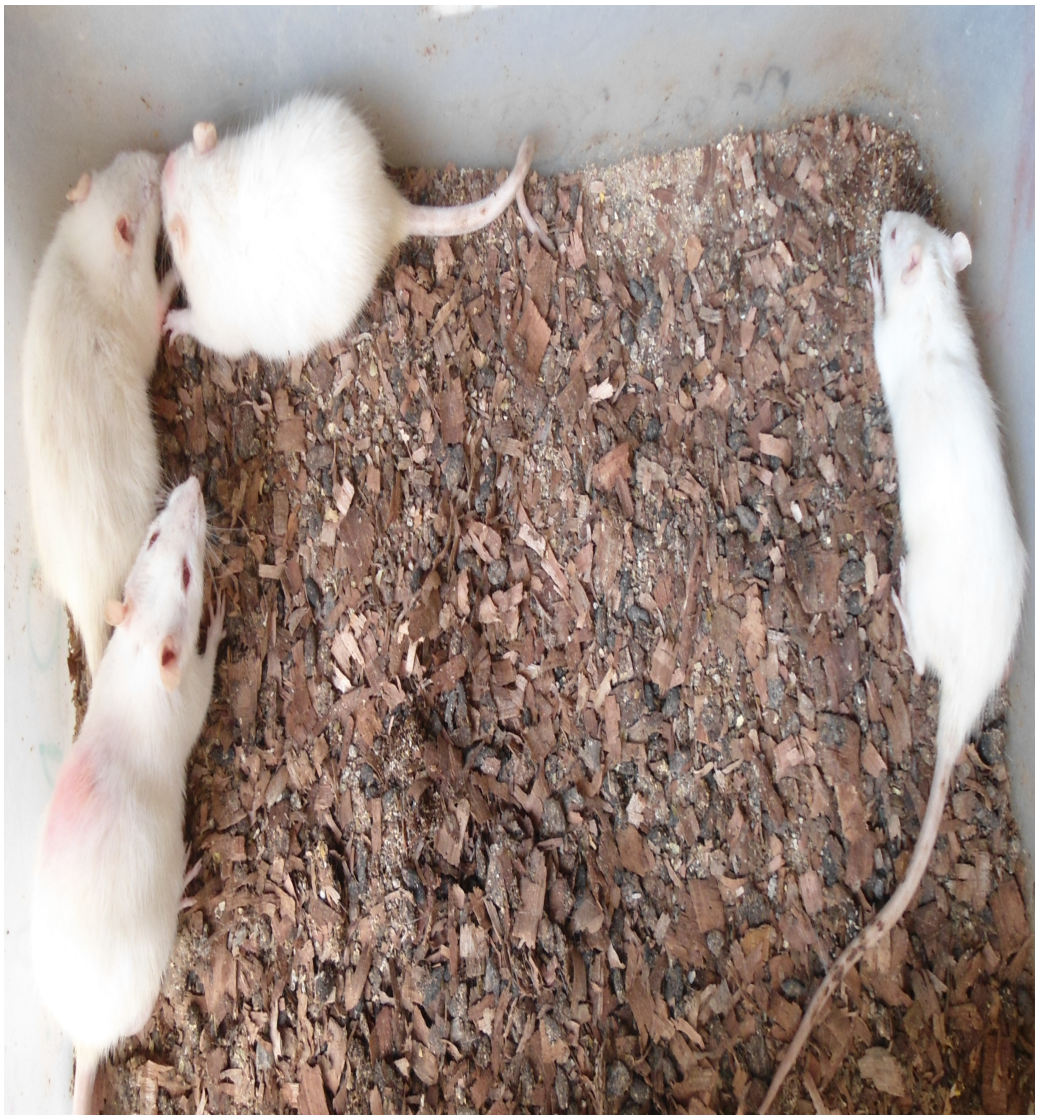
APPENDIX XXIV

Group II: Animals loaded with glucose treated with500 mg/kg/b.wt of PN

APPENDIX XXV

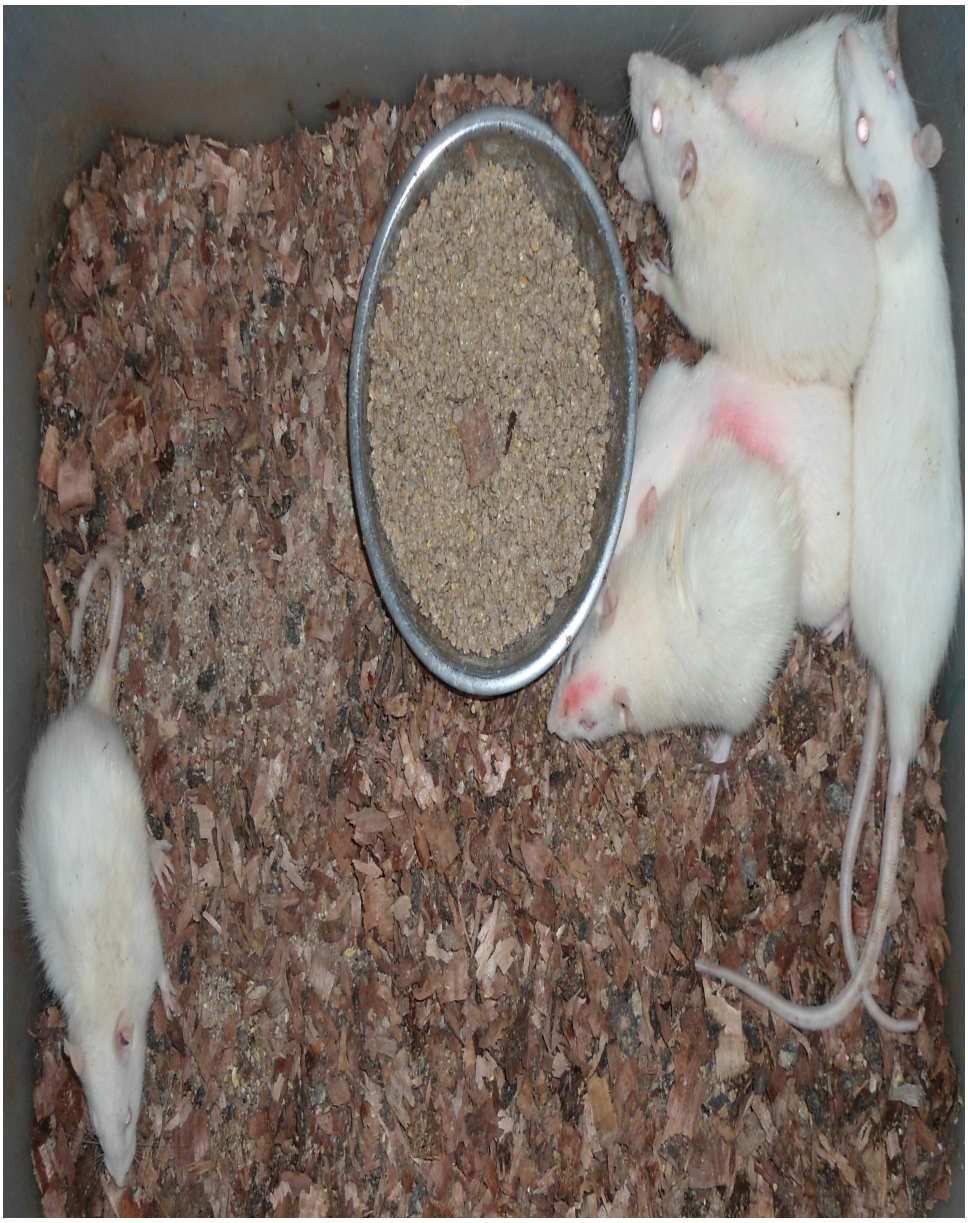
Group III: Animals loaded with glucose treated with 500 mg/kg/b.wt-of PB

APPENDIX XXVI



Group IV: Animals loaded with sucrose treated with1000 mg/kg/b.wt of BS,

APPENDIX XXVII



Group V: Animals loaded with sucrose treated with 1000 mg/kg/b.wt of PN,

APPENDIX XXVIII

Group VI: Animal loaded with sucrose treated with1000 mg/kg/b.wt of BS,

APPENDIX XXIX

Samplee collection site (Bode market)

APPENDIX XXX

Downloading of enzymes from Protein Data Bank (PDB)

APPENDIX XXXI

My host and the lab group

APPENDIX XXXII

COMSATS Institute of Information Technology

presents

Research Productivity Award 2015

to

Raji Akeem Adewale

in appreciation of his/her contributions in Research

Prof Dr Raheel Qamar (T.I.)
Dean Research, Innovation & Commercialization

Prof Dr Izhar Hussain (T.I.)
Secretary Board of Advanced Studies & Research

Award received at CIIT-COMSAT

APPENDIX XXXII

Preparation of 2.0 M HCl solution

The 2.0 M HCl solution was made by diluting 16.0 mL of the concentrated HCl in 84.0 mL of water (distilled). The mixture was careful vortex and stored in a reagent bottle prior to use.

2.0 M methanolic sodium hydroxide

2.0 M methanolic sodium hydroxide solution was made by dissolving 8.0 g of NaOH in methanol (100 mL). The resulting solution was kept in a reagent bottle at room temperature

Stock preparation of seed extracts for antioxidant activity

10.0 mg each of the seed crude extracts was diluted in 1.0 mL of dimethyl sulfoxide (DMSO, Sigma Aldrich) and stored at -10 ^{o}C until needed.

Preparation of DPPH solution

A 330 µM DPPH stock solution was made in methanol. 1.3 mg of DPPH was weighed into 10.0 mL of methanol in amber bottle covered with aluminum foil and kept at 4 ^{0}C.

Ascorbic acid solution

1.7 mg/mL of ascorbic acid (Sigma Aldrich) which is equivalent to 10.0 mM concentration was made in methanol and kept in an eppendorf wrapped with aluminum foil at -4 ^{0}C until further use.

Preparation of 100.0mM KH_2PO_4 (pH 7.2) assay buffer

The buffer solution of KH_2PO_4 in a molar concentration of 100.0 mM was made in 100.0 mL volumetric flask by dissolving 1.360 g of the salt with distilled water. The solution was adjusted to pH of 7.2 and then made up to the mark by the addition of distilled water.

0.1 % (w/v) Ferric chloride

0.1 % $FeCl_3$ solution was made by weighing 0.01 g of the salt into distilled water (10.0 mL). The resulting solution was wrapped with aluminum foil and kept in dark at room temperature until use.

Potassium ferricyanide ($K_3(Fe(CN)_6$) (1.0 % w/v)

The ($K_3(Fe(CN)_6$) 1 % (w/v) solution was prepared by dissolving 0.1 g of the complex in 10.0 mL of distilled water. The solution was prepared in an amber bottle and further wrapped with aluminum foil and kept in a dark place at room temperature before use.

10 % (w/v) trichloroacetic acid (TCA)

Trichloroacetic acid (TCA) solution of 10.0 % w/v was made by dissolving 10.0 g of TCA in 100.0 mL of distilled water. The solution was vortex and store at room temperature prior to the application.

Preparation of 10.0 % (v/v) Folin-Ciocalteu reagent solution for total phenols content

Folin-ciocalteu reagent solution of 10 % (v/v) was made by diluting 10.0 mL of the stock reagent in 90.0 mL of distilled water in a standard volumetric standard that was later transferred into an amber bottle wrapped with the aluminum foil. The solution was kept in a dark place at room temperature until require.

6.0 % w/v sodium carbonate solution

6.0 g of Na_2CO_3 was weighing into 100 mL of distilled water in a flask. The solution was vortex, made up to the mark and then stored in a reagent bottle at room condition.

Preparation of gallic acid solution

2.0 mg of gallic acid was weighed into 10.0mL flask solubilised by methanol. Six different concentrations of the gallic acid were further made in the range of 12.5 μg/mL to 200 μg/mL for the preparation of the calibration curve.

Preparation of stock solution of quercetin for total flavonoids content

A stock solution of quercetin was made by dissolving 2.0 mg of the compound in 10.0 mL of methanol. From this, six different concentrations of quercetin solutions were prepared in the range of 12.5-200 μg/mL for the preparation of the calibration curve.

10.0 % (w/v) aluminum chloride

10.0 % (w/v) solution of $AlCl_3$ was made in 100.0 mL of distilled water, and vortexed to obtain homogenous solution. The solution was kept at 25 ^oC in a dark place.

67.0 mM disodium hydrogen phosphate assay buffer

A buffer solution of 67.0 mM disodium hydrogen phosphate buffer (pH 7.4) was made by dissolving 237.8 mg of the salt with distilled water; the pH was adjusted to 7.4 and made up to the mark of the volumetric flask.

Bovine serum album (BSA) solution

BSA solution was prepared by dissolving l0.0 mg of the bovine serum album in 67.0 mM of Na_2HPO_4 buffer solution (pH 7.4) and kept at 4 ^oC in an amber bottle.

Homogenisation buffer solution for the extraction of ALR1 enzyme

The homogenisation buffer used for the isolation of ALR1 enzyme was prepared by the combination of 709.9 mg of disodium phosphate salt (10 mM), 42.78 g of sucrose (0.25 M), 292.0 mg of EDTA (2 mM) and the addition of 5 µL of β-mercaptoethanol (2.5 mM) in a 500.0 mL of standard volumetric. 500.0 mL of distilled water was gradually added to monitor the pH at 7.2.

ALR1 dialyse buffer solution

The dialysed buffer for ALR1 enzyme was prepared from the composition of 1.4196 g of 10 mM disodium phosphate salt, 584.0 mg of 2 mM EDTA and 5.0 mL of 2 mM β-mercapto ethanol in a litre standard volumetric flask. To get the final buffer of pH 7.2, one litre of distilled water was added and the pH then adjusted at room temperature.

Disodium phosphate homogenisation buffer solution (pH 7.2)

This buffer was prepared by mixing 3.54 mg of 10 mM Na_2HP04, 14.0 mg of 2 mM EDTA and 5 µL of 2 mM β-mercaptoethanol in a 250 mL volumetric flask with distilled with further adjustment of the pH to 7.2 kept for the lens homogenization.

Dialyse 50.0 mM NaCl buffer solution

1.46 g of sodium chloride salt (NaCl, Sigma) was dissolved in 500.0 mL of distilled water and the pH was adjusted to 6.2 that was kept at room temperature until further require.

Preparation of protein solution

A stock solution of protein in the concentration of 10 mg/mL was prepared from Bovine serum album (BSA). From the stock solution, a series of six different standard solutions of the protein in the range of 0.01-1.00 mg/mL were made in 50.0 mL standard volumetric flasks.

ALR1 enzymatic activity

The activity of ALRI and ALR2 enzymes was spectrophotometrically determined by the consumption of NADPH at 340 nm expressed as optical density S^{-1} protein $^{-1}$ due to NADPH oxidation catalyzed by ALRI and ALR2 enzymes. ALR1 activity was assayed

spectrophotometrically in a reaction mixture containing 20 µL of 100 mM sodium phosphate buffer (pH 6.2), 70 µL of dialyzed ALR1 enzymatic solution, 40 µL of 50 mM of D, L-glyceraldehyde, 50 µL of NADPH and 20 µL of distilled water in a total of 200 µL. All the reagents mixture, except the substrate was incubated at 37 ^{o}C for 5 mins, the substrate was then added for the initiation of the reaction which was monitored for 5 mins.

ALR2 enzymatic activity

ALR2 activity was determined at 37 ^{o}C in a reaction medium containing 100 mM sodium phosphate buffer (pH 6.2) 70 µL of dialyzed ALR2 enzymatic solution, 40 µL of 50 mM of D, L-glyceraldehyde, 50 µL of NADPH (0.5 mM) and 20 µL of distilled water to make a total volume 200 µL in a microplate reader. The content was incubated for 5 mins followed by the addition of NADPH for the initiation of the reaction which was further incubated for 3 mins and monitored for 5 mins. To obtain 100.0 mM dibasic sodium phosphate buffer solution, 354.9 mg of the phosphate salt was added to 25.0 mL standard flask, and 25.0 mL of distilled water was added, vortex to obtain homogenous solution. The pH of the buffer solution was then adjusted to 6.2 by gradual addition of 25.0 mL of the distilled water.

Glyceraldehyde (substrate)

4.50 mg of glyceraldehyde was measured and put in 1.0 mL of distilled water and kept at 4 ^{o}C until required in the assay.

20.0 mM NaOH (pH 12.3)

This solution was made by mixing 3.99 mg of NaOH with 5.0 mL of distilled water and pH was adjusted to 12.3 for subsequent use.

Preparation of 0.5 mM NADPH (co-factor)

0.5 mM of NADPH was prepared by weighing 0.4 mg of the salt which was dissolved in 1.0 mL of 20.0 mM of NaOH (pH 12.3) in an amber bottle kept in the fridge at a temperature of 4 ^{o}C, until further use in the assay.

Preparation of 100.0 mM of dibasic phosphate buffer (pH 6.2)

To obtain 100.0 mM dibasic sodium phosphate buffer solution, 354.9 mg of the phosphate salt was added to 25.0 mL standard flask, and 25.0 mL of water was introduced, vortex to get uniform solution. The pH of the buffer solution was then adjusted to 6.2 by gradual introduction of 25.0 mL of the distilled water.

Glyceraldehyde (substrate)

4.50 mg of glyceraldehyde was measured and added into in 1.0 mL of distilled water and kept at 4 ^{o}C until required in the assay.

20.0 mM NaOH (pH 12.3)

This solution was made by mixing 3.99 mg of NaOH with 5.0 mL of distilled water and the pH was adjusted to 12.3 for subsequent use.

Preparation of 0.5 mM NADPH (cofactor)

0.5 mM of NADPH was made by accurate weighing pf 0.4 mg of the salt which was dissolved in 1.0 mL of 20.0 mM of NaOH (pH 12.3) in an amber bottle kept in the fridge at 4 ^{o}C, until further use in the assay.

Preparation of 10.0 mM of valproic acid

The stock drug contains 500.0 mg/5mL of valproic acid and a required concentration of 1.66 mg/mL of the drug was made to give concentration of 10.0 mM of the valproic acid used in the assay.

70.0 mM phosphate buffer (pH 6.8)

Preparation of 70.0 mM dibasic sodium phosphate (Na_2HPO_4 BDH-Analar) buffer was made by dissolving 993.72 mg of the dibasic salt in 100.0 mL of distilled water using standard volumetric flask, pH of the solution was adjusted to 6.8 and made up to the mark, kept at the temperature of 4 ^{o}C.

10.0 mM *P*-nitrophenyl α-D-glucopyranoside solution

P-nitrophenyl α-D-glucopyranoside solution of 10.0 mM concentration was prepared in a 1.5 mL eppendorf by taken 3.012 mg of the substrate into 1.0 mL of 70.0 mM of dibasic sodium phosphate buffer (pH 6.8) and kept at 4 ^{o}C until further use.

Preparation of stock solution of acarbose

A standard solution of an acarbose contain 1.2 mg in 200 µL of methanol was made and also kept in the fridge at 4 ^{o}C.

70.0 mM phosphate buffer (pH 6.8)

Preparation of 70.0 mM dibasic sodium phosphate (Na_2HPO_4 BDH-Analar) buffer was made by dissolving 993.72 mg of the dibasic salt in 100.0 mL of distilled water using volumetric flask, the resulting solution p^{H} was adjusted to 6.8 and made up to the mark, kept at 4 ^{o}C.

Preparation of 10.0 mM *P*-nitrophenyl β-D-glucopyranoside (substrate)

10.0 mM of the substrate was made by measuring 3.012 mg of the powder in 1.0 mL assay buffer solution (70 mM) of pH 6.8. The solution was put in an eppendorf and kept at a temperature of 4 ^{o}C until required for assay.

Enzyme buffer solution

The buffer solution required for the preparation of the enzymes was made from the combination of 104.26 mg of morpholinepropanesulphonic acid (Mops, 10 mM) with 18.612 mg of EDTA (1.0 mM) in a 100.0 mL standard flask. The solution was made top up to the mark of the flask by monitoring the pH at 6.8 and kept until needed in the assay.

Assay buffer solution preparation

The assay buffer was a mixture of 100.0 mM of Trismonomethane and 5.0 mM $CaCl_2$ in 100 mL standard flask with adjusted pH of 7.0.

Substrate preparation

10.0 mM of the substrate was prepared by dissolving 2.09 mg in 1.0 mL of the assay buffer (mixture of Trismonomethane (100.0mM) and $CaCl_2$ (5mM) of 7.0 pH.

Preparation of 2.5 units of α-glucosidase enzyme

2.5 U/mL of α-glucosidase enzyme was made in 70.0 mM of dibasic sodium phosphate buffer of pH 6.8 by weighing 1.0 mg of the enzyme. The required units of the enzyme required for the assay were furthered made by diluting 22.0 µL of the enzyme stock solution into 978.0 µL of the buffer which was kept at -4 ^{o}C.

Preparation of β-glucosidase enzyme unit (2.0 U/mL)

2.0 units of β-glucosidase enzyme was prepared from sweet almond by weighing 1.0 mg of the powder enzyme into 1.0 mL of buffer solution of 70.0mM and pH 6.8 in an eppendorf and kept in the fridge at 4 ^{o}C

Preparation of 0.24 M KOH

0.24 M solution of potassium hydroxide (KOH) was prepared from 1.4 g of potassium KOH dissolved in distilled water (250.0 mL), the solution was then vortex and kept at room temperate until use.

100.0 mM KH_2PO_4 buffer (pH 8.0)

3.4 g of potassium dihydrogen phosphate salt was made in 250 mL of distilled water in a standard volumetric flask to make a concentration of 100.0 mM. A previous prepared

0.24 M KOH solution was then used to adjust the buffer pH to 8.0 and the solution was kept in a reagent bottle at 4 ^{o}C prior to use.

Enzyme preparation

Pancreatic porcine lipase enzyme was made by dissolving 5.0 mg of the powder in 10.0 mL of the enzyme buffer solution (MOPs) of pH 6.8 in an eppendorf and kept at 4 ^{o}C in the fridge

15-lipoxygenase enzyme inhibition assay

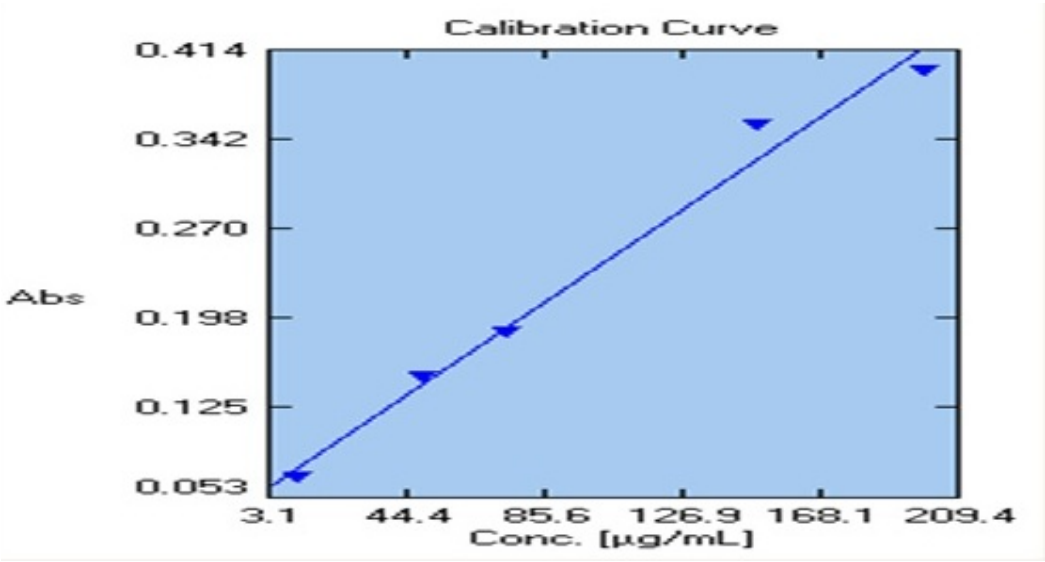
Preparation of linoleic acid (substrate)

1.0 M sodium hydroxide was made by dissolving 4.0 g of the salt in 100.0 mL of distilled water in a volumetric flask. The substrate was prepared from the combination of 8.75 mg of linoleic acid with 17.5 mg of tween 20, sonicated for 40 mins at 40.0 ^{o}C, and then followed by the introduction of 75.0 µL of 1.0 M NaOH and 500.0 µL of distilled water in an amber bottle. The solution was transferred into eppendorf and kept in the fridge at the temperature of -20 ^{o}C until require in the assay. The working solution of the substrate was made from the stock by dissolving 100.0 µL of the stock solution in 900.0 µL of distilled water to make 0.25 mM as the required per well concentration in the assay.

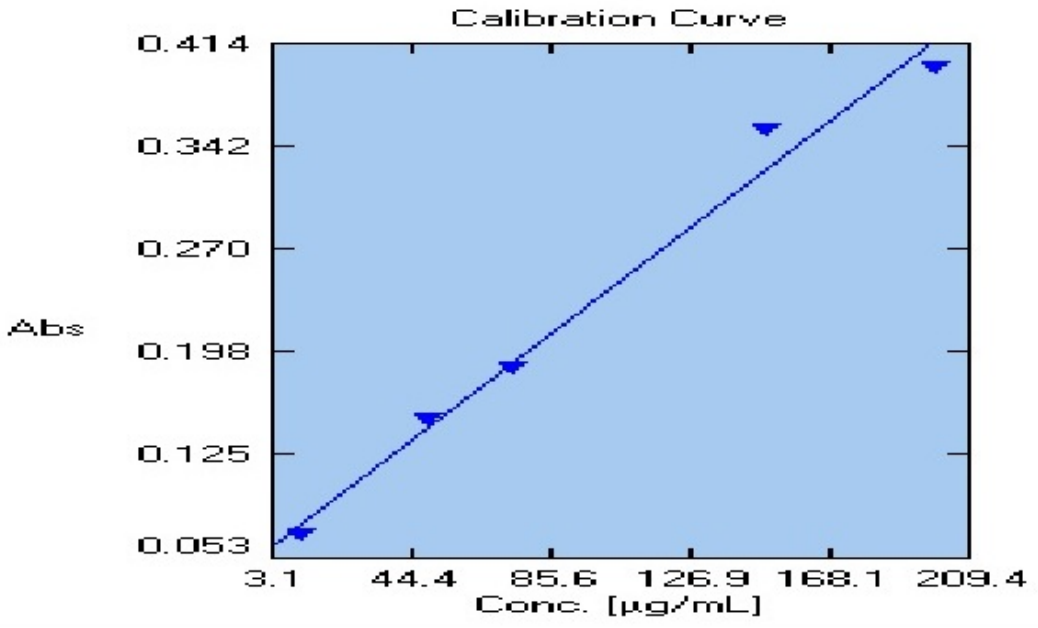
3.8.5.2 Preparation of Lipoxygenase (LOX) enzyme

The stock solution of lox enzymes (soybean from Sigma-Aldrich) was prepared by dissolving 0.50 mg of the enzyme in 1.0 mL of 100.0 mM of KH_2PO_4 buffer solution (pH 8.0) in an eppendorf. From the stock solution with 110850 units of the enzymes, a working unit of 42.5 per well in the assay were made by the dilution of the stock solution with the buffer and kept at -20 ^{o}C prior to the assay.

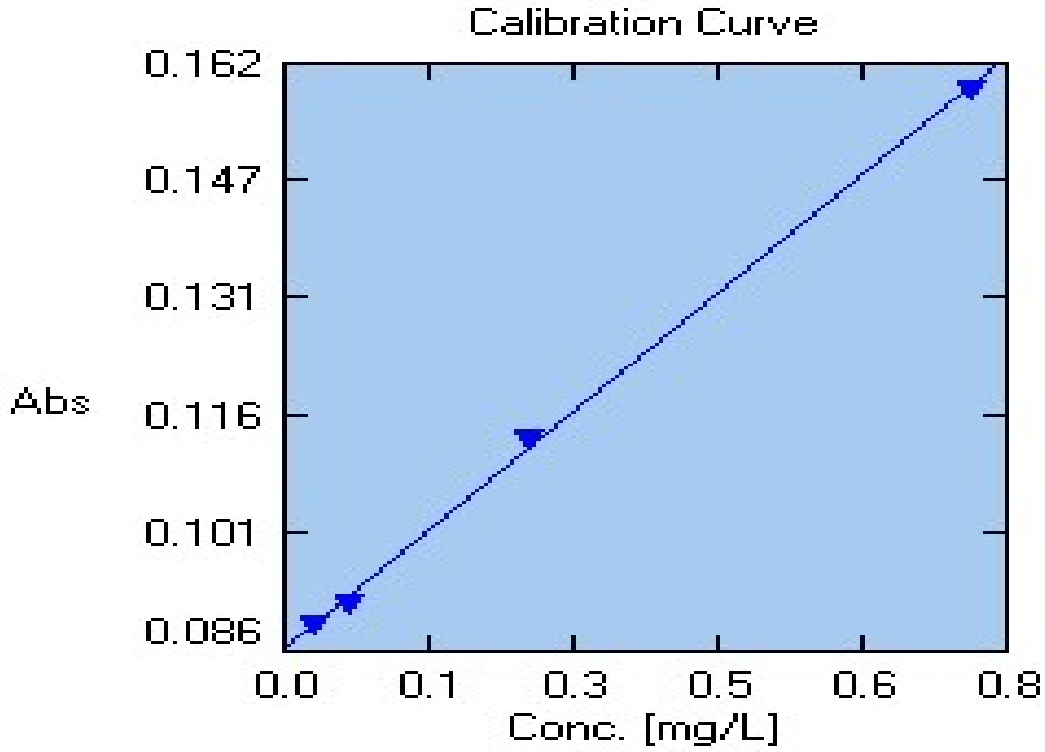
APPENDIX XXXIII



Gallic acid calibration curve for total phenol content determination

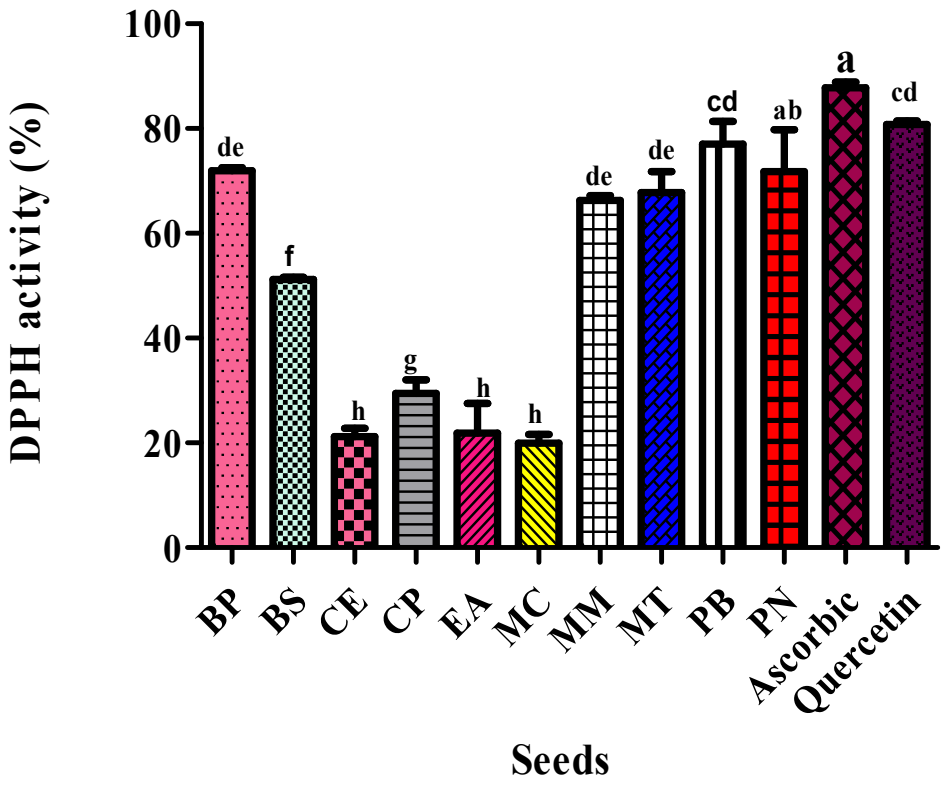


Quercetin calibration curve for total flavonoids content determination

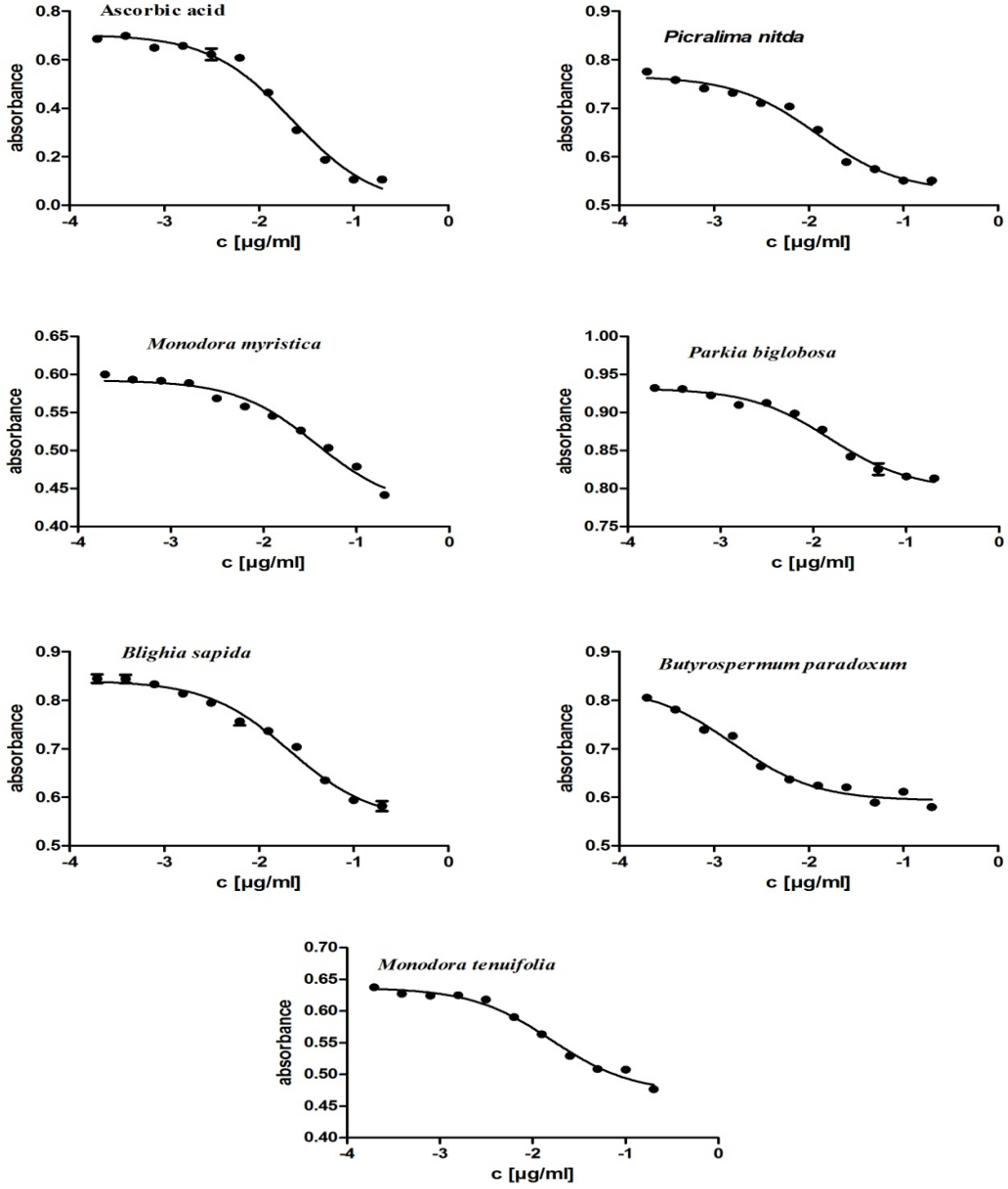


Calibration curve for ALR1 and ALR2 protein estimation

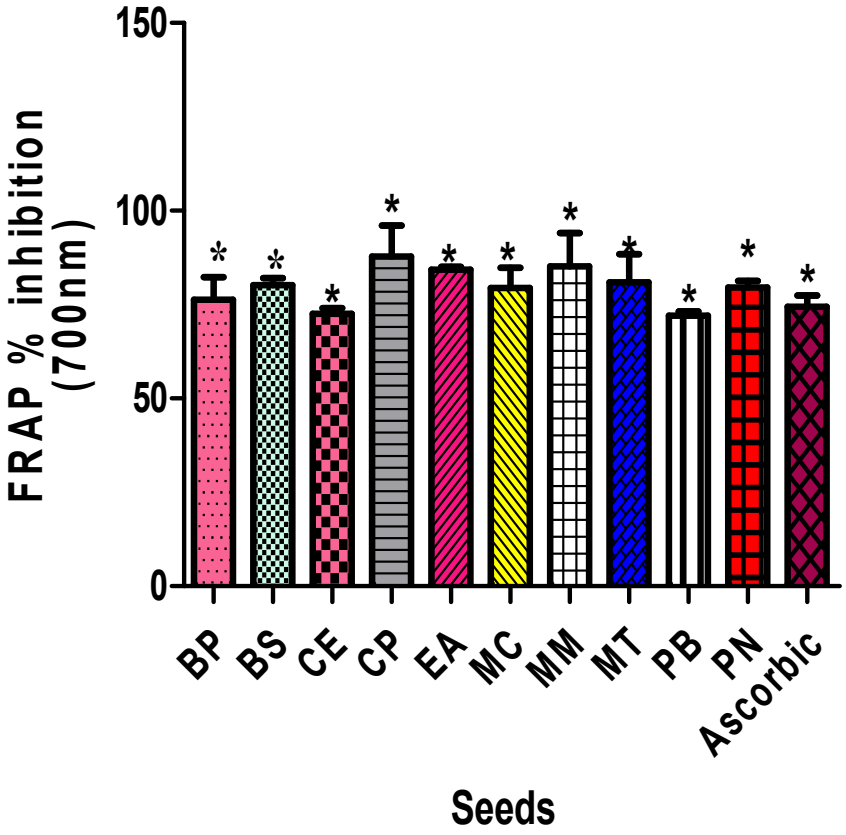
Group photograph of diabetic mice and non diabetic control groups

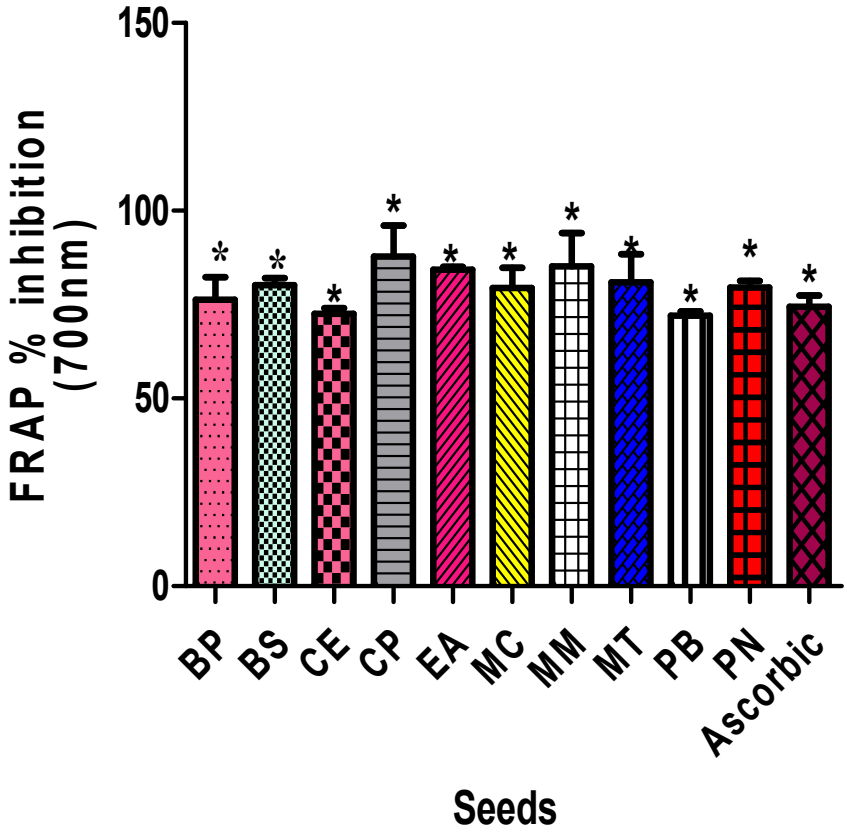


DPPH inhibition (%) of the seed extract and ascorbic acid (positive control). Different alphabets on the error bar indicate significant ($p<0.05$) difference between the seed extracts and the control

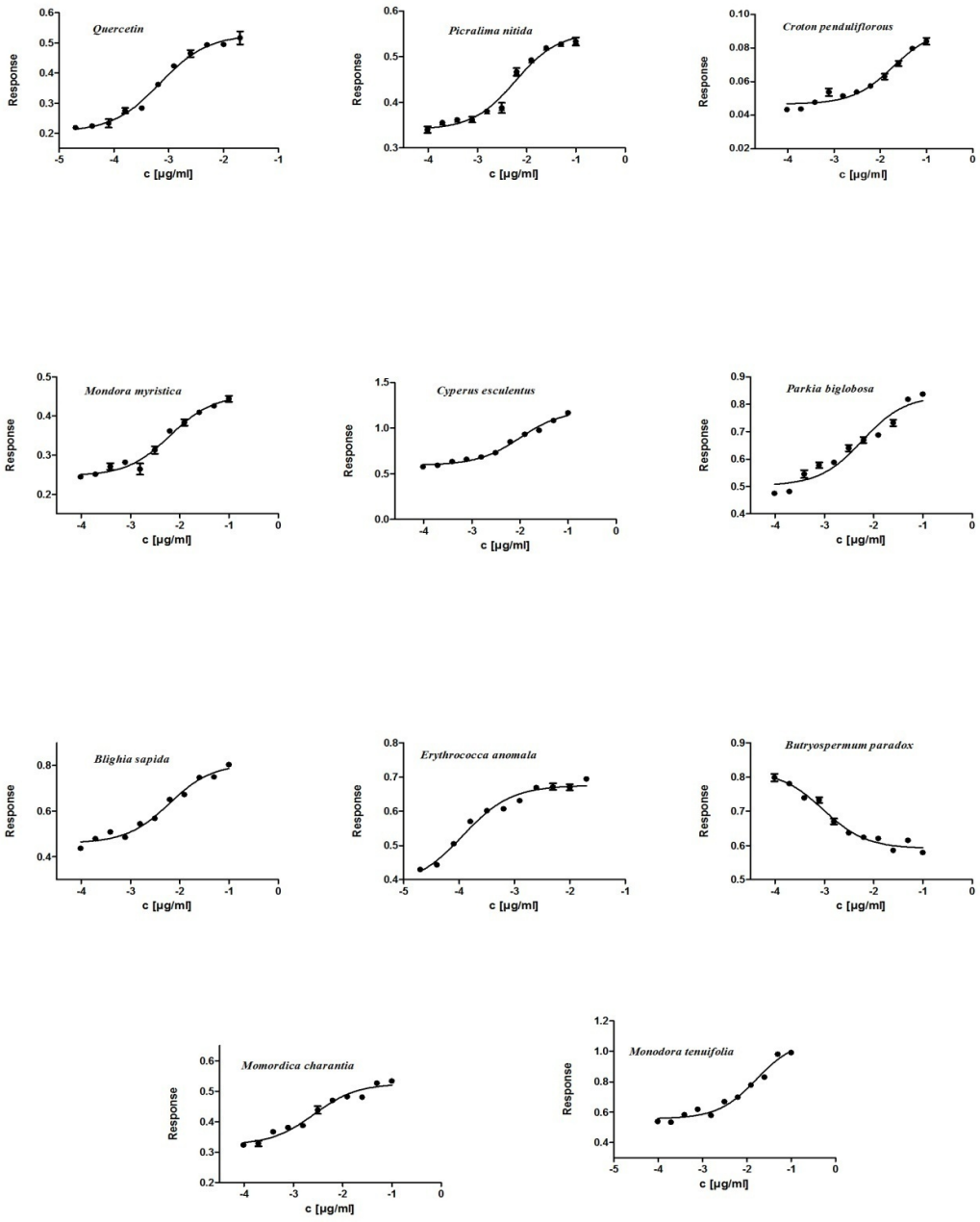


The plots of dose-dependent curves for DPPH at varying concentrations of the seed extracts represented by the values -4, -3, -2, -1 and 0 on the x-axis

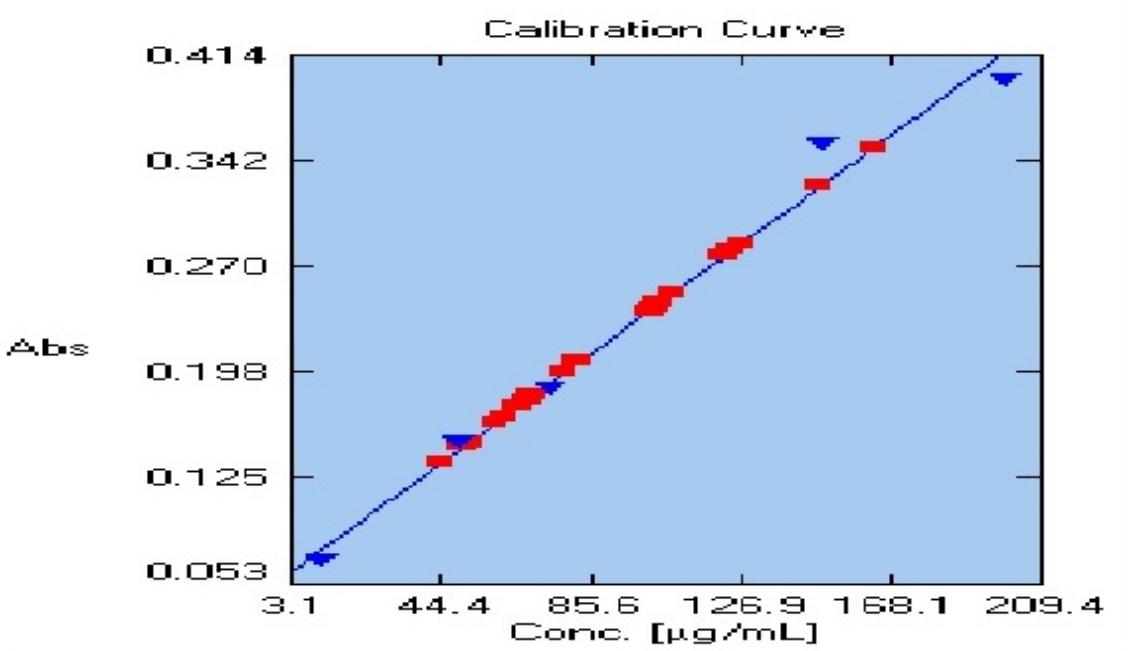

FRAP % inhibition (700nm)
150
100
50
0
BP
BS
CE
CP
EA
MC
MM
MT
PB
PN
Ascorbic
Seeds



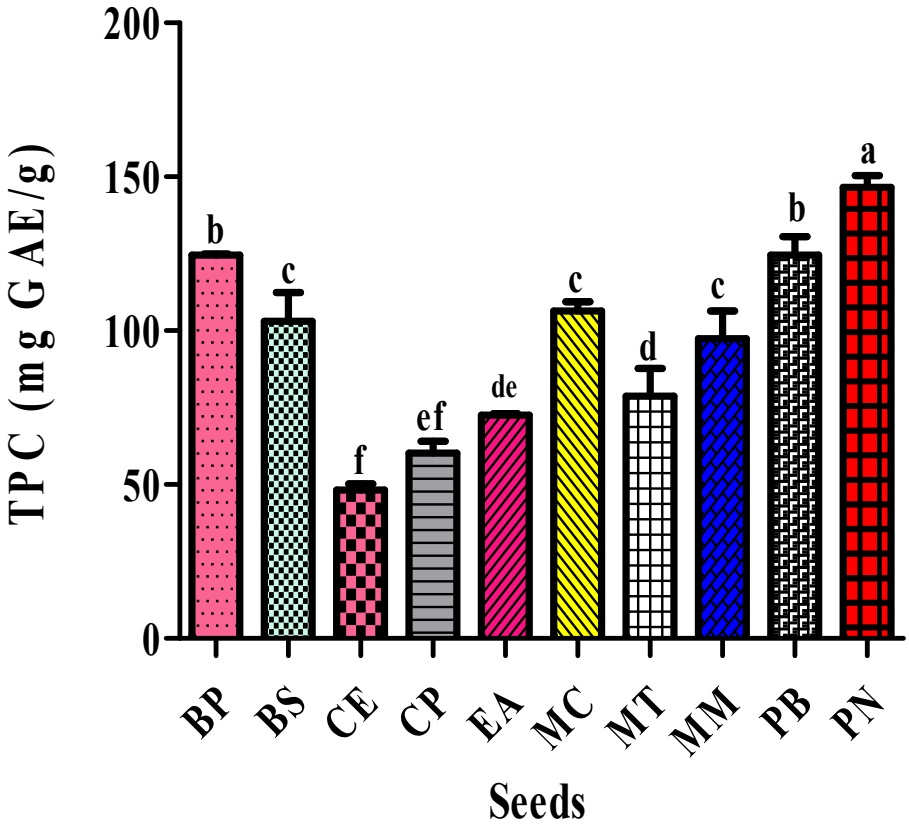
Seed extracts and ascorbic acid (positive control) FRAP activity (%). The same asterisks on the error bar indicate no significant ($p<0.05$) difference between the seed extracts and the control



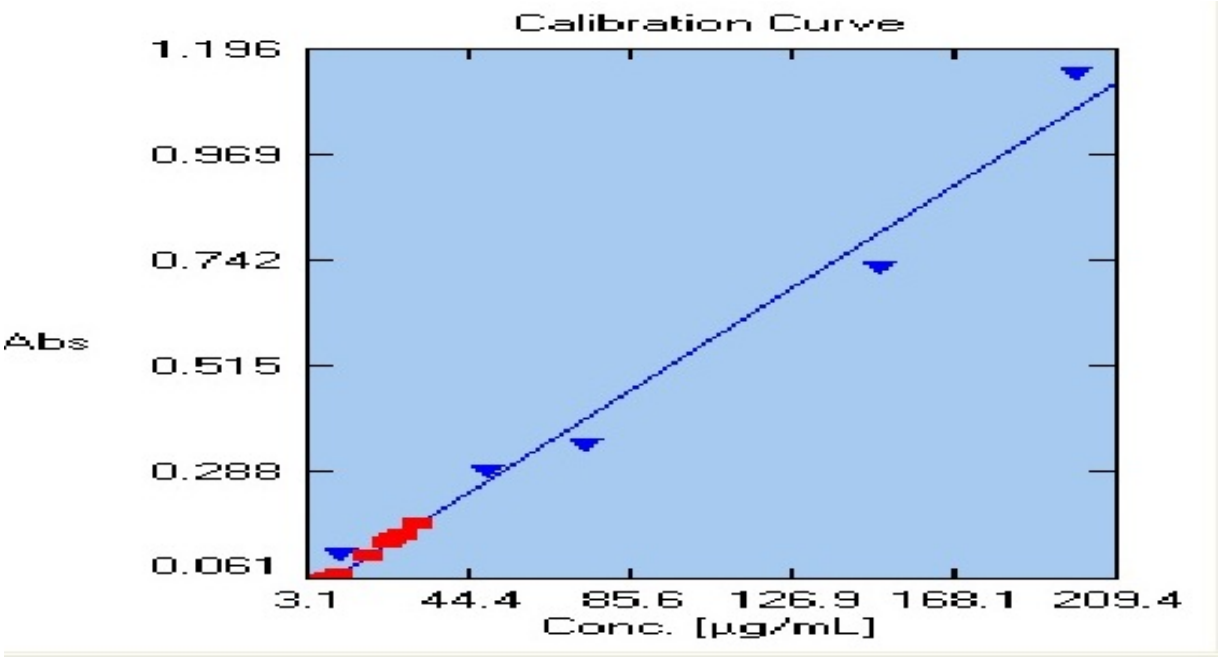
The plots of dose-dependent curves for FRAP at varying concentrations of the seed extracts represented by the values -4, -3, -2, -1 and 0 on the x-axis. Values are the mean of standard deviation of triplicate experiments



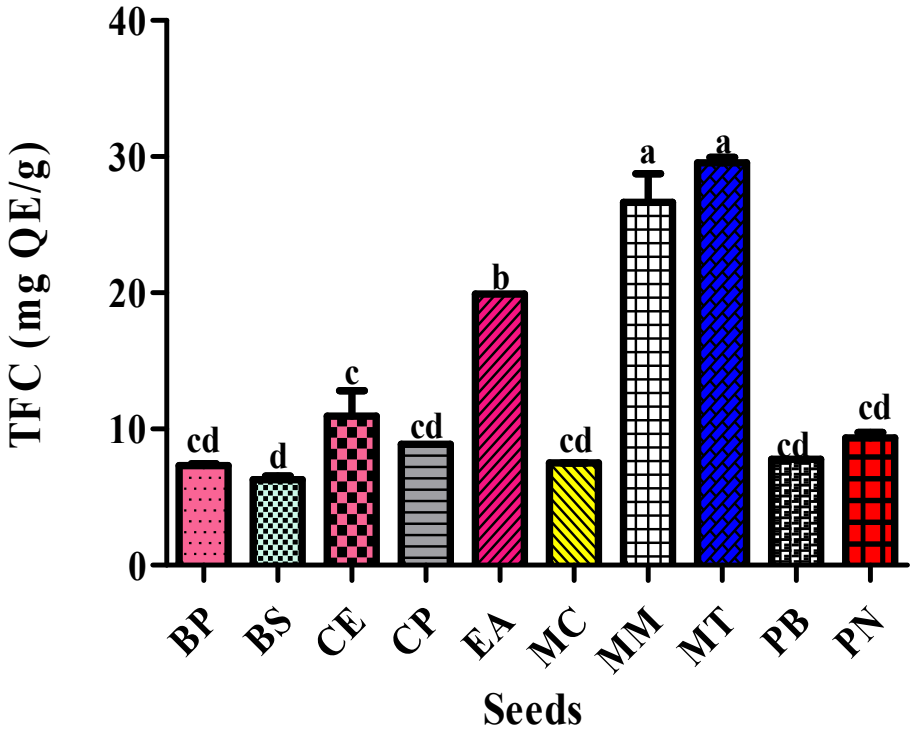
Total phenol contents of the seed extracts estimated from quercetin calibration curve



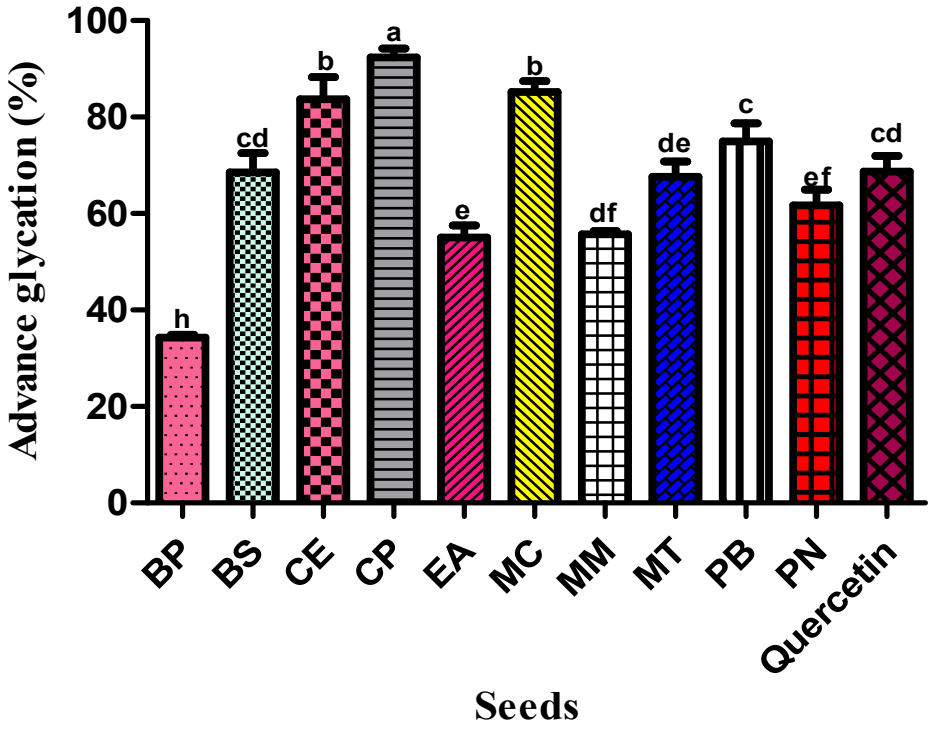
: **Total phenolic contents of the seed extracts. Results are expressed as mean±SD of three independent experiments in triplicate. Error bars sharing a common superscript are not significantly different at *p<0.05***



Total flavonoids contents of the seed extracts estimated from quercetin calibration curve



Total flavonoids contents of the seed extracts. Results are expressed as mean±SD of three independent experiments in triplicate. Error bars sharing a common superscript are not significantly different at *p<0.05*



Inhibition of advance glycation endproducts (%) by the seed extracts and quercetin (positive control). Different alphabets on the error bar indicate significant (*p<0.05*) difference between the seed extracts and control

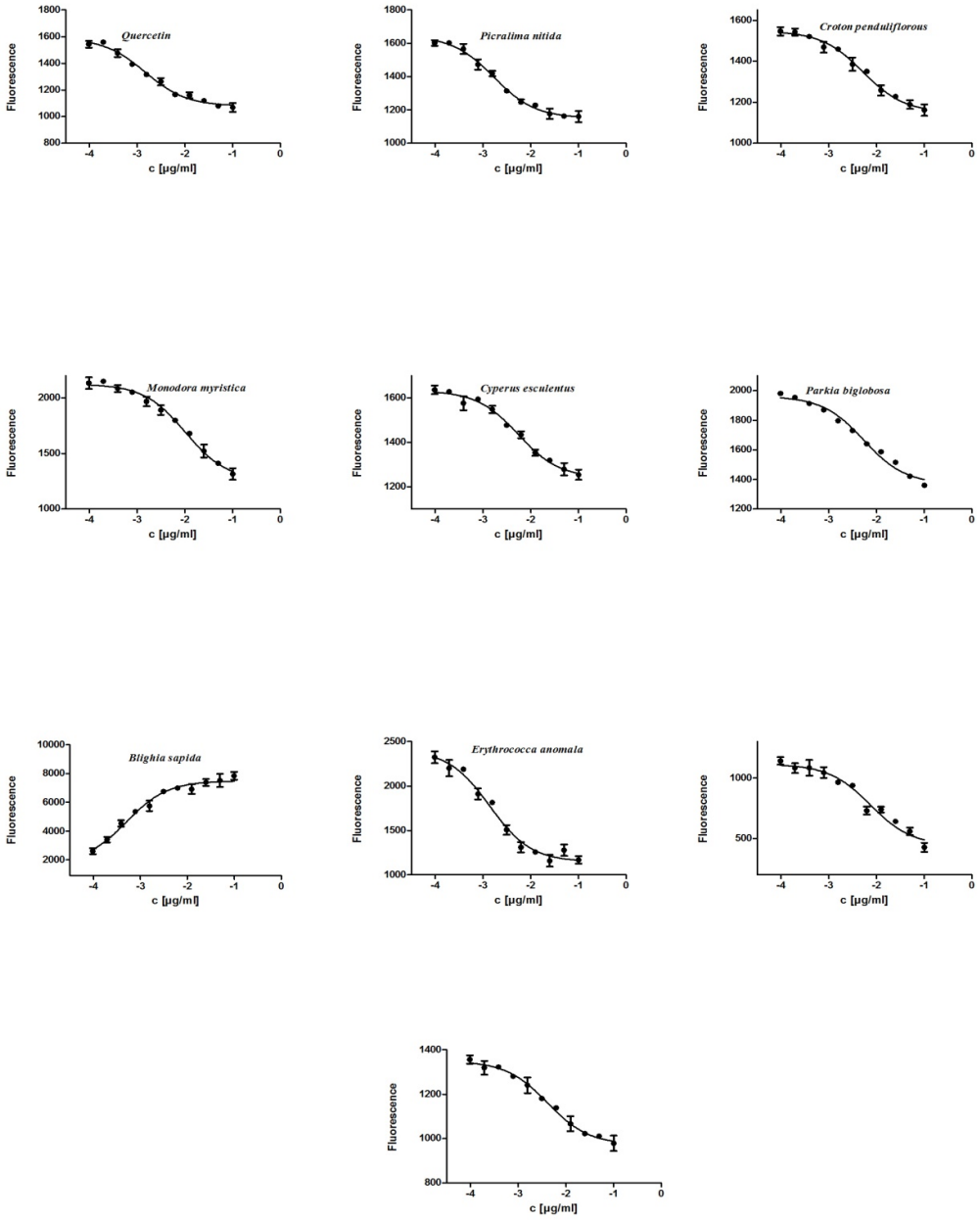
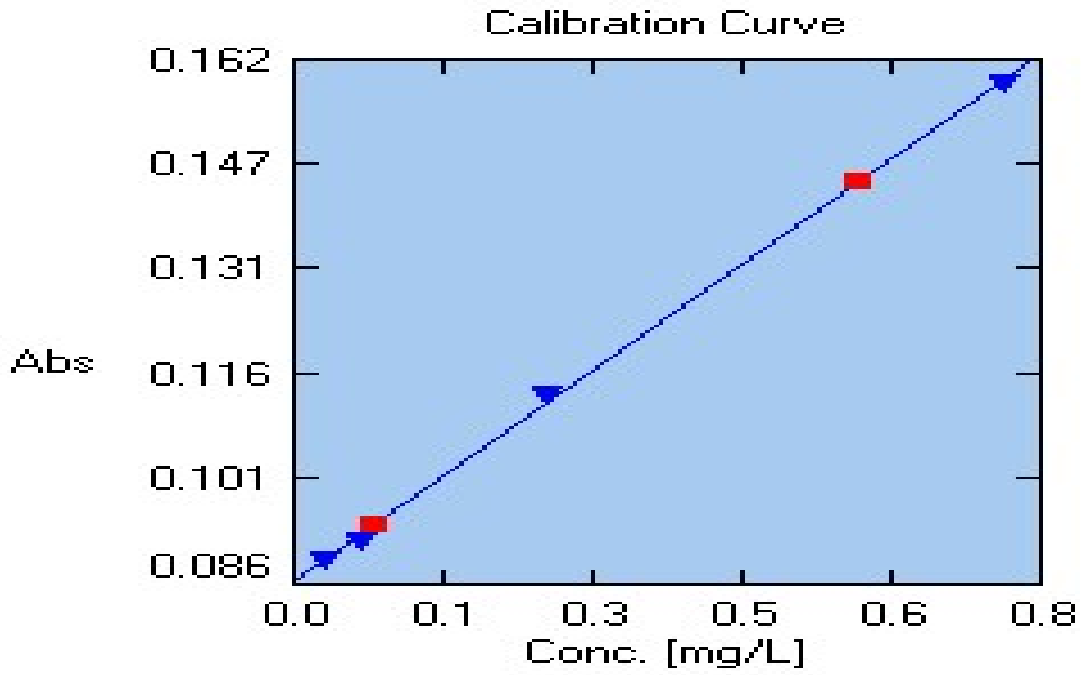
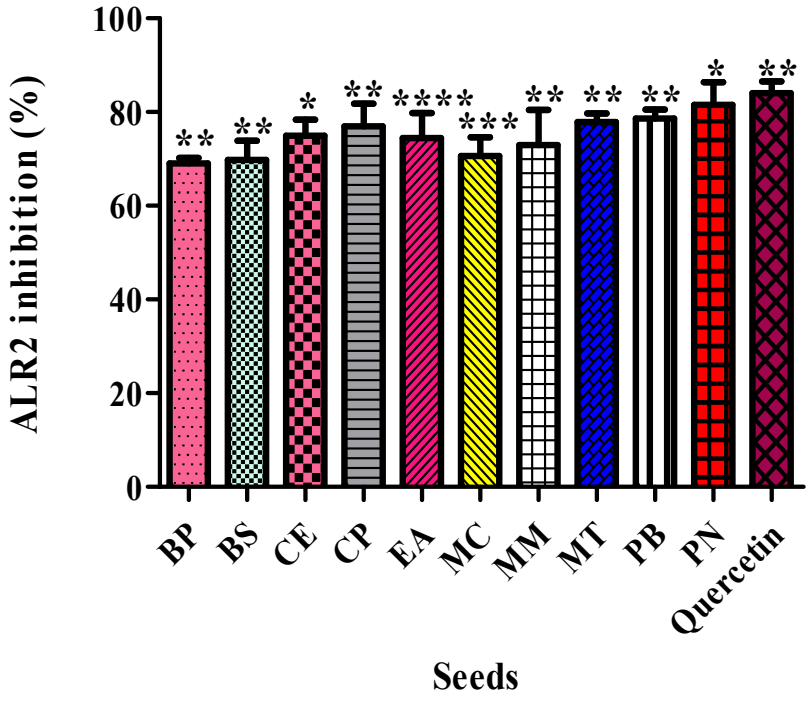


Figure 4.10: **The plots of dose-dependent curves for advance glycation endproducts at varying concentration of the seed extracts between 1.0-0.0078 mg/mL for the values of -4, -3, -2, -1, and 0 on the x-axis. Values are the mean of standard deviation of triplicate experiments**



Protein estimation in the crude enzymes of ALR1 and ALR2



: **Inhibition of ALR2 (%) by the seed extracts and quercetin (positive control). Different asterisks on the error bar indicate significant (*p<0.05*) difference between the seed extracts and the control**

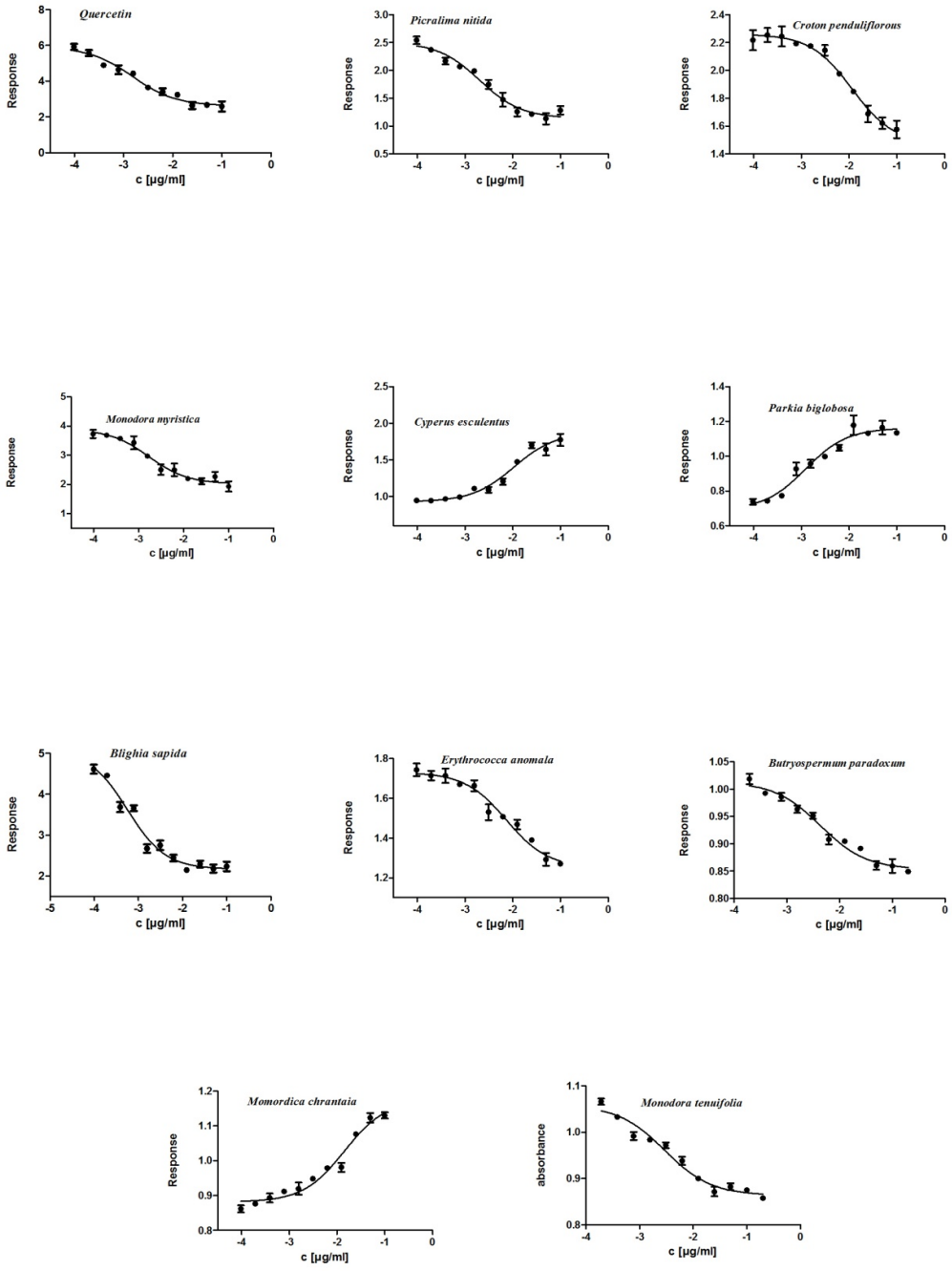
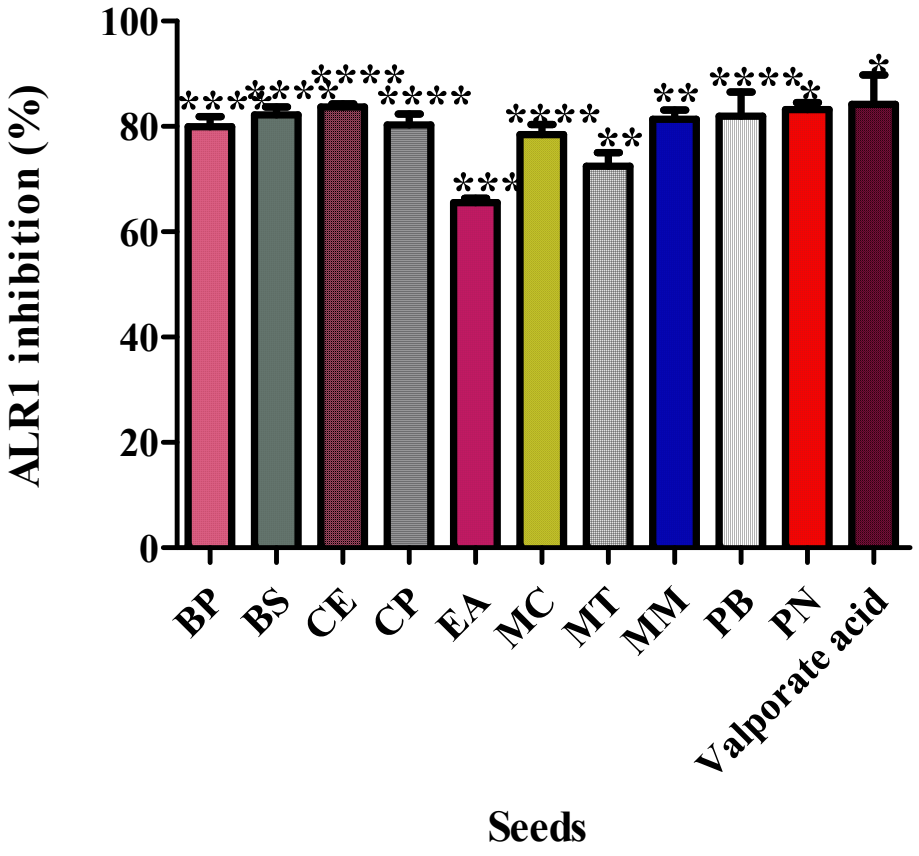
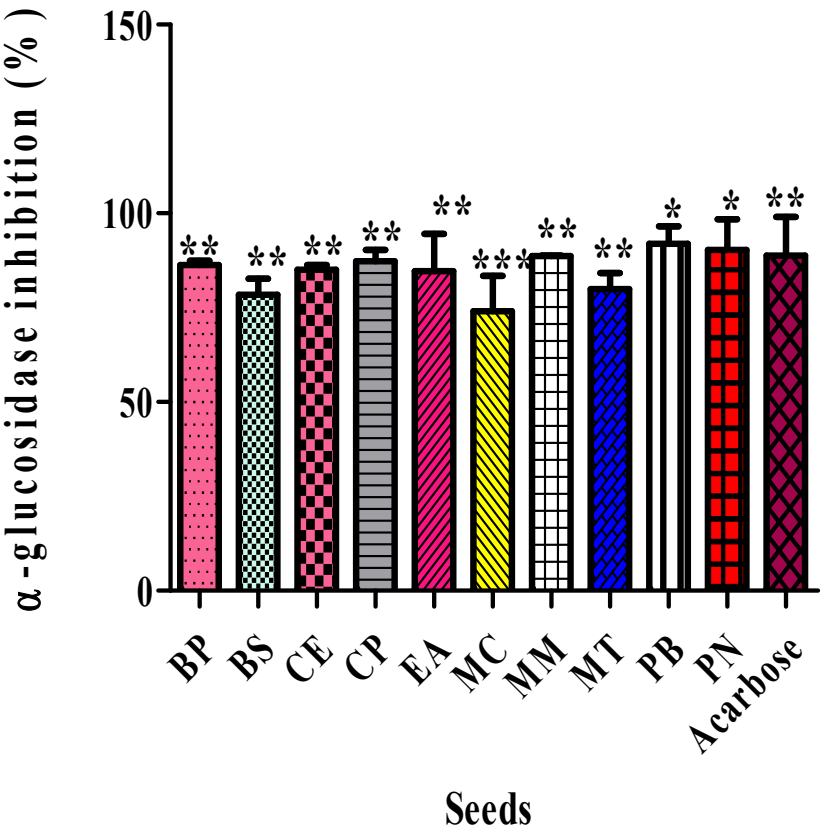


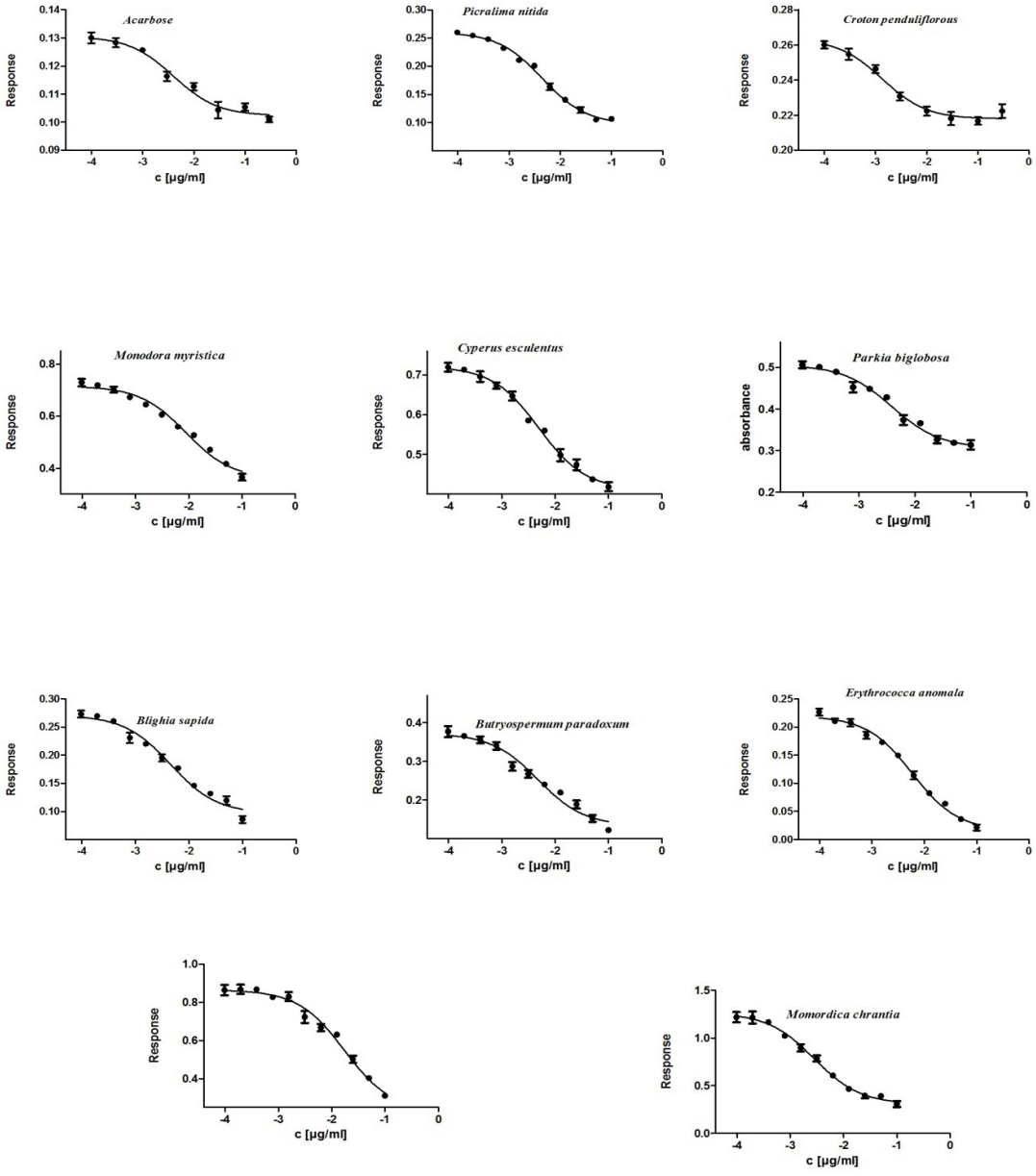
Figure 4.16: **The plots of dose-dependent curves for ALR2 at varying concentrations of the seed extracts between 1.0-0.0078 mg/mL for the values of -4, -3, -2, -1 and 0 on the x-axis. Values are the mean of standard deviation of triplicate experiments**



hibition of ALR1 (%) by the seed extract and valporate acid (positive control). Different asterisks on the error bar indicate significant (*p<0.05*) difference between the seed extracts and the control



Inhibition of α-glucosidase enzyme (%) by the seed extract and acarbose (positive control). Different asterisks on the error bar indicate significant (*p<0.05*) difference between the seed extracts and the control



The plots of dose-dependent curves for α-glucosidase enzyme at varying concentrations of the seed extracts between 1.0-0.0078 mg/mL for the values of -4, -3, -2, -1 and 0 on the x-axis. Values are the mean of standard deviation of triplicate experiments

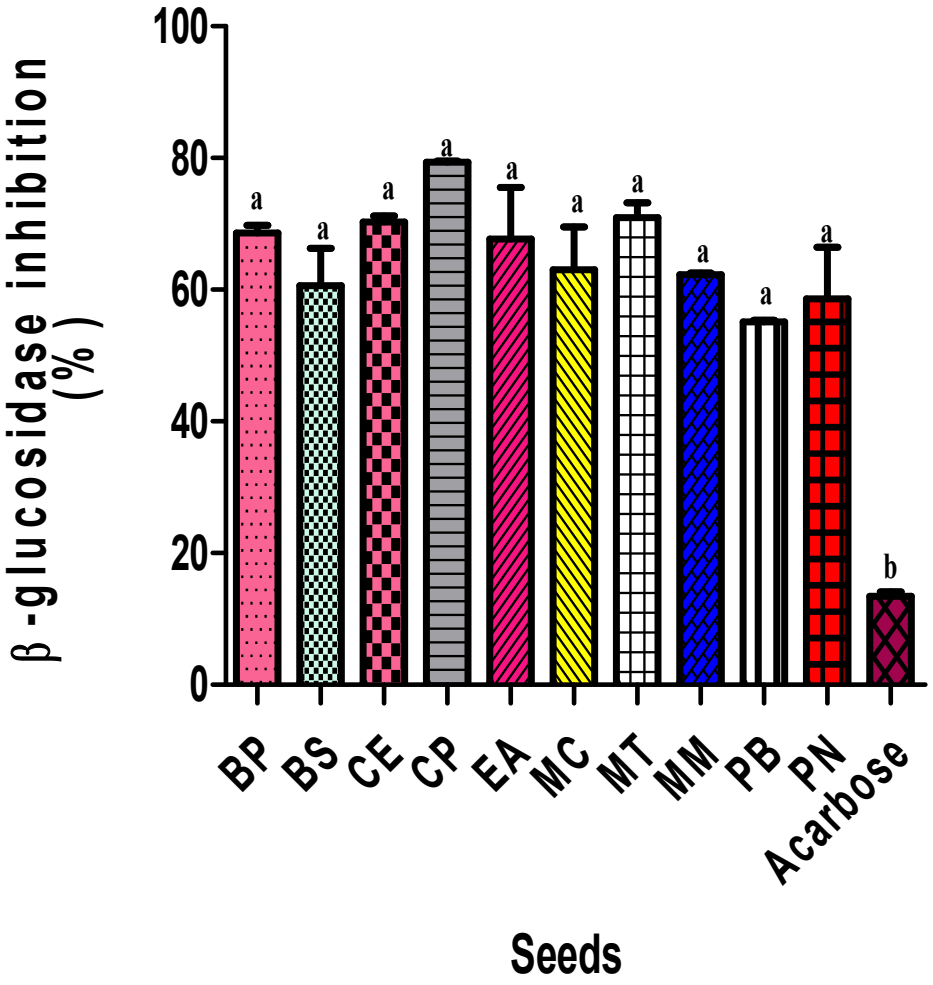


Figure 4.23: **Inhibition of β-glucosidase enzyme (%) by the seed extracts and acarbose (positive control). Different asterisks on the error bar indicate significant (*p<0.05*) difference between the seed extracts and the control**

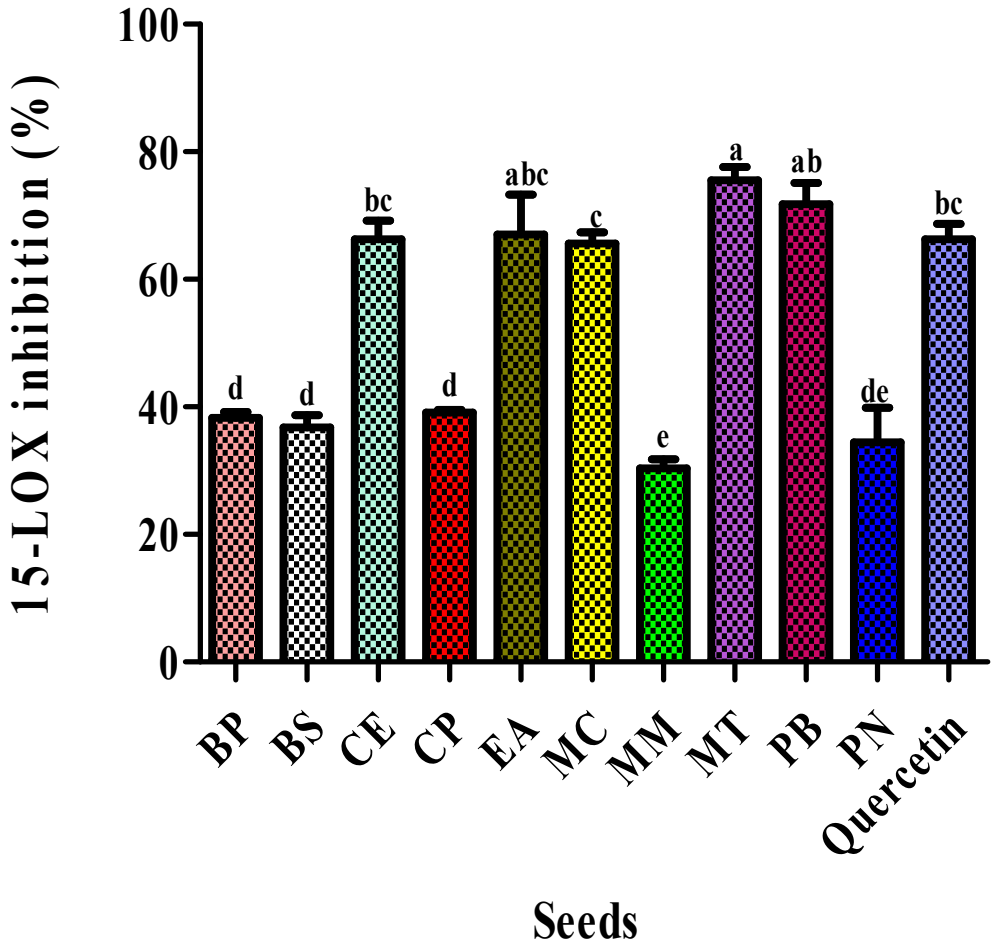
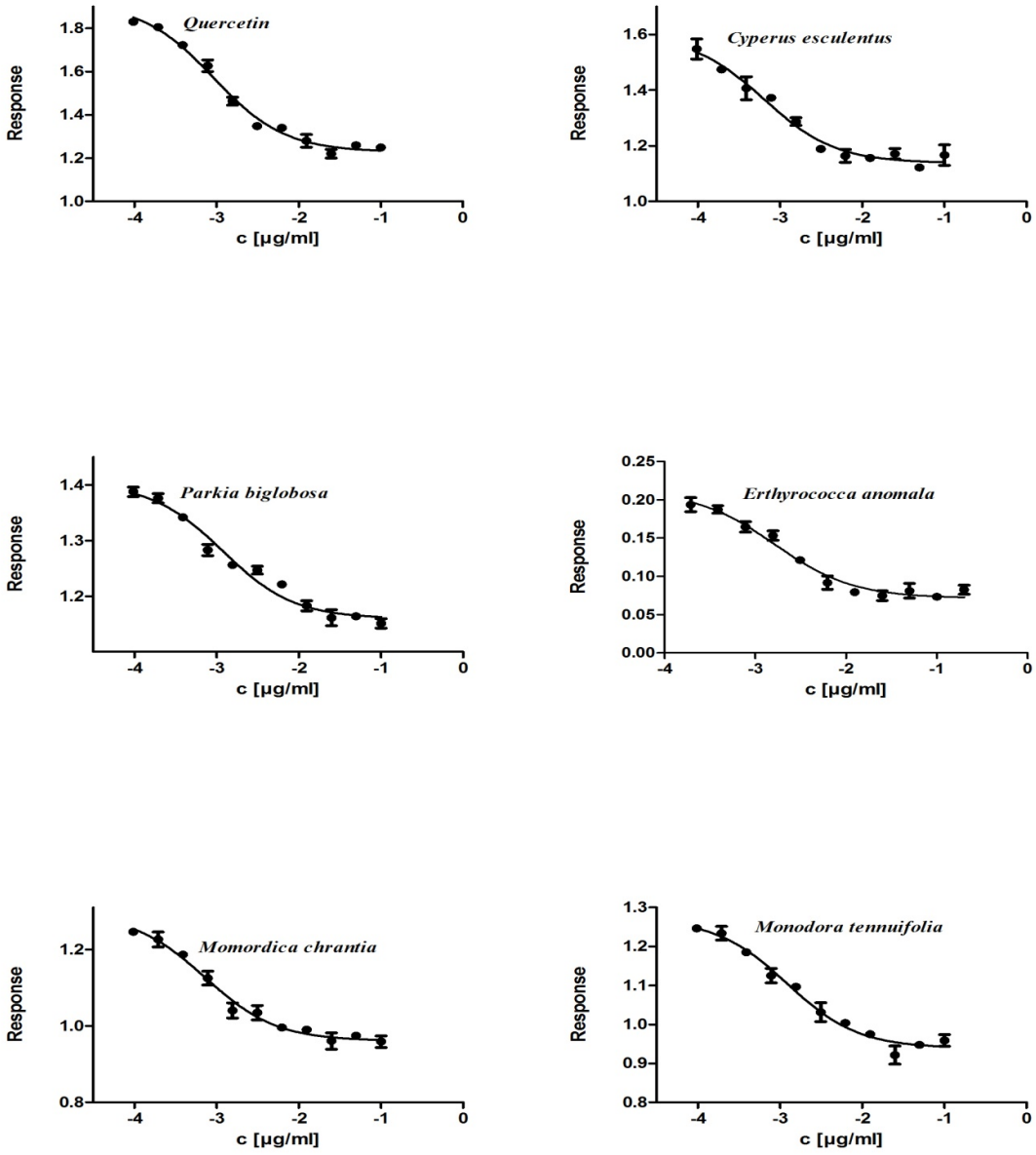
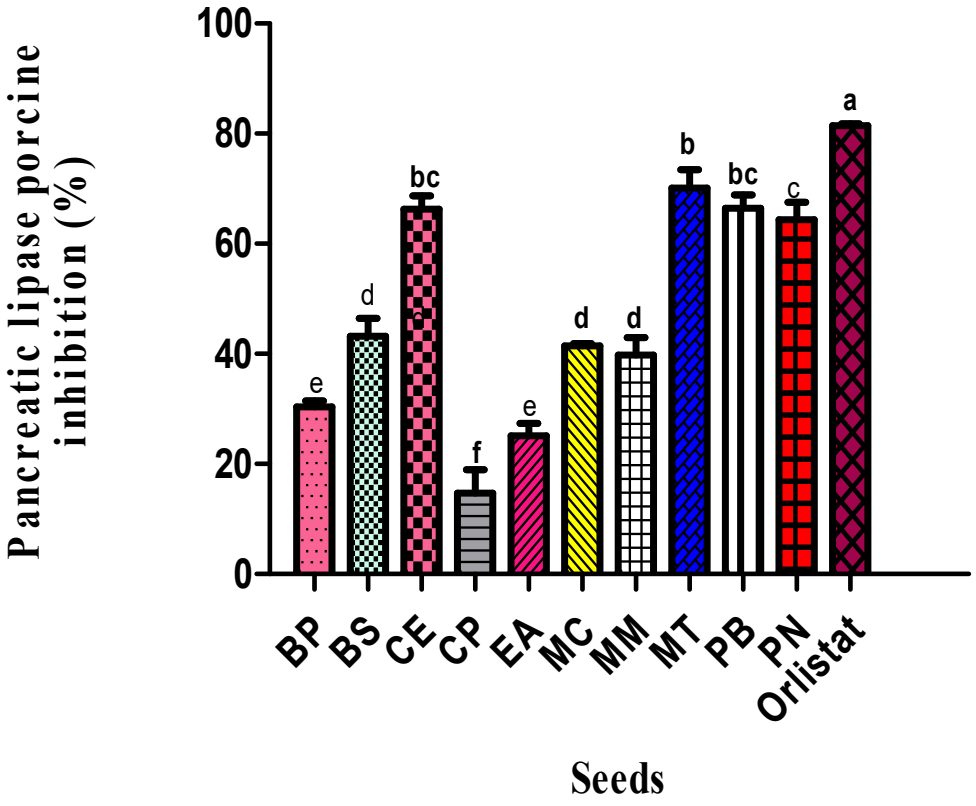


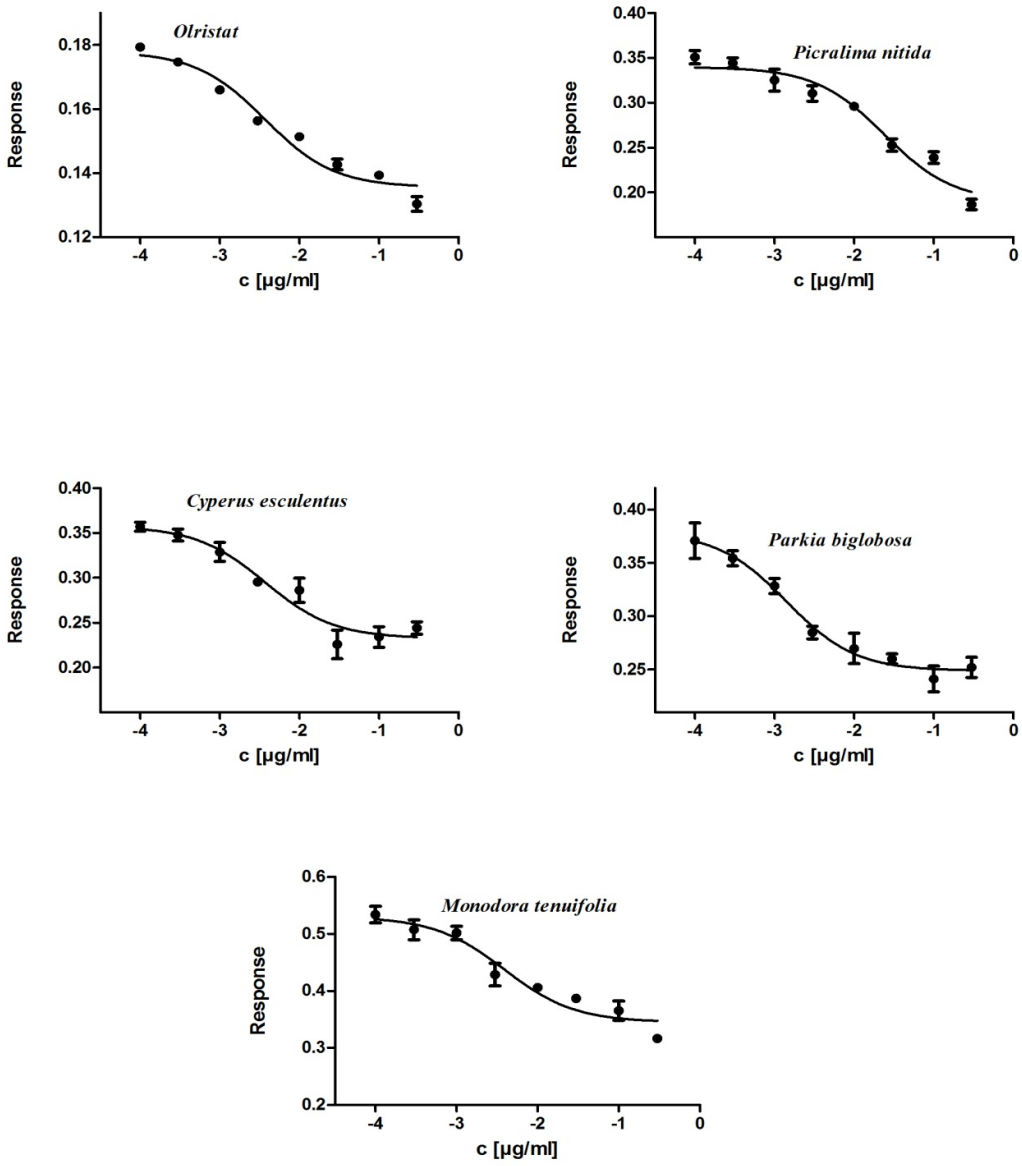
Figure 4.24: **Lipoxygenase enzyme inhibition (%) by the seed extracts and quercetin (positive control). Different alphabets on the error bar indicate significant (*$p<0.05$*) difference between the seed extracts and the control**



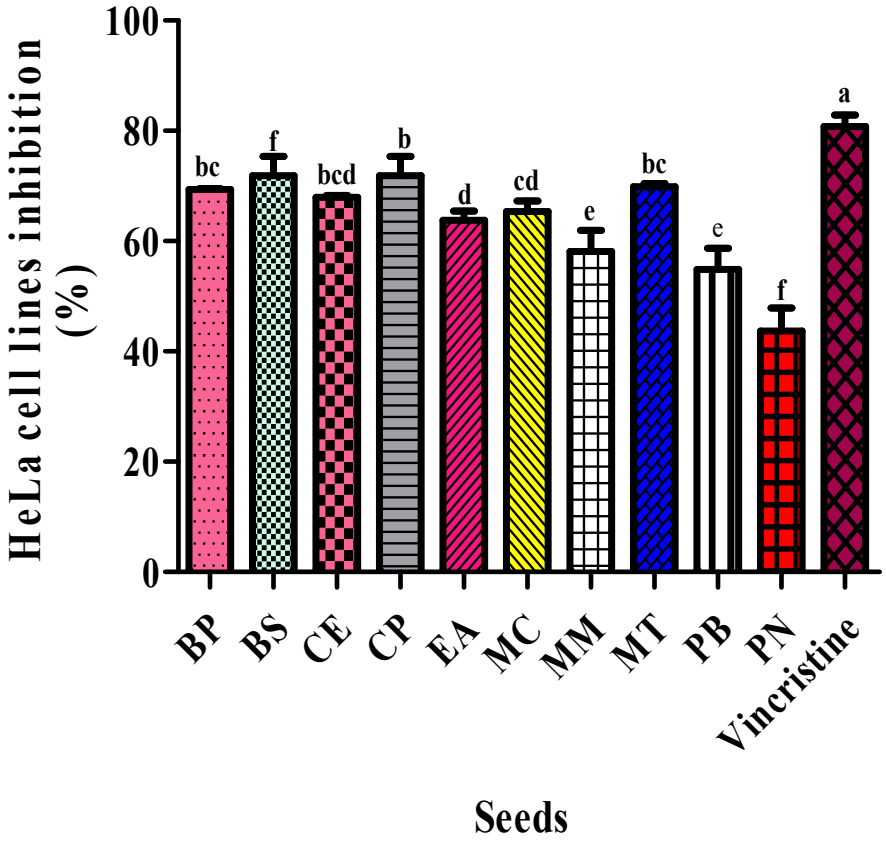
: **The plots of dose-dependent curves for lipoxygenase enzyme at varying concentrations of the seed extracts between 1.0-0.0078 mg/mL for the values of -4, -3, -2, -1 and 0 on the x-axis. Values were the mean of standard deviation of triplicate experiments**



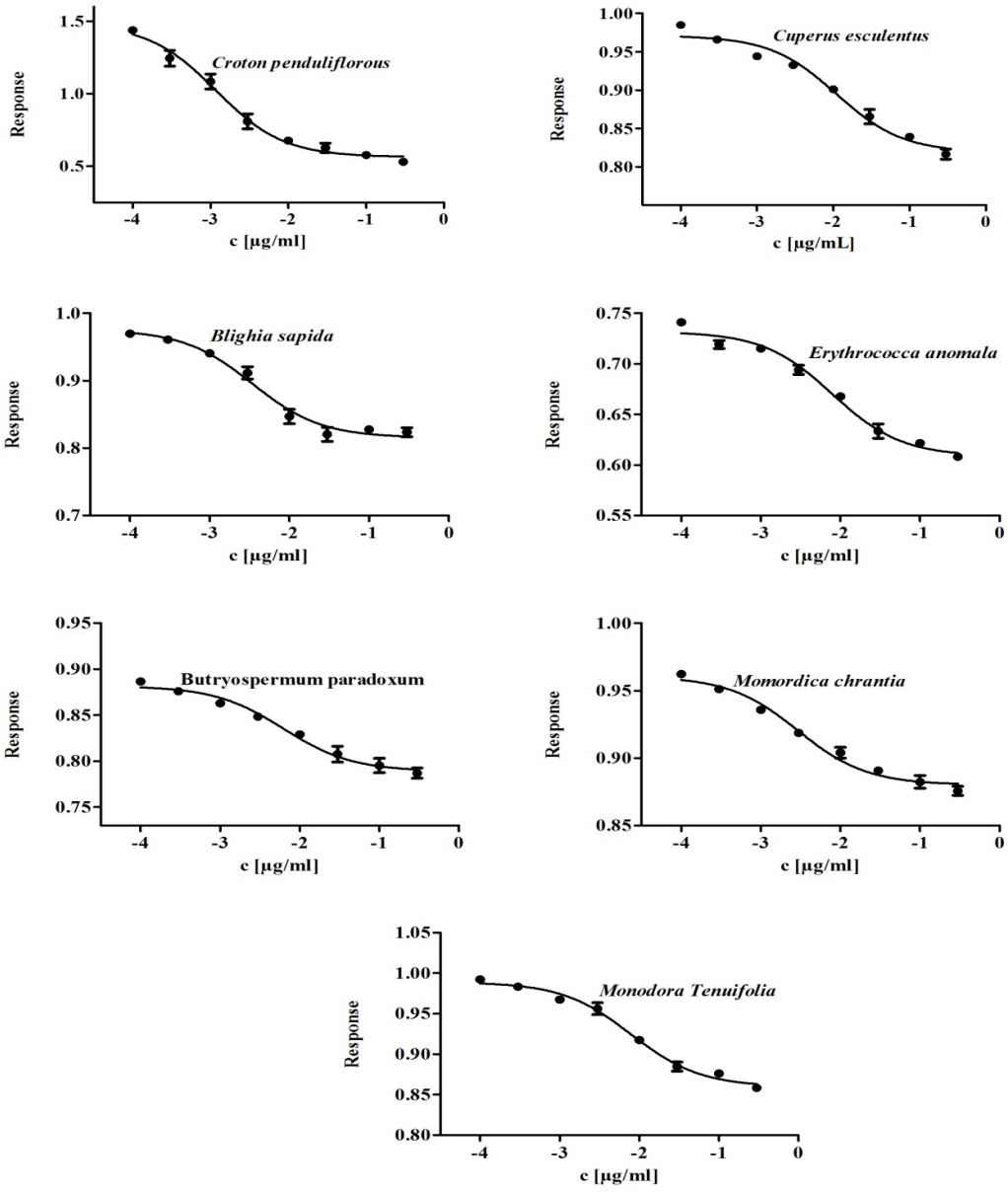
Inhibition of pancreatic lipase porcine (%) by the seed extracts and orlistat (positive control). Different alphabets on the error bar indicate significant (*p<0.05*) difference between the seed extracts and the control



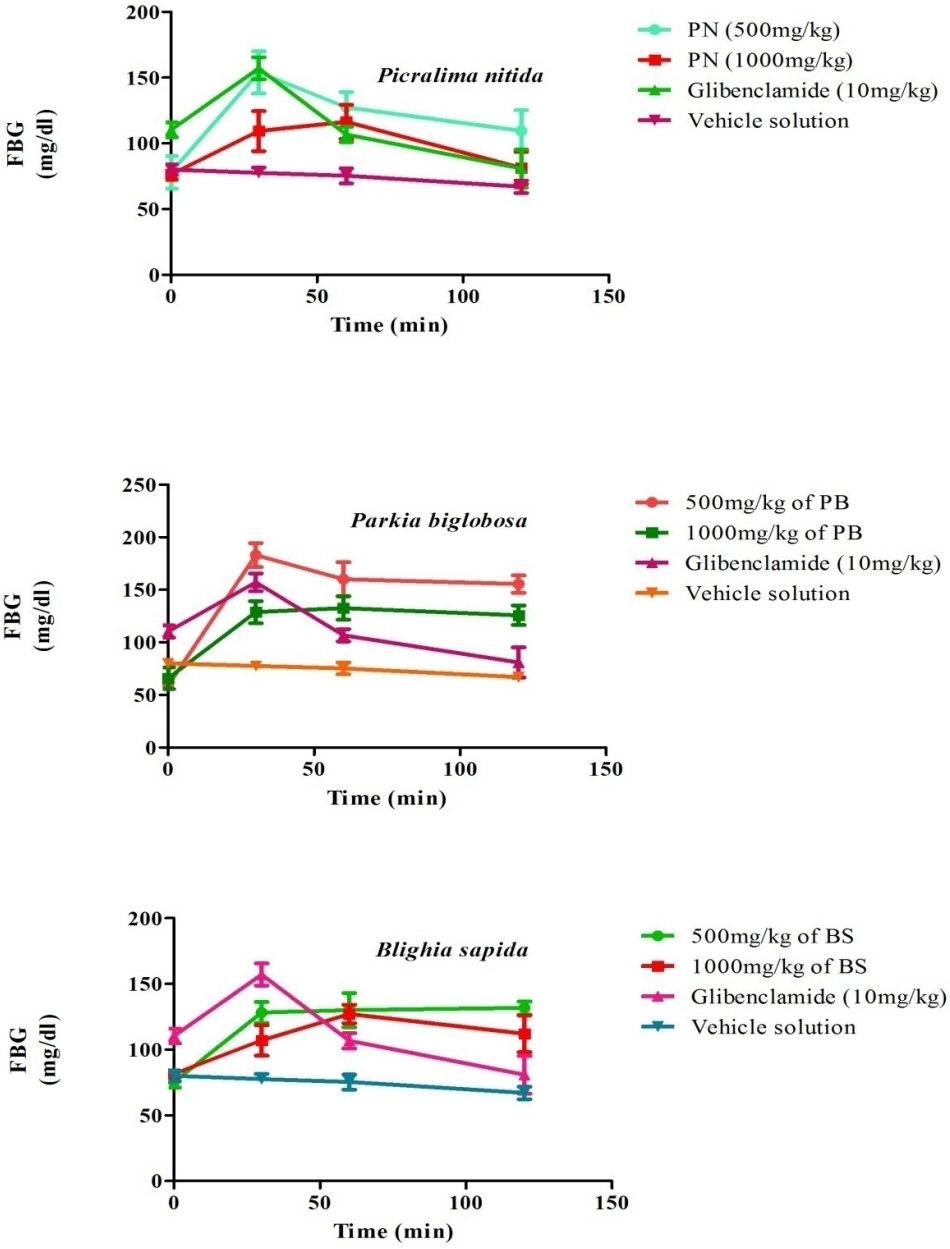
The plots of dose-dependent curves for pancreatic porcine lipase enzyme at varying concentration of the seed extracts between 1.0-0.0078 mg/mL for the values of -4, -3, -2, -1 and 0 on the x-axis. Values are the mean of standard deviation of triplicate experiments



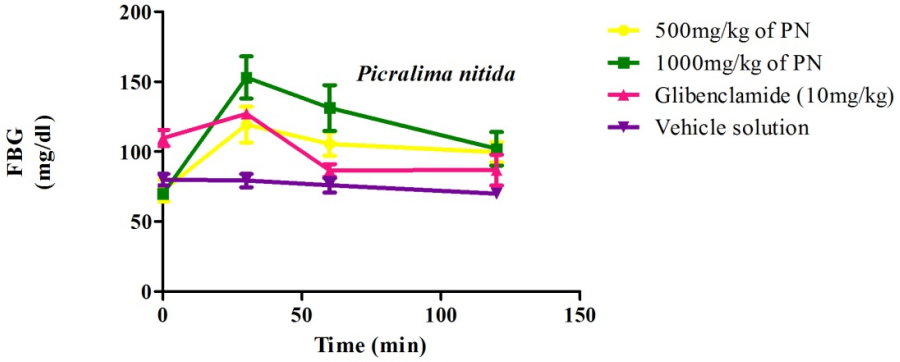
Inhibition of HeLa cell lines (%) by the seed extracts and vincristine (positive control). Different alphabets on the error bar indicate significance (*p<0.05*) difference between the seed extracts and control

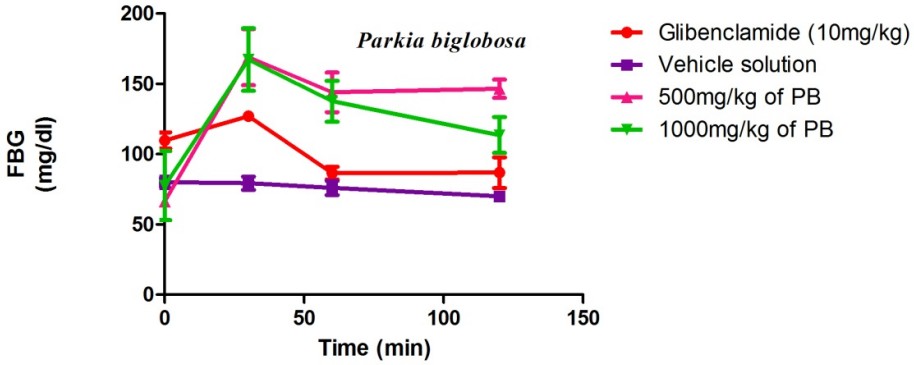


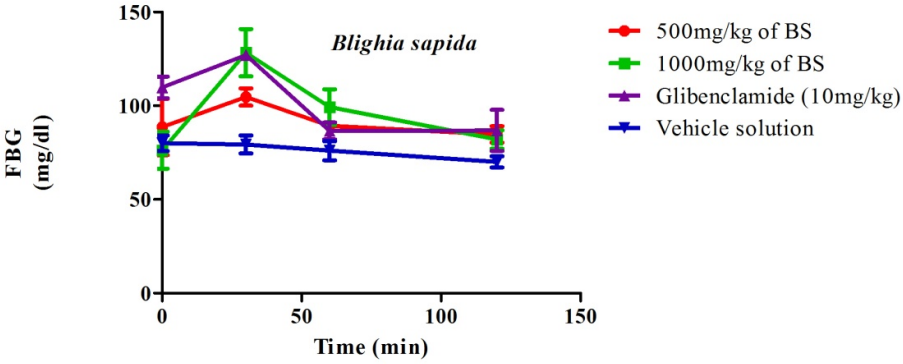
The plots of dose-dependent curves for HeLa cell lines at varying concentration of the seed extracts between 1.0-0.0078 mg/mL for the values of -4, -3, -2, -1 and 0 on the x-axis. Values are the mean of standard deviation of triplicate experiments

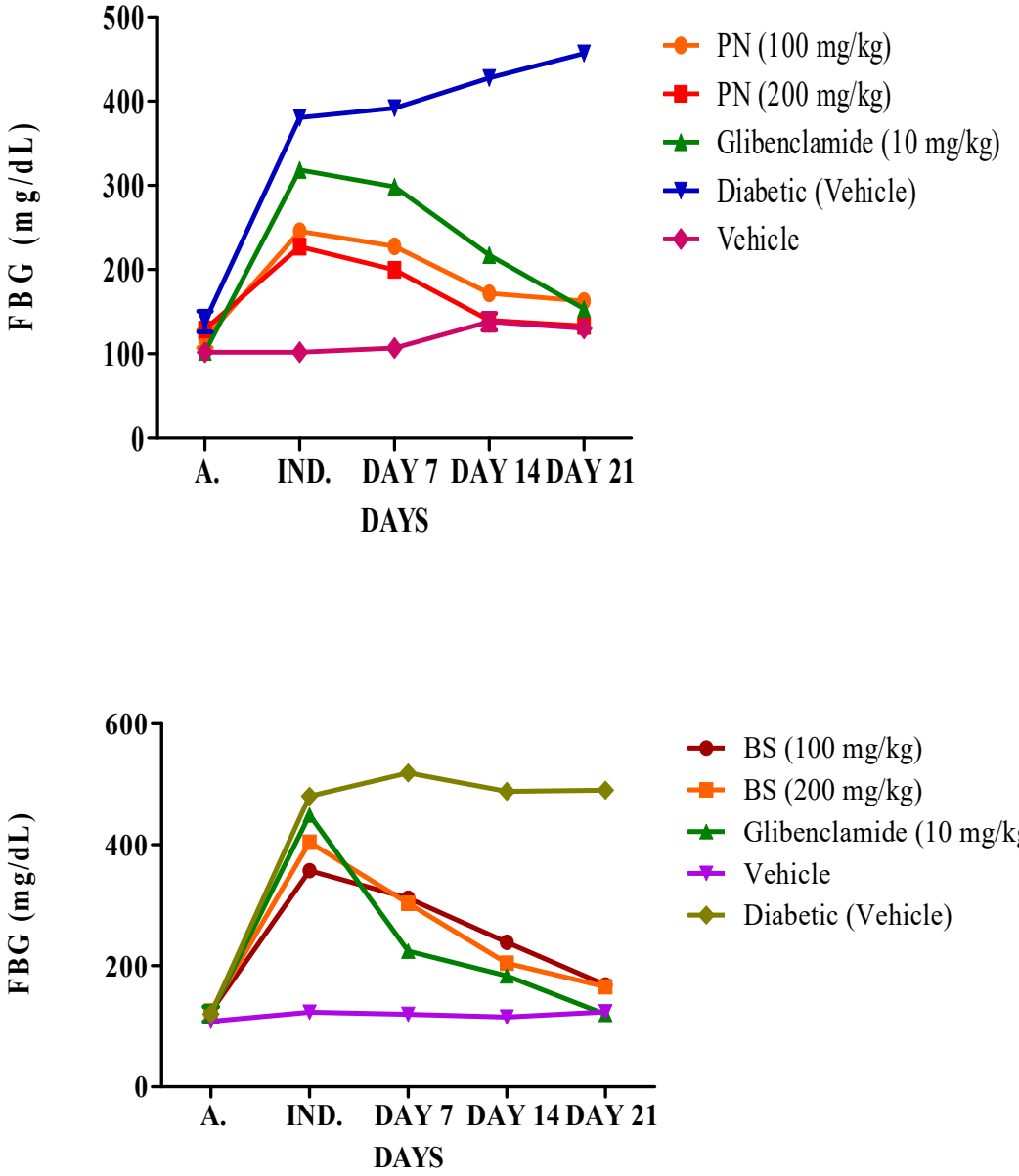


Effect of oral administration of PN, PB and BS at doses of 500 and 1000 mg/kg on blood glucose levels of albino rats loaded with 2 g/kg of glucose. Values are expressed as mean±SD; significances at *p<0.05* was compared to the control

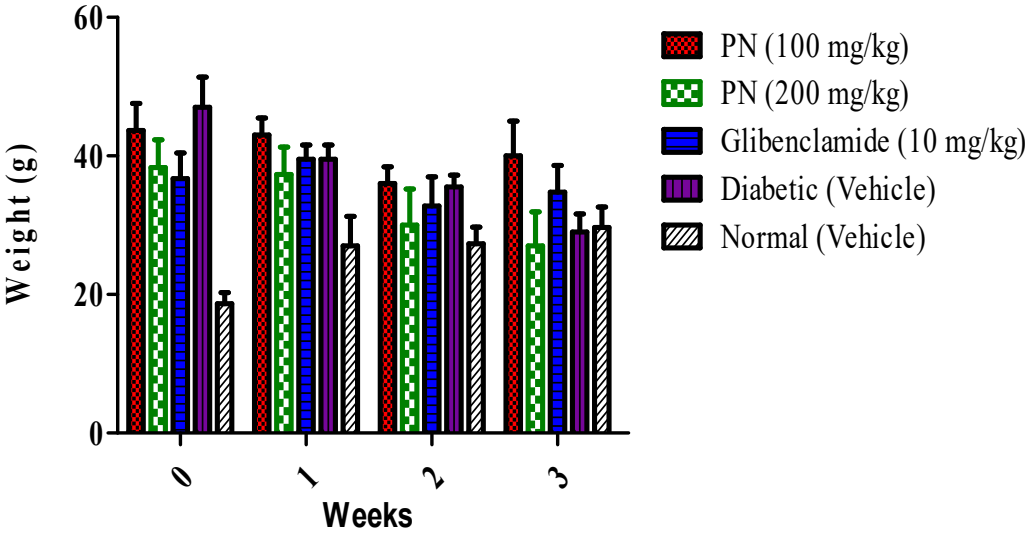

Picralima nitida
FBG (mg/dl)
Time (min)
0
50
100
150
200
500mg/kg of PN
1000mg/kg of PN
Glibenclamide (10mg/kg)
Vehicle solution

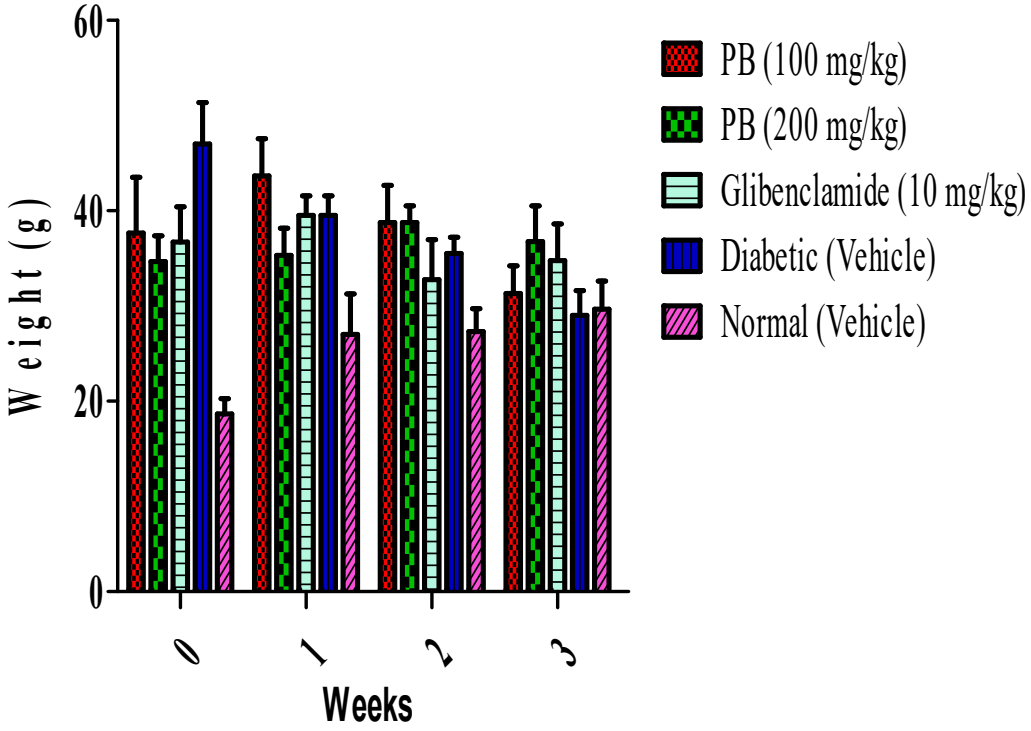

Parkia biglobosa
FBG (mg/dl)
Time (min)
0
50
100
150
200
Glibenclamide (10mg/kg)
Vehicle solution
500mg/kg of PB
1000mg/kg of PB

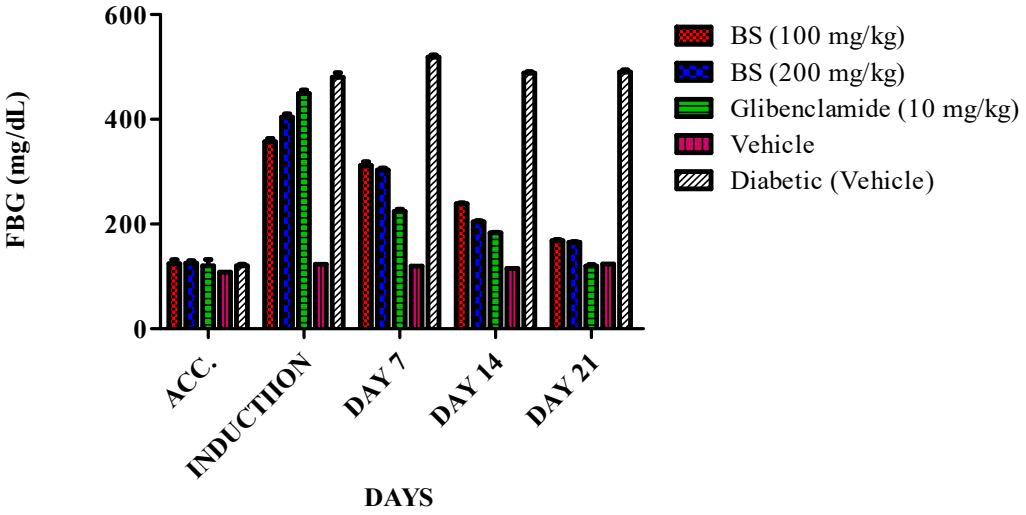

Blighia sapida
FBG (mg/dl)
Time (min)
0
50
100
150
500mg/kg of BS
1000mg/kg of BS
Glibenclamide (10mg/kg)
Vehicle solution

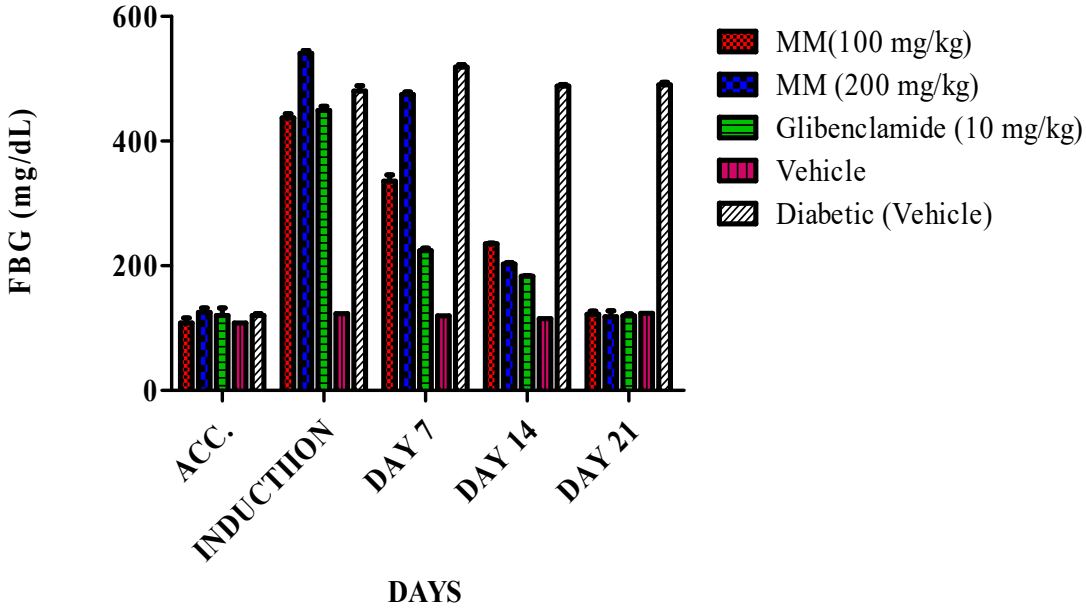


Effects of PN and PB seed extracts on fasting blood glucose of diabetic mice


60
40
20
0
Weight (g)
0
1
2
3
Weeks
PN (100 mg/kg)
PN (200 mg/kg)
Glibenclamide (10 mg/kg)
Diabetic (Vehicle)
Normal (Vehicle)


60
40
20
0
Weight (g)
0
1
2
3
Weeks
PB (100 mg/kg)
PB (200 mg/kg)
Glibenclamide (10 mg/kg)
Diabetic (Vehicle)
Normal (Vehicle)





Effects of PN and BS seed extracts on mean body weight of diabetic mice